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The Effect of *Tinnas Grewia* on Hemoglobin Levels in the Females of White Rats

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Abstract

The present work aimed at studying the effect of *Tinnas grewia*, on raising the level of hemoglobin, in female albino rats and to compare the effect of haemojet drug for the treatment of anemia. Twenty-five adult female albino rats, weighting 150±10g were used in this study, and dividing into the following groups (each 5 rats). Groups 1 and 2 were negative and positive control . Group 2 was positive control group fed on basal standard diet and orally administrated daily 3 cm of haemojet. From groups 3 to 5 fed on basal standard diet and orally administrated daily at doses 0.5.1 and 1.5 cm of *Tinnas grewia* extract. The mean value of hemoglobin, WBC in group 3 & group 4 which were fed on *Tinnas* by different levels were significantly lower than haemojet group, While, they were significantly higher in group 5 than haemojet group. The mean values of RBC in group 4 and 5 showed significant higher values as compared to haemojet group; while, it didn't show significant difference between group 3 and haemojet. At the end we recommend drinking *Tinnas grewia* extract, because it is improving the level of hemoglobin in the blood and treating anemia.

Keywords*: Tinnas grewia,Anemia, Hemoglobin, liver function,*

Introduction

Anemia is a common nutritional deficiency disorder and global public health problem which affects both developing and developed countries with major consequences for human health and their social and economic development. According to WHO reports, one third of the global populations (over 2 billion) are anemic due to imbalance in their nutritious food intake. It was estimates that even among the South Asian countries, India has the highest prevalence of anemia (Shubham et al., 2020).

There are several types and classifications of anemia. The occurrence of anemia is due to the various red cell defects such as production defect (aplastic anemia), maturation defect (megaloblastic anemia), defects in hemoglobin, synthesis (iron deficiency anemia), genetic defects of hemoglobin, maturation (thalassemia) or due to the synthesis of abnormal hemoglobin, (haemoglobinopathies, sickle cell anemia and thalassemia) and physical loss of red cells (hemolytic anemias) (El-kenawy, 2019). Iron deficiency anemia is an advanced stage of iron depletion. It occurs when storage sites of iron are deficient and blood levels of iron cannot meet daily needs. Blood hemoglobin, levels are below normal with iron deficiency anemia (Abbas, 2020).

Tinnas grewia, it is a fruit producing deciduous tropical shrub or tree, widespread in semi-arid and sub humid tropical climates. The wild shrub is the main source of the growing commercial demand for the fruit. It is considered a prime candidate for domestication and commercialization as new crop for the semi-arid regions of the Sudan (Venkatesan et al., 2019).

Tinnas grewia, is a multithemed small shrub up to two meters tall usually rounded but generally battered and untidy due to browsing. Bark is smooth, grey, and very fibrous so that twigs are hard to break. The leaves are oval and the tip is pointed or rounded. The edge of the leave is toothed. The vein network is very clear below. Alternate, almost circular in outline, 1.5 - 4 cm in diameter, Margins toothed and prominently tri-nerved at the base, stipules are conspicuous, up to 4mm. long, filiform, pubescent, falling early. It has been the subject of much global interest in research and development as it might be the solution of worldwide standing problem such as iron deficiency anemia (Venkatesan et al., 2019).

Tinnas grewia, fruit seed preparation is safe. They showed no toxic effects in rats. As normal rats were used, the plant preparations did not demonstrate hematinic efficacy on blood constituents especially hemoglobin, concentration, red blood cells count, iron and iron binding capacity, in spite of their high contents of iron and the presence of vitamin, which enhance iron absorption (Sulieman and Mariod, 2019).

The present study was designed to investigate the effect of *Tinnas grewia*, on blood CBC, kidney functions and liver enzymes in rats.

Materials and methods

Materials:

Plant materials: *Tinnas grewia*, were obtained from Sudan country it is a dray cereal that does not need refrigeration .

Rats and diets: Twenty-five adult female albino rats, weighting 150±10g, from rats laboratory at Faculty of Home Economic, Menoufia university, Shebin al kom, were used in this study

Methods

Preparation of plant extracts:

Tinnas grewia, used in this study were purchased from the Market of Seeds and Grains in Omdurman, Sudan. The plants were authenticated by the National Council for Research, Sudan.Ripen fruits of the plant were washed and allowed to dry at room temperature. 500 grams of the fruits were soaked in distilled water placed in a 1-liter beaker with the volume completed to 1 litre. The beaker was covered with aluminum foil and left overnight in the refrigerator at 4° C. The macerated fruits were then filtered through a coarse sieve. The filtrate was then collected in 1liter volumetric flask and concentrated in a water bath at 30° C till the volume reached 500 ml. A stock solution of the concentrate prepared was equivalent to one gram of the fruit in one ml of solution. The stock solution was stored in the refrigerator at 4° C for administration to rats within a maximum period of two days. The intended doses were freshly prepared daily before dosing to rats by dilution from the stock solution using distilled water. (Elhassan and Yagi, 2010)

Experimental Design:

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin El-kom. Rats were housed in wire cages in a room temperature 25° C and kept under normal healthy conditions.

Rats were divided into the following groups (each 5 rats), groups 1and 2were negative and positive control. Groups 2was positive control group fed on basal diet and orally administrated daily 3cm of haemojet. Groups 3to 5 fed on basal diet and orally administrated daily at doses(0.5.1.1.5) cm of *Tinnas grewia*, extract.

Biological Evaluation:

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG%), efficiency ratio (FER), and organs/ body weight were calculated according to (Chapman et al., 1959).

Hematological analyses:

Were performed using Beckman coulter LH750 (Germany/U.S.A) Hemoglobin (Hb):
(Hb) determined in whole blood samples by coulter electronic counter model CD 1800 specimen (coulter hematology system) hemoglobin, was determined according to the method described by (Lewis and Dacie, 1965).

Counting of red blood corpuscles (RB Cs):

Red blood corpuscles were de termined according to the method described by(Castle and Engberg,2006)

Determinations of platelet count:

Serum platelet count was determined as (103/cmm)according to the method described by (Daly, 2011).

Determination of liver functions:

Determination of serum alkaline phosphates (ALP): Enzymatic calorimetric determination of alkaline phosphates was carried out according to (Belfield and Goldberg, 1971).

Estimation of serum glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminase (GPT): GOT and GPT activities were measured according to method described by (Tietz, 1976) by following reaction.

Determination of renal functions:

Estimation of urea: Urea was determinate according to the enzymatic method of (Patton and Crouch 1977).

Estimation of creatinine in serum: Creatinine was determinate according to kinetic method of (Henry 1974).

Estimation of Uric Acid: The intensity of this red color formed is proportional to the uric acid concentration in the sample (Schultz, 1984).

Statistical Analysis:

Data was examined for normality of distribution using one sample Kolmogorov-Simirnov Test. Values at ($P \leq 0.05$) were considered to be statistically significant according to **(Snedecor and Cochran, 1989).**

Results and Discussion

The mean value of hemoglobin, (HB), white blood count (WBC), PLT, HCT and RBC of rats treated at different levels of *Tinnas* extract are shown in table (1). It could be noticed that the mean value of HB, WBC, PLT, HCT and RBC of control (+) group were mostly significantly higher than control (-) group, the best HB, WBC, PLT, HCT and RBC was recorded for rats fed *Tinnas grewia*, 1.5 cm when compared to control (-) group. The obtained results agree with the traditional use of the fruits of *Tinnas grewia*, in Sudan (Northern and Southern parts of Kordofan region) to improve hemoglobin, and the general health status and lactation of breastfeeding mothers, as they are given in the form

of nashi (a porridge prepared from *Tinnas grewia*, fruits sweetened drink by the addition of custard and flour).

Table (1): hemoglobin, (g/dl), WBC (*103/cmm), PLT(*103/cmm), HCT (g/dl) and RBC (cells/mcL) of negative control (1), positive control (2), and all treated groups as affected by Tinnas grewia, extract

Variables	G(1) Negative control (-ve)	G(2) Haemojet group	Tinnas grewia, extract			LSD (p≤ 0.05)
	Normal	Haemojet3 cm	G(3) 0.5cm	G(4) 1cm	G(5) 1.5cm	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
HB (g/dl)	11.55±0.46	15.5a±0.7	11.6b±0.4	12.3b±0.3	16.0a±0.6	1.11
% change of negative control	_____	+49.42	+10.99	+18.80	+53.8	—
% change of positive control	-33.07	_____	-25.71	+20.54	+2.95	—
WBC(103/cmm)	5.9b±0.3	9.7a±1.14	6.63b±1.30	7.73b±1.40	10.2a±0.55	1.78
% change of negative control	_____	+36.9	+7.29	+13.07	+72.88	—
% change of positive control	-38.98	_____	-31.40	-20.03	+5.48	—
PLT(*103/cmm) per microliter	820b±84.6	1075.6a±72.5	939ab±45.7	961ab±89.4	1098a±32.0	131.0
% change of negative control	_____	+31.17	+14.51	+17.19	33.9	—
% change of positive control	-23.76	_____	-12.64	-10.65	2.1	—
HCT (g/dl)	41.53a±2.02	45.4a±1.60	41.43a±3.9	40.7a±1.65	43.9a±2.1	4.77
% change of negative control	_____	+9.31	-0.240	-1.998	+5.85	—
% change of positive control	-8.52	_____	-8.74	-10.35	-3.17	—
RBC (cells/mcL)	6.96a±0.38	7.52a±1.158	7.52a±0.61	7.60a±0.68	8.53a±0.48	1.12
% change of negative control	_____	-8.04	+8.13	+9.28	+22.55	—
%change of positive control	-0.074	_____	+0.079	+13.44	+13.43	—

Means in the same row with different letters indicate sig. difference.

While the obtained results are not in agreement with the study of Ahmed (2006) who found that WBC and PLT for male and female rats' groups showed no significant differences between mean values of all hematological parameters for tested animals at different dose levels compared with control group and normal physiological values of the sex, age and strain. Cui et al., (2018) found that HCT for male and female rats' groups showed no significant differences between mean values of all hematological parameters for tested animals at different dose levels compared with control group and normal physiological values of the sex, age and strain. Also, Lei et al., (2008) reported that, iron relates closely to the generation and maturation of RBC. Studies of iron deficient rats have shown that erythroid burst colony forming units (BFU-E), erythroid colony forming units (CFU-E) colony number, and cell number in each colony was significantly decreased in comparison with normal rats.

The mean value of alkaline phosphatase (ALP), serum GPT, GOT and urea, creatinine and UA (mg/dl) of rats which treated with different level *Tinnas* are shown in table (2). It could be noticed that the mean value of ALP of control (+) group was significantly lower than control (-) group. But serum GPT, GOT and urea were higher than control (-) group, the best ALP, serum GPT, GOT and urea was recorded for rats fed *Tinnas grewia*, when compared to control (-) group.

Table (2): Fasting serum ALP (u/L), GPT (u\l), GOT and urea (mg/dl) of negative control (1), haemojet group (2), and all treated groups as affected by Tinnas grewia, extract

Variables	G(1) Negative control (-ve)	G(2) Haemojet group	Tinnas grewia, extract			LSD (p≤0.05)
	Normal	Haemojet3 cm	G(3) 0.5cm	G(4) 1cm	G(5) 1.5cm	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
ALP (U/L)	239a±24.24	118.3c±19.85	174.3b±11.5	100c±7.21	172b±19.07	33.4
%change of negative control	_____	-59.61	-40.50	193	-41.29	—
%change of positive control	147.162	_____	47.32	-15.49	45.36	—
GPT (ALT) U/L	42.33ab±1.52	44.33ab±2.51	36b±3	40ab±5.56	48a±5.56	7.964
%change of negative control	_____	+4.72	-14.95	-5.50	+13.39	—
%change of positive control	+4.51	_____	-14.27	-9.76	+8.27	—
GOT (AST) U/L	197b±61.37	240.66a±14.05	181b±11	181b±7.2	252a±10.53	23.44

Variables	G(1) Negative control (-ve)	G(2) Haemojet group	Tinnas grewia, extract			LSD (p≤0.05)
	Normal	Haemojet3 cm	G(3) 0.5cm	G(4) 1cm	G(5) 1.5cm	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
%change of negative control	_____	+34.44	+ 1.117	+ 1.117	+ 40.78	—
%change of positive control	-25.62	_____	-24.790	-24.790	+4.712	—
Urea (mg/dl)	47.33ab±3.055	56a±3.60	41.6b±3.78	55a±2.64	45.66±5.50	7.574
%change of negative control	_____	2.49	-11.97	16.20	-352	—
%change of positive control	-15.48	_____	-25.60	-1.78	-18.46	—
Creatinine	0.64a±0.03	0.696a±0.35	0.7a±0.065	0.6a±0.05	0.7a±0.05	0.100
%change of negative control	_____	+8.75	+12.5	+3.125	+8.75	—
%change of positive control	-8.045	_____	+3.44	-5.17	0	—
UA	1.623b±0.23	1.81a±0.17	1.57ab±0.3	1.16b±0.0	1.4ab±0.08	2.158
%change of negative control	_____	11.52 +	-3.26	0	11.89-	—
%change of positive control	-11.52	_____	-13.25	-37.23	181+	—

Means in the same row with different letters indicate sig. difference.

Weight of liver, kidney, heart and lungs for negative control (1), haemojet group (2), and some treated groups as affected by *Tinnas grewia*, extract.

Results of table (2) illustrate the mean value of liver, heart, lungs and kidneys weight (g) of rats fed on *Tinnas* by different level. It could be noticed that the mean value of liver, heart and lungs of control (+) group was higher than control (-) group, but kidneys' weight was significantly lower than control (-) group.

Ahmed (2006) found that in order to evaluate the safety of the aqueous extracts of the fruit of *Tinnas grewia*, liver functions tests should be determined. This due to the fact that liver is the most common site of toxic injury due to receiving about half of its blood supply from the portal vein, which drain blood from the gastrointestinal tract, and as a result toxic substances are absorbed into the portal blood and transported directly to the liver which is the organ most commonly involved with intoxication and detoxification.

Also, Aiello et al., (2016) found that in order to evaluate the safety of the aqueous extracts of the fruit of *Tinnas grewia*,, kidney functions tests were done. Aqueous-soluble compounds tend to be excreted by the kidney, so kidneys are also secreting urine for the purpose of elimination of metabolic wastes. Therefore, the safety of the aqueous extract of the fruit of *Tinnas grewia*, has further been confirmed, through the determination of some serum biochemical parameters which have shown normal physiological values and no significant differences between the tested groups compared with control groups for all the estimated parameters. But the results are not in agreement with the study of Ahmed, (2006) who found that there were no significant (P> 0.05) differences between the various groups in body weights of rats at any time of weight determination. Weights of livers, kidneys, hearts and spleens of male and female rats of the different groups, showed no significant (P>0.05) differences between groups respectively.

Table (3): Weight of liver (g), kidney (g), heart (g) and lungs (g) for negative control (1), haemojet group (2), and some treated groups as affected by Tinnas grewia, extract

Variables	**G(1) Negative control (-ve)**	**G(2) Haemojet group**	**Tinnas grewia, extract**			**LSD (p≤0.05)**
	Normal	**Haemojet3 cm**	**G(3) 0.5cm**	**G(4) 1cm**	**G(5) 1.5cm**	
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	
Liver (g)	3.796a±0.64	3.044±0.002	3.02a±0.5	3.266a±0.7	3.046a±0.5	0.839
%change of negative control	_____	-19.81	-20.44	-13.96	-19.75	—
%change of positive control	+95.42	_____	-96.35	-96.062	-96.32	—
Kidney (g)	0.79a±0.117	0.696a±0.040	0.7a±0.10	0.73a±0.09	0.6a±0.04	0.161
%change of negative control	_____	-13.50	-2.531	-7.21	+22.78	—
%change of positive control	+13.505	_____	+10.63	+5.31	-12.35	—
Heart (g)	0.33a ±0.030	0.4a±0.036	0.4a±0.04	0.33a±0.06	0.33a±0.04	0.093
%change of negative control	_____	+20.120	+17.117	0	0	—
%change of positive control	-16.75	_____	-2.56	-20.120	-20.120	—
Lungs (g)	0.72a±0.06	0.73a±0.096	0.5bc±0.0	0.45c±0.05	0.63ab±0.0	0.115

Variables	G(1) Negative control (-ve)	G(2) Haemojet group	Tinnas grewia, extract			LSD (p≤0.05)
	Normal	Haemojet3 cm	G(3) 0.5cm	G(4) 1cm	G(5) 1.5cm	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
%change of negative control	_____	-1.38	-26.38	-36.67	-12.083	—
%change of positive control	-1.36	_____	-27.39	-37.53	-13.28	—

Means in the same row with different letters indicate sig. difference.

The mean value of FER, FI and BWG of rats fed on *Tinnas* by different level is shown in table (4). Data show that the mean value of FER of control (+) group was significantly higher than control (-) group, but the BWG was significantly lower than control (-) group.

Table (4): Food efficiency ratio (FER), feed intake (FI) and BWG of negative control (1), haemojet group (2), and all treated groups as affected by Tinnas grewia, extract.

Variables	G(1) Negative control (-ve)	G(2) Haemojet group	Tinnas grewia, extract			L.S.D (p ≤ 0.05)
	Normal	Haemojet3 cm	G(3) 0.5cm	G(4) 1cm	G(5) 1.5cm	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
FER	0.07c±0.01	0.273b±0.025	0.7a±0.03	0.25b±0.0	0.73a±0.0	0.058
%change of negative control	_____	+290	+914.28	+275.14	+942.85	—
%change of positive control	+64.28	_____	+160.073	-8.42	+167.39	—
FI	5.13b±0.821	6b±1	16a±2	5.33b±1.5	15a±1	2.158
%change of negative control	_____	+16.95	+211.98	+3.89	+192.39	—
%change of positive control	-94.87	_____	+166.66	-11.16	+150	—
BWG %	6.31c±0.296	5.033d±0.126	14.3b±0.7	6.8c±0.20	15.3a±0.1	0.997
%change of negative control	_____	-20.23	+631	+8.24	-20.23	—
%change of positive control	+25.37	_____	+18.412	+35.70	0	—

Means in the same row with different letters indicate sig. difference.

The results are the agreement with that of (Chen et al., 1984) and (Thakur et al., 2019) considering feeding of hepatic rats on certain plants. but the results disagreed with the finding of (Waynforth and Flecknell, 1980) who found that no significant differences observed between the weights of organs of treated animals compared with the control groups and normal physiological wet weight in grams/100 g body weight. Also, Ahmed, (2006) found there were no significant differences in animals body weights in tested groups as compared with control groups of the female Wistar rats. At necropsy, three months after oral dosing, all the internal organs of tested and control groups exhibited normal texture and the appearance of all the inspected organs revealed no gross postmortem changes

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تأثير القضيم على مستوى الهيموجلوبين فى إناث الفئران البيضاء

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قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر

الملخص العربي
يهدف البحث الحالي إلى دراسة تأثير القضيم في رفع مستوى الهيموجلوبين في إناث الفئران البيضاء ومقارنة تأثير عقار الهيموجيت في علاج فقر الدم. تم استخدام خمسة وعشرون أنثى بالغة من الفئران البيضاء ، وزنها 150 ± 10 جرام في هذه الدراسة وتم تقسيمها إلى المجموعات التالية (كل مجموعة 5 فئران). المجموعات 1 و 2 كانت سلبية وإيجابية. المجموعة الثانية كانت إيجابية و تتغذى على الغذاء الأساسي وتعطى يومياً 3 سم من الهيموجيت عن طريق الفم. أما المجموعات من 3 إلى 5 التي تغذت على نظام غذائي قياسي وتناولت مستخلص نبات القضيم عن طريق الفم يوميًا بجرعات15,10,5سم ، كانت القيمة المتوسطة للهيموجلوبين وكرات الدم البيضاء في المجموعة 3 والمجموعة 4 التي تم تغذيتها على القضيم بمستويات مختلفة أقل بكثير من مجموعة الهيموجيت بينما كانت أعلى بشكل ملحوظ في المجموعة 5 عن مجموعة الهيموجيت. أظهرت القيم المتوسطة لكرات الدم الحمراء في المجموعة 4 و 5 قيمًا أعلى معنوية مقارنة بمجموعة الهيموجيت؛ بينما لم يظهر فرق كبير في المجموعة 3 و الهيموجيت.. في النهاية نوصي بشرب مستخلص القضيم ، لأنه يحسن مستوى الهيموجلوبين في الدم ويعالج فقر الدم

الكلمات المفتاحية: القضيم، الانيميا، الهيموجلوبين، وظائف الكبد،