Study The Probable Effects of *Tinnas grewia* Extract on Complete Blood Count and Serum Antioxidant Enzymes Levels in Rats

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Abstract

The aim of this study is to investigate the probable effects of *Tinnas grewia* extract (**TGE**) on complete blood cells and serum antioxidant enzymes level in rats by the dose of (2,4,6 and 8ml orally/day). Thirty 30 male albino rats about (150±10g) were used in this study, and then divided into five equal groups each (6 rats). The first one considers a control negative group that fed on a basal diet all the time during the experiment, the other groups (24 rats) were fed on a stander diet + the Oral doses of TGE for (4 weeks). Complete blood cells count levels (HB, HCT, **RBCs** and **PLT**) showed significant increases compared with the negative control group. **WBCs** showed a decrease. For antioxidant enzymes (**SOD**, **CAT**, **GSH** and **GPx**) were significantly increase comparing with control (-ve).

Keywords: *Tinnas grewia*, Wight blood cells, Red blood cells, Hemoglobin, Antioxidant enzymes

Introduction

Grewia tenax is a plant that belongs to the family of *Tiliaceae*. It is an important species as it is a good source of fiber, fuel, lumber and a domain of usual drugs that repair some critical diseases and an anti-microbial properties. *Grewia tenax* is a tree spread in Africa and Asia. The plant parts are rich in amino acids, minerals and some pharmacological constituents **(Sulieman and Mariod , 2019).** There are a lot of active components that have been secluded from different species of this plant such as alkaloids, flavonoids, triterpenoids, steroids, lignans, organic acids, phenolic compounds, glycosides , lactones and anthocyanin. The extracts from the various plants, which are expectantly secure and exhibited some biological effects such as anti-oxidant, anti-bacterial, hepatoprotective action , anti-inflammatory, anti-emetic, anti-malarial and anti pyretic activities **(Wali *et al.*, (2012).**

It is used as a curative plant in a lot of countries for medical purposes **(Khemiss *et al.*, 2006)** and **(Gebauer *et al.*, 2007).** *G. tenax* is a multipurpose plant species that are used for cured some diseases because it has antibiotic effects **(Sharma and Patni, 2012).** *G. tenax* fruits contain a lot of nutrients like vitamins, minerals and amino acids. Also, some bioactive compounds, such as flavonoids, phenolic compounds, tannins and anthocyanins (**Zia-Ul-Haq *et al.*, 2013).** *G.tenax* is used as traditional medicine to cure the wounds, burning, allergic ailments, itching, gastric ulcers and hepatic disorders **(Khadeer *et al.*, 2009)** and **(Khadeer *et al.*, 2010).** And for, bone strengthening, tissue healing, anaemic children and improving fertility in females **(Sharma and Patni, 2012).** All plant parts of *G.tenax* have different medical effects for example, fruit possess

antioxidant and radio protective activates. Leaves have anticancer, antiemetic activities and antimicrobial action, Stem possesses anti-inflammatory and analgesic activities **(Zia-Ul-Haq *et al.*, 2013).**

Plant medicines are integral therapy that uses a lot of plants to avoid disorders in various countries all over the world as therapeutic agents in traditional medicines (**Kumar *et al.*, 2012).** But there is not enough review of the literature for their probable toxic and side effects, so we need more searches about this point **(Monfared, 2013).**

Anaemia is a widespread nutritional deficiency disease and secular as a big health problem which that affects developed countries. According to the reports of WHO, one-third of the universal populations more than two billion are anaemic because of the imbalance in their feed intake from nutritious **(Shubham *et al.*, 2020).**

There are some kinds of anaemia such as:

- Deficiency production of red cells (aplastic anaemia).
- Deficiency in haemoglobin synthesis (iron deficiency anaemia).
-Deficiency of maturation (megaloblastic anaemia).
- Genetic deficiency of haemoglobin maturation (thalassemia).
- Synthesis of abnormal haemoglobin (haemoglobinopathies).
- Physical loss of red cells (haemolytic anaemia's) **(El-kenawy, 2019)** and **(Abbas, 2020)**

Materials and Methods

Materials:

Plants: Fresh *Tinnas grewia* were gained from Sudan.

Rats and diets: Thirty male albino rats weighting 150±10g were used in this study.

Chemicals: for biochemical analysis of serum kits were purchased from Gama Trade Company for Chemicals, Cairo, Egypt. The basal diet is prepared as the following: **(**10% protein, 10% corn oil, 1% vitamin mixture, 4% mineral mixture, 0.2% choline chloride, 0.3% methionine, 5% cellulose, and 69.5% corn starch) **(Campbell,1963)** . The vitamin mixture was according to **(Hegested *et al.*, 1941)**, and the salt mixture was according to **(Drury and Wallington, 1980).** Cholesterol containing diet was prepare by 2% cholesterol to the basal diet.

Methods:

Preparation of plant extract:

Ripened fruits of **TG** were purchased from Omdurman for the seeds in Sudan. The fruits were authenticated by the National Council for Research, Sudan, then, washed well. 500g of the fruits were soaked in 1 liter of distilled water, in a beaker with the volume completed to 1 liter. The beaker was enveloped with foil and left about 12h at 4 o C. Then, filtered by a coarse screen. The extract was collected in the flask (1 liter)and concentrated in a bath of water at 30^{o}C till the volume of 500 ml. prepared extract was equal (one gram from the fruit in one ml of extract). The extract was stored stored at 4^{o} C, then for given to rats a maximum of two (2) days. Calculated doses which giving to the rats were prepared by dilution using distilled water daily **(Elhassan and Yagi, 2010).**

Experimental Plane:

- The experiential part was accomplished in the biological laboratory of the Faculty of Home Economics - at Menoufia University. Rats were kept at 25^{0}C, and 40-60% humidity in wire cages.

Thirty 30 male albino rats were used, fed on a basal diet + water for one week as acclimatization period then, divided into equal 5 groups as follows: group (1) was kept as a negative control. Groups (2 to 5) were

fed on basal diet for the prepared extract daily at doses of 2, 4, 6, and 8 ml.

- At the end of the experiment time (four weeks) and after fasting for 12 h rats were sacrificed, blood samples were taken from the portal vein into clean and dry centrifuge tubes for serum separation, and blood samples were centrifuged for ten minutes at 3000 rpm. And serum frozen at – 20 ºC until chemical analysis **(Drury and Wallington, 1980)**.

Biochemical Evaluation:

Hematological tests**:** were completed using Beckman coulter LH750 Germany/ U.S.A.

- **Determination of total leucocyte count (WBC):**

WBC (total and differential) was determined according to **(Koda-Kimble *et al.*, 2001).**

- **Determination of differential count of white blood cells:**

WBC leukocytes are divided into two groups, the polymorph nuclear leukocytes (Neutrophils, Eosinophil's, and Basophils) and the Mononuclear Leukocytes (Monocytes and Lymphocyte). Leukocytes are a part of the body's defense system; they respond immediately to foreign invades by going to the site of involvement. The differential count of white blood cells was determined according to **(Mathy and Koepke, 1974)**

- **Hemoglobin, (Hb):**

Hemoglobin was determined in whole blood according to **(Lewis and Dacie, 1965)**

- **Red Blood Corpuscles count (RB C):**

R.B. Cs corpuscles were determined according to **Lubsandorzhiev, (2006).**

- **Platelet Count Determination:**

Serum **PLT** was determined according to **Daly, (2011).**

- **Determinations of hematocrits:**

Serum hematocrits were determined as % according to **Purves *et al.,(*2004).**

Statistical tests:
Data were presented as mean ± SD.. ANOVA (Analysis of Variance) at P≤0.05 **(SAS, 2006).**

Results:

Haemoglobin and Haematocrit of rats which received different levels of *Tinnas grewia* extract

Table (1) illustrates the effect of **TGE** on haemoglobin and haematocrit levels of healthy rats. Tabulated data showed that there were significantly increase in mean values of **HB** and **HCT** of all treatments. It could be noticed that the highest values for **HB** and **HCT** were recorded for group 5 (rats received 8ml **TGE**) by the per cent of (34.67 and 45.57 % respectively) compared with the control.

Table (1) Haemoglobin and Haematocrit of rats which received different levels of *Tinnas grewia* extract

Groups	HB (g/dl) Mean ± SD	% change of control negative	HCT (%) Mean ± SD	% change of control negative
(G1) : Control	10.5 ^{e} ± 0.1	------	28.5 ^{e} ± 0. 1	------
(G2): TGE (2ml)	10.85 ^{d} ± 0.49	3.33	31.66 ^{d} ± 0.11	11.08
(G3): TGE (4ml)	12. 43 ^{c} ± 0.098	18.38	37.5 ^{c} ± 0.05	31.57
(G4): TGE (6ml)	13.78 ^{b} ± 0.111	31.23	38.9 ^{b} ± 0.1	36.49
(G5): TGE (8ml)	14.15 ^{a} ± 0.015	34.67	41.49 ^{a} ± 0.156	45.57
LSD (p ≤ 0.05)	0.166	------	0.235	------

Means with different litters in the same column are significantly (p ≤ 0.05) different.

Platelet (PLT), White Blood Cells (WBCs) and Red Blood Cells (RBCs) (cm) of rats which received different dosages **of *Tinnas grewia* extract**

Table (2) illustrates the effect of **different dosages** on (**PLT**), (**WBCs**) and (**RBCs**) (cm) of healthy rats. Data illustrated that there were significant increases in values of **PLT** and **RBCs** of all treated groups compared to the control The highest value was for group 5 (rats received 8cm **TGE**) by the percent of the increase (47.71 and 91.35% respectively) compared with the control, For **WBCs**, it could be observed a significant decrease in the mean values of all treated groups. The lowest value was for group 4 (rats received 6ml **TGE)** compared with the control.

Table (2) Platelet (PLT), Wight Blood Cells (WBC) and Red Blood Cells (RBC) (cm) of groups which received different dosages of ***Tinnas grewia*** **extract**

Groups	PLT (10^3cm)	% change of control negative	WBC (10^3cm)	% change of control negative	RBC(10^6cml)	% change of control negative
	Mea ± SD		Mean ± SD		Mean ±SD	
(G1): Control	555.33 ^{e} ±1.52	------	11.23 ^{a} ± 0.11	------	2.89 ^{d} ± 0.59	-----
(G2): TGE (2ml)	632.33 ^{d} ± 2.52	13.86	10.17 ^{b} ± 0.07	- 9.43	3.8 ^{c} ± 0.01	31.48
(G3): TGE (4ml)	650.33 ^{c} ± 3.51	17.12	9.81 ^{c} ± 0.08	- 12.64	4.41 ^{b} ± 0.03	52.59
(G4): TGE (6ml)	700.66 ^{b} ± 3.05	26.17	8.27 ^{e} ± 0.02	- 26.35	4.66 ^{b} ± 0.12	61.24
(G5): TGE (8ml)	820.33 ^{a} ± 3.51	47.71	8.51 ^{d} ± 0.09	- 24.22	5.53 ^{a} ± 0.11	91.35
LSD ($p \leq 0.05$)	5.959	--------	0.154	-------	0.513	-------

Means with different litters in the same column are significantly ($p \leq 0.05$) different.

Superoxide Dismutase (SOD u/ml) and Catalase (CAT ng/ml) of rats that **received different** dosages **of *Tinnas grewia* extract**

Table (3) showed the effect of **TGE** on **SOD** and **CAT** of healthy rats. For (**SOD**) data showed increases in mean values of all treatments

compared with control without any significant differences except for group (5) which was increased by the percent of 41.03%) the lowest value was for the group (2) which was increased by the percent of 1.83%). For **CAT**. There were significantly higher means of **CAT** of all treated groups compared with the control. The highest value was for the group (5) by the percent of the increase (82.20%) compared with the control.

, and the lowest value was for the group (2) by the percent (39.82%).

Table (3) Superoxide Dismutase (SOD u/ml) and Catalase (CAT ng/ml) of rats which received different dosages **of *Tinnas grewia* extract**

Groups	SOD (u /ml)	% change of control negative	CAT (ng/ml)	% change of control negative
	Mean ± SD		Mean ± SD	
(G1): Control	**136.5 ^{b} ± 0.5**	**-----**	**2.26 ^{e} ± 0.11**	**-----**
(G2): TGE (2ml)	**139.00 ^{b} ± 0.23**	**1.83**	**3.16 ^{d} ± 0.06**	**39.82**
(G3): TGE (4ml)	**143. 89 ^{b} ± 3.94**	**5.41**	**3.49 ^{c} ± 0.16**	**54.42**
(G4): TGE (6ml)	**151.53 ^{b} ± 5.17**	**11.02**	**3.71 ^{b} ± 0.06**	**64.16**
(G5): TGE (8ml)	**192. 5 ^{a} ± 32.91**	**41.03**	**4.12 ^{a} ± 0.04**	**82.30**
LSD ($p \leq 0.05$)	**26.05**	**-----**	**0.192**	**----**

Means with different litters in the same column are significantly ($p \leq 0.05$) different.

Glutathione (GSH u/ml) and Glutathione Peroxidase (GPx ng/ml) of rats which received different dosages of *Tinnas grewia* extract

Table (4) showed the effect of **TGE** diet on the GSH and **GPx** of healthy rats. The obtained data illustrated a a significantly higher in means of **GSH** and **GPx** of all treatments except group (2) of **GSH** which

showed a significant decrease compared with a significant. The highest value was for group 5 (rats received 8ml **TGE**) by the percent of the increase (25.21 and 17.57 % respectively) compared with the control

lowest value was for group 2 (rats received 2 ml **TGE** by the percent of (-1.07 and 1.86 % respectively) comparing with the control.

Table (4) Glutathione (GSH u/ml) and Glutathione Peroxidase (GPx ng/ml) of rats which received different levels of *Tinnas grewia* extract

Groups	GSH (ng /ml) Mean ± SD	% change of control negative	GPx (ng /ml) Mean ± SD	% change of control negative
(G1): Control	**119 ^{d} ± 0.11**	**-----**	**124.3 ^{d} ± 0. 28**	**-----**
(G2): TGE (2ml)	**117.72 ^{e} ± 0.25**	**- 1.07**	**126.62 ^{d} ± 2.87**	**1.86**
(G3): TGE (4ml)	**128.00 ^{c} ± 0.56**	**7.56**	**135 ^{c} ± 0.5**	**8.61**
(G4): TGE (6ml)	**132 ^{b} ± 0. 50**	**10.92**	**141. 20 ^{b} ± 0.49**	**13.59**
(G5): TGE (8ml)	**149 ^{a} ± 0.30**	**25.21**	**146.15 ^{a} ± 0.17**	**17.57**
LSD ($p \leq 0.05$)	**0.454**	**-----**	**2.391**	**-----**

Means with different litters in the same column are significantly ($p \leq 0.05$) different.

Discussion

In this study, supplemented rats with **TGE** for 28 days cussed some improvement in most haematological parameters. Also, significant for RBCs and platelets count. Trivial increases were observed increase was observed for HB. These results was found to be in the same line with **(Al-Said *et al.*, 2011)** who found that oral dosages (250 and 500 mg/kg/rat) of ethanol extract of *G.tenax* for 3 weeks increased haemoglobin levels significantly. And this may be due to the amounts of iron and amino acids in fruits in different species of *Grewia* such as *G. tenax*, *G. flavescens* and *G. villosa* **(Mohammed Elhassan and Yagi, 2010).**

In addendum, it was indicated that different dosages of *G. tenax* extract fruit for four weeks cussed significantly lower **PLT** count. Also, the same observation has been related to some herbal medicine **(Cheesbrough, 2005).** Significant increases in the **PLT** count and megakaryocytes observed in rats that received an aqueous.

extract of *G. tenax* fruit for one week (**Deutsch and Tomer, 2006).**

Supporting with ethanolic extract (E.X) of *G. tenax* fruit + formalin makes improvement in **Hb**, **RBCs** and **PCV** compared with mice which received formalin only. Also, amelioration in **PLT** count was observed. The improvement in most haematological parameters of groups treated with *G. tenax* + formalin may be due to the antioxidant contents of G. *tenax* extract. Supplementation with *G. tenax* E.X fruits improved the levels of haemoglobin in rats. These obtained results support the main use of *G. tenax* ethanol extract fruits with the exception of anaemic conditions **(Al- Said *et al.*, 2011).** Antioxidant compounds of different species of *G. tenax* are known to initiate the oxidative damage effects **(Ramesh *et al.*, 2010).**

Conclusion

The used plan extract in this study has an effective and improved CBC analysis and antioxidant enzymes. The obtained results supported the suppositions that this plant has a lot of bioactive compounds which are able to promote blood parameters. So, we recommended more interest and consumption of this plant as an extract in our diets by (10-20 ml/day).

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المجلة العلمية للتربية النوعية والعلوم التطبيقية
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دراسة التأثيرات المحتمله لمستخلص القضيم على صورة الدم ومستويات الانزيمات المضاده للأكسده في فئران التجارب

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الملخص العربي

تهدف هذه الدراسه الى توضيح تأثير مستخلص القضيم على مكونات صورة الدم ومستوى الانزيمات المضاده للأكسده لفئران التجارب عند تلقيها جرعات فمويه مختلفه (٨,٦,٤,٢ سم) من المستخلص بصوره يوميه لمدة ٤ أسابيع. وقد تم استخدام(٣٠) ثلاثون فأرا من ذكور الألبينو يتراوح وزنهم من ١٥٠ الى ١٦٠ جم فى هذه الدراسه وتم تقسيمهم الى خمس مجموعات متساويه (كل منهما ستة فئران), المجموعه الأولى تمت تغذيتها على الغذاء الأساسى طوال مدة التجربه كمجموعه ضابطه سالبه للمقارنه, أما باقى المجموعات فقد تم تغذيتهم على الغذاء الأساسى بالأضافه الى جرعات المستخلص الفمويه يوميا ٨,٦,٤,٢ سم يوميا على التوالى للمجموعات ٥,٤,٣,٢طوال فترة التجربه. وقد أظهرت النتائج المتحصل عليها على وجود ارتفاع معنوى فى مستويات الهيموجلوبين , الهيماتوكريت , كرات الدم الحمراء والصفائح الدمويه للمجموعات المتلقيه للمستخلص محل الدراسه مقارنة بالمجموعه الضابطه السالبه , بينما لوحظ انخفاض فى مستوى كرات الدم البيضاء. وبالنسبه لمستويات الانزيمات المضاده للاكسده (سوبر أكسيد ديسميوتيز, الجلوتاثيون, الجلوتاثيون بيروكسيديز و الكاتاليز) فقد أظهرت النتائج ارتفاع معنويا مقارنة بالمجموعه الضابطه السالبه.

الكلمات المفتاحية : االقضيم ,الهيموجلوبين, كرات الدم البيضاء, كرات الدم الحمراء, الانزيمات المضاده للأكسده