HISTOLOGICAL AND PHYSIOLOGICAL ALTERATIONS RESULTED FROM AQUEOUS EXTRACT OF *GREWIA TENAX* ADMINISTRATION IN SWISS ALBINO MICE

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ABSTRACT

Recently in Libya and many other countries, *Grewia tenax* was found to be used traditionally for treatment a variety of diseases as anemia. Thus, it is possible to develop a drug to treat different types of anemia. The objective of this study was to investigate the effect of repeated high dose of aqueous extract of *Grewia tenax* (leaves & fruits) on some hematological parameters (HB , PCV, RBCs ,WBCs & Platelets counts) and some biochemical parameters including serum activities of ALT or SGPT, AST or SGOT, creatinine and urea for testing liver and kidney functions. As well as, the present work aimed to study the histological effects of aqueous extract of *Grewia tenax* administration on liver and kidney tissue. Data were statistically analyzed using student-t test. Selected organs (liver& kidney) from both control and treated animals were examined microscopically for histopathological changes. The results showed that, administration of aqueous extract of *Grewia tenax* induced significant ($p<0.05$) increase in Hb concentration, PCV % and RBCs count. On the other hand, a significant ($p<0.05$) decrease in WBCs count was recorded as compared to control group. However, no significant change in platelets counts. Biochemical parameters revealed significant increase in treated group when compared to control. Microscopic examination revealed signs of pathological alterations which were more pronounced in the liver. This study indicates the possible utility of aqueous extract of *Grewia* in treatment of some blood diseases characterized by decreasing hemoglobin concentration and RBCs count. However, these data were not considered sufficient to assess the safety of this natural plant

Key words: Toxicity aqueous extract of *Grewia tenax*, histopathological, hematological, hepatic and renal functions, mice.

INTRODUCTION

Grewia tenax is a tree spread in African and Southeast Asiatic continent. It belongs to the *Tileacea* family. It is known by its utilization as a medicinal plant **(Khemiss et al.,2006).** Grewia is widely used in traditional Indian medicine to cure jaundice, biliousness, dysentery and the diseases of blood as well as it heal chronic wounds, gastric ulcers, burning sensation, itching and other allergic ailments ***(Khadeer et al., 2009 and Khadeer et al., 2010).***

Hepatoprotective activity of the methanolic extract and the isolated constituents were evaluated against CCl (4) which-induced hepatotoxicity in rats.

The treatment with methanolic extract, EHGL and GAGL at oral doses of 100, 150 and 60 mg/kg respectively with concomitant intraperitoneal injection (1 ml/kg) of CCl(4). Significant reduction in the elevated plasma levels of aminotransferases, alkaline phosphatase as well as incidence of liver necrosis was recorded with the CCl(4)-injection. Histology of liver tissues treated with the extract of Grewia showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration as similar to the normal control (Khadeer et al., 2010). Sisodia et al., 2008 reported that the post treatment of Swiss albino mice with Grewia (700 mg/Kg. body wt. /day for 15 consecutive days) protect liver and blood against irradiation.

Hence, the current study was aimed to evaluate the toxicity properties of repeated dose of aqueous extract of Grewia in *Swiss albino* mice based on evaluation of biochemical and hematological changes as well as pathological changes in liver and kidney tissues.

MATERIALS & METHODS

EXPERIMENTAL ANIMALS:

Male Swiss albino mice (Mus musculus, 2n= 40) weighing 20-27gm and 12 weeks old were used in this study. They were obtained from experimental animal house Arab Tebia University. The animals were randomly selected and kept in their cages for 5 days prior to experiment for acclimatization in the laboratory conditions.

The animals were housed in air conditioned room at 22±2 °C and maintained in metal cages with regular light dark cycle (photoperiod of 12hr/days) and free access food (commercial pellet) and tap water ad libitum.

Preparation Of Plant Extract:

Grewia tenax (leaves & fruit) were purchased from a local herb grocery (EL Badia Libya). The plant were air dried and milled into powder (5gm of the dried plant was mixed with 200 ml of distilled water) and

left the mixture overnight at 20-22°C. The mixture was then filtered through four folds of cheesecloth. Fresh preparation was used every day, according to the method of **Somda et al. (2007).**

EXPERIMENTAL PROTOCOL:
The mice were randomly divided into 2 groups of 10 animals each. Group I received buffered normal physiological saline (0.2ml/mouse once daily orally for 10 successive days) and served as control, whereas mice in group II received aqueous extract of Grewia at the dose of 2000mg/kg b.w./day for 10 successive days (i.e. 0.2ml of extract/mouse/day).

Body weight: Initial and final body weight were measured and the changes in the mean gain in body weight between the successive intervals was estimated.

Hematological and Biochemical Studies:

Twenty four hours after the end of experimental period (10 days) unanaesthetized mice from both control and experimental groups were humanely scarified by slaughtering. The peripheral blood samples were collected from the neck blood vessels into clean dry, sterile container containing EDTA (1mg/ml fresh blood). Uncoagulated blood samples were used for hematological analysis. All measurements were examined within two hours after blood collection. RBCs count, hemoglobin content, haematocrit value, platelets count and total white blood cell count were counted and calculated according to **Dacia and Lewis, 1995).**

For biochemical parameters, the blood samples were collected into free uncoagulated containers and allowed to clot for 2 hr at room temperature. They were centrifuged at 3000 rpm for 10 minutes and the supernatant serum was collected in Eppendorf capped sterile tubes and utilized for estimation various biochemical parameters. Serum activities of alanine aminotransferas (ALT or GPT) and asparatate aminotransferease (AST or GOT) were determined according to the method recommended by Reitman and Frankel (1957). Serum creatinine and urea were determined according to procedures of Henry (1974) and Fawcett and Scott (1960) respectively.

Histopathological Examination:

The rats were examined daily, including weekends, for deaths and illness. Animal were autopsied at the end of the experimental period. Visceral organs were examined grossly in all the autopsied animals. The portions of livers and kidneys were fixed in 10% neutral buffered formalin, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. Sections of 5um thickness were stained with Ehrlich

haematoxylin and eosin (H&E) according to Bancroft& Gamble (2002). Histological specimens were examined by light microscopy and histopathological lesions were recognized in the H&E staining sections and then photographed.

STATISTICAL ANALYSIS:-

Data obtained was analyzed using t test. The results were presented as means ± standard deviation (SD). P value < 0.05 was considered statistically significant.

RESULTS & DISCUSSION:

Administration of aqueous extract of Grewia at a dose of 2000mg/kg b.w. did not result in any mortality. However, no significant changes in the behavior and external feature were noticed.

Aqueous extract of Grewia exhibited significant increase in the body weight gain as compared to control group. It was noticed that the final body weight of treated animals increased by 9.20 % while the finale body weight of control mice increase by 1.65 % than their initial body weight (table I).

Table (I): Effect of aqueous extract of Grewia on Body weight gain

Treatment	Initial body weight (g)	Final body weight (g)	The mean of the changes in body weight (%)
Control	20.85± 2.27	21.20±3.00	1.65 %
Grewia	21.o7±1.78	23.01±3.621*	9.20%*

Each value represents the mean of body weight of survival in each group.
*significant as compared to Initial body

Hematological and biochemical Results:

The hematological results of control and treated mice are given in table II and Fig (1). Administration of aqueous extract of Grewia induced a marked increase in haemolglobin conce-ntration, PCV, RBCs, while no significant change in the platelet count and significant decrease in the total WBC count were noticed comparing to control values. Similar observation was noticed by Reem (2009) who study the effect of aqueous extract of Grewia on rats with hemorrhagic anemia and iron-deficient anemia.

Table (II): Effect of aqueous extract of Grewia on hematological parameters in mice

Treatment	Hb (g/dl)	PCV (%)	RBCs (10^6/mm^3)	PI (10^3/mm^3)	WBCs (10^3/mm^3)
Control	12.49± 0.09	41.21±0.29	7.16±0.31	7.14±0.21	6.39±0.80
Grewia extract	14.2±0.14*	46.07±0.47*	8.79±0.13*	6.92 ±0.12	5.4±0.13*

Each value represent mean of ten values ± SD
*significant as compared to control group
Hb: Hemoglobin concentration
PCV: packed cell volume. RBCs: Red blood cells PI: platelets
WBCs: White blood cells

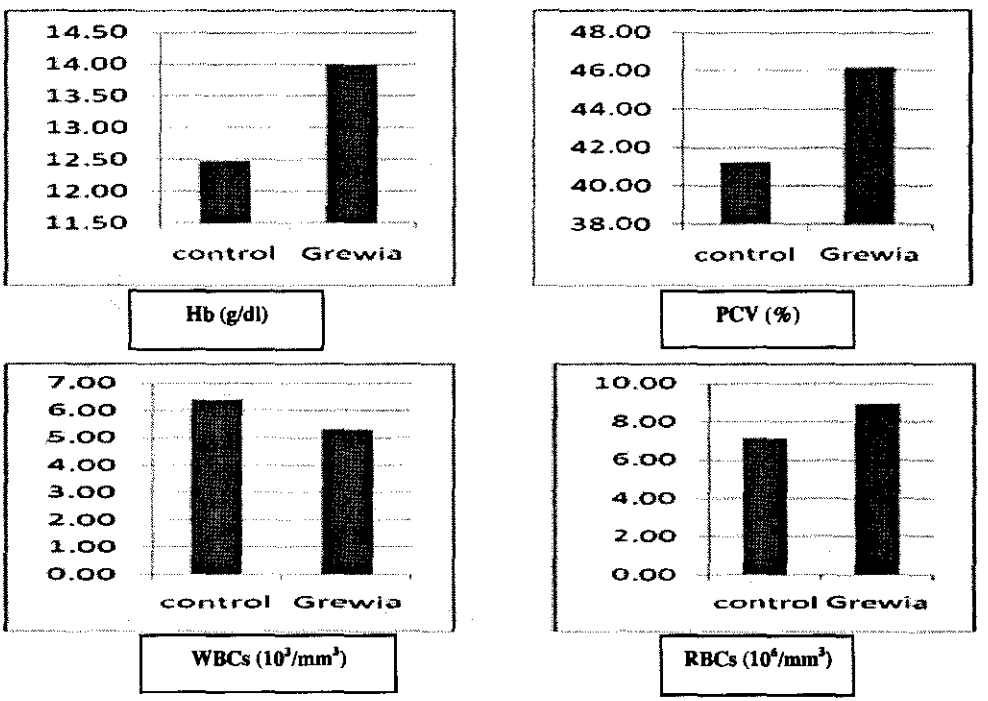


Fig. (1): Effect of aqueous extract of Grewia on hematological parameters in mice

The serum biochemical analysis presented in table III and Fig (2) indicated that the treatment with aqueous extract of Grewia induced marked changes in the liver and kidney functions as detected by significant elevation of serum activity of ALT and AST compared to control group. This may be attributed to severe damage in hepatocytes (Hayes, 2006). Significant increase ($p < 0.05$) in creatinine and urea levels was recorded in animals treated with aqueous extract of Grewia when compared to the control. Hence, the possibility of renal injuries could be suspected

Table (III): Effect of aqueous extract of Grewia on hepatic and renal functions.

Treatment	ALT or SGPT (Iu/L)	AST or SGOT (Iu/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	35.0±1.0	33.0±1.0	25.0±1.0	0.09±0.1
Grewia	36.09±1.1 *	33.9±1.04*	30.05±0.1*	1.1±0.6 *

Each value represent mean of ten values ±SD
ALT: alnine aminotransferase AST: aspartate aminotransferase
*Significant as compared to control group

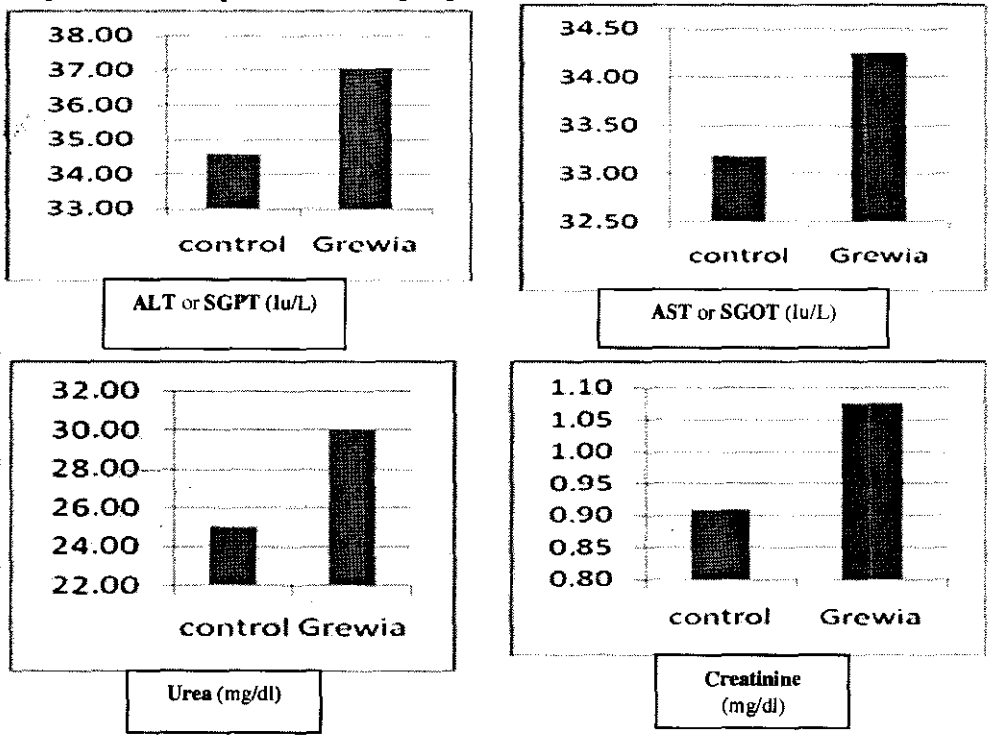


Fig (2): Effect of aqueous extract of Grewia on hepatic and renal functions

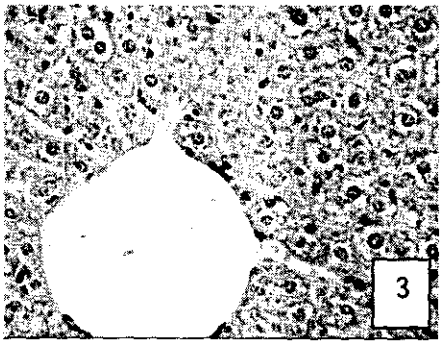
Pathological Findings:

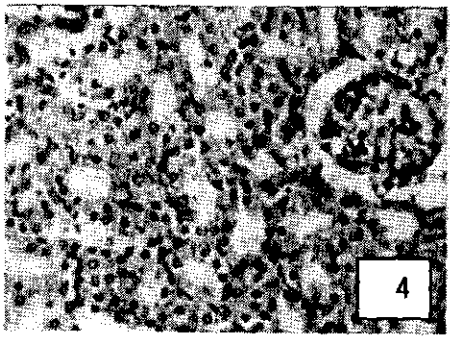
Pathological changes were not observed in all animals of control group. However, mild pathological alterations in aqueous extract of Grewia treated animals were observed. These animals showed pale liver; in addition obvious hypertrophied spleen and mild congestion in heart in some animals were evident

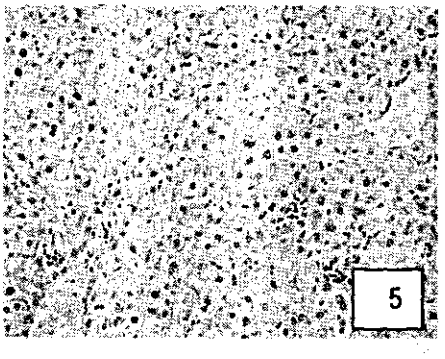
Histopathological Results:

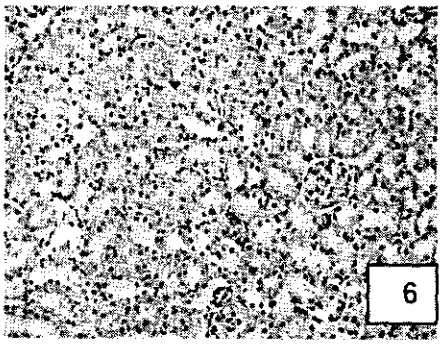
No signs of pathological alterations could be detected in the liver and kidney of control mice (Fig. 3&4), Histopathological examination revealed that repeated high dose administration of Grewia extract induced focal pathological alterations in liver. The changes were more pronounced in the cytop-

lasm.Degenerated hepatocytes with high vacuolated cytoplasm were frequently observed. In addition, hepatocytes with pyknotic or karyolytic nuclei were seen. Many inflammatory cells infiltrations were also noticed (Fig 5). The kidney of mice treated with Grewia extract showed hydropic degeneration with cloudy epithelial cell lining the convoluted tubules. However, most glomeruli appeared intact with normal feature (Fig.6). Our data is supported by Khemiss et al. (2006) who noticed histopathological and cytotoxic signs in gut of rat treated with high dose (20-30 mg/ml) of aqueous extract of Grewia tenax fruit.









Figures (3 &4): Liver & kidney of control mice showing normal architecture (H&E stained,org. mag.X400).

Figure (5):Liver of mice treated with aqueous extract of Grewia showing degenerated hepatocytes with vacuolated cytoplasm, many inflammatory cells infiltration (H&E stained,org. mag.X200).

Fig.(6): kidney of mice treated with aqueous extract of Grewia showing hydropic degeneration in renal tubules (H&E stained, org. mag.X200).

CONCLUSION:

The therapeutic properties of aqueous extract of Grewia in the treatment of some hematological problems require its further exploration involving laboratory and clinical investigations. So further studies may still needed to establish which dose levels of aqueous extract of Grewia are effective but not toxic in mice until these data is available. It is concluded that the available data are insufficient to support the safety of this extract. Therefore, such doses may be not safe for daily repeated administration and causing some histopathological alteration that lead to disturbance in liver and kidney functions.

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الملخص العربي

التغيرات النسيجية و الفسيولوجية الناتجة عن تجريع المستخلص المائي لنبات القضيم في الفئران السويسرية البيضاء

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يستخدم نبات القضيم شعبيا في علاج كثير من الأمراض مثل الأنيميا ولهذا تهدف هذه الدراسة إلى بيان تأثير هذا وبعض المعايير الكيميائية Hb, RBCs, WBCs, & Plateletes لقياس وظائف النبات على مكونات الدم وكذلك تهدف الدراسة إلى بيان تأثير المستخلص المائي الكبد و الكلية لنبات القضيم على نسيج الكبد و الكليةز GOT, GPT , Creatinine & urea وأستخدم في هذه الدراسة عدد عشرون من ذكور الفئران البالغة و قسمت إلى مجموعتين المجموعة الأولى أعطيت عن طريق الفم المستخلص المائي لنبات القضيم (2000 مليجرام/كجم من وزن الجسم) (0.2 مل من المستخلص المائي جرعة واحدة يوميا لمدة عشرة أيام متتالية)، المجموعة الثانية (الضابطة) أعطيت جرعة مكافئة من محلول فسيولوجي. أظهرت النتائج أن المستخلص المائي لنبات القضيم ,PCVأدى إلى ارتفاع معنوي (ذو دلالة إحصائية) في كلا من نسبة الهيموجلوبين، عدد خلايا الدم الحمراء بينما أدى إلى نقص في عدد الصفائح الدموية ونقص معنوي في عدد خلايا الدم البيضاء وتغييرات معنوية في وظائف الكبد والكلي وأظهرت النتائج الهستوباثولوجية حدوث تغيرات في نسيج الكبد والكلى و كانت أكثر وضوحا فى الكبد عنها فى الكلى.

تشير هذه الدراسة أن نبات القضيم ممكن أن يكون علاج واعد لكثير من أمراض الدم خاصة تلك التي تؤدى إلى نقص في نسبة الهيموجلوبين وعدد خلايا الدم الحمراءو مع ذلك هذه المعلومات لا تعتبر وافيه لتدعيم هذا النبات كعلاج طبيعي آمن لأمراض الدم مثل الأنيميا ويلزم له كثير من الدراسات الأكلينيكية والمعملية للتوصل إلى الجرعة التي يمكن أن تؤدى إلى تحسين صورة الدم بدون حدوث خلل في نسيج الكبد والكلى ولا يجب أن يستخدم بطريقة عشوائية في العلاج الشعبي.