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سعادة أ. د. رئيس تحرير المجلة المصرية للدراسات المتخصصة المحترم

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تحية طيبة وبعد،،،

يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي الثامن للمجلات للعام 2023.**

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Effects of Bidens Pilosa L Extract on Complete Blood Count and Serum Antioxidant Enzymes Levels in Rats

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Effects of Bidens Pilosa L Extract on Complete Blood Count and Serum Antioxidant Enzymes Levels in Rats

Dr. Batoul N. A. Mohammed

Abstract

The aim of this study is to investigate the effects of Bidens Pilosa L extract (BPE) on complete blood picture and serum antioxidant enzymes level in rats by the dose of (3, 6 and 9ml orally/day). Thirty tow male albino rats about (170±10g) were used in this study, and then divided into four equal groups each (8rats). 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) was fed on a basal diet + the Oral doses of B. Pilosa for (4 weeks). Complete blood cells count levels (HB, HCT, RBCs and PLT) showed significant increases compared with the control group. WBCs showed a decrease. For antioxidant enzymes (SOD, CAT, GSH and GPx) were significantly increase comparing with control.

Keywords: lBidens Pilosa L , Red blood cells, Wight blood cells, Antioxidant enzymes, Hemoglobin

ملخص:

العنوان : تأثير مستخلص الحسيكة على صورة الدم الكاملة ومستويات الإنزيمات المضادة للأكسدة في مصل الدم في الجرذان

المؤلفون : بتول ناصر عبد الله محمد

تهدف الدراسة لفحص التأثيرات المحتملة لمستخلص نبات الحسيكة على صورة الدم الكاملة والأنزيمات المضادة للأكسدة في مصل الدم في الجرذان بجرعة (3،6 و 9 مل فموياً / يوم). استخدم في هذه الدراسة أثنان وثلاثون فأراً ألبينو ذكراً بوزن حوالي (170±10 جم)، تم تقسيمهم إلى أربعة مجموعات متساوية لكل منها (8 فئران). اعتبرت المجموعة الأولى مجموعة ضابطة تغذت على نظام غذائي أساسي طوال فترة التجربة، أما المجموعات الأخرى (24 فأراً) فقد تم تغذيتها على نظام غذائي ثابت + الجرعات الفموية من مستخلص نبات الحسيكة لمدة (4 أسابيع). أظهرت مستويات العد الكامل لخلايا الدم (HB، HCT، كرات الدم الحمراء وPLT) زيادات معنوية مقارنة مع المجموعة الضابطة.كما أظهرت كرات الدم البيضاء انخفاضا و بالنسبة للأنزيمات المضادة للأكسدة (SOD، CAT، GSH وGPx) كانت هناك زيادة معنوية مقارنة المجموعة الضابطة .

الكلمات الدالة : نبات الحسيكة ، خلايا الدم الحمراء ، خلايا الدم البيضاء ، الإنزيمات المضادة للأكسدة ، الهيموجلوبين

Introduction

Bidens Pilosa L generally known as blackjack, hairy beggar-ticks and Spanish needle belonging to the family Asteraceae. It is a herbaceous medicinal plant domestic to South America that nowadays is widely world spread **(Oliveira et al., 2004).** *B. Pilosa* is an annual, erect herb growing up to 1.5 m tall with minutely hairy stems.. It has yellow or white flower heads, and narrow long ribbed black seeds **(Ashafa and Afolayan, 2009).** In many countries of the world traditional medicines used different parts of B. pilosa in form of juice, decoction ,powder, or taken orally have been reportedly used to treat hepatitis, inflammation, hypertension, stomach disorders, and digestive disorders **(Bartolome et al., 2013 and Silva et al., 2014).** Furthermore, the leaves are eaten as a vegetable **(Odhav et al., 2007 and Orech et al., 2007).** Studies of B. pilosa plant extracts have shown it has antidiabetic **(Chien et al. 2009),** antiulcerogenic **(Alvarez et al., 1999),** antitumor **(Kviecinski et al., 2011),** immunosuppressive and anti-inflammatory **(Pereira, et al., 2020),** antihypertensive **(Leandre et al., 2008),** anti-leukemic **(Chang et al., 2001)**, antibacterial **(Lawal et al., 2015),** 4 hepatoprotective **(Yuan, et al., 2008; Kviecinski et al., 2011),** and antioxidant **(Krishnaiah et al., 2021)** effects. B. pilosa is an remarkable source of phytochemicals and 201 compounds have so far been particular from this plant, including 70 aliphatics (36 polyynes), 60 flavonoids, 25 terpenoids, 13 aromatics, 8 porphyrins, 19 phenylpropanoids, and 6 other compounds **(Silva et al., 2014).** Phytochemical studies of B. pilosa L. leaf extract contains of many polyacetylenes, glycosides and flavonoids, **(Hoffmann and Hölzl, 2019),** essential oils and terpenes **(Zollo et al., 1995)** with anti-microbial and anti-inflammatory properties **(Wong-Leung, 2018).**

Anemia 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 anemic because of the imbalance in their feed intake from nutritious **(Shubham et al., 2020).**

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).**

Therefore, this study proposes to evaluate the effects of the ethanolic extract of the *Bidens Pilosa L* extract (BP) on complete blood cells and serum antioxidant enzymes level in rats.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *B. Pilosa L* . collected from the fields at El-Beheira Governorate. Leaves identified by Flora and Phytotaxonomy Researchers Department, belonging to Horticultural Research Institute, Agricultural Research Center.

Experimental Animals:

Thirty tow male albino rats about (170±10g) were purchased from the Animal House of the National Research Center, Dokki, Egypt.

Methods:

Preparation of Plant Extract:

Leaves of *B. Pilosa* were washed with running tap water and air dried. The air dried leaves were grinded into fine powder and kept in a tightly closed container at room temperature for further use. The extract was prepared by soaking 500 g of powdered *B. Pilosa* leaves in one liter of a solvent composed of 700 ml ethanol 95% and 300 ml distilled water at room temperature for 24 hours with stirring. The infusion was filtered by a piece of double layer gauze. The filtrates evaporated using a rotary evaporator at 40℃ under vacuum **(Muralidharan and Srikanth, 2009).**

Chemical analysis:

Moisture content, total protein, fat, fiber, and ash were determined in dried powder of *B. Pilosa* leaves according to the methods outlined in **A.O.A.C (2006),** while the carbohydrates content will be calculated by difference.

Experimental Design:

Rats were fed on basal diet prepared according to **Reeves et al. (1993)** and water was provided ad libitum. Rats were left to accommodate for one week before experimental use. After the period of adaptation, the rats divided into equal 4 groups as follows: group (1) was kept as a control. Groups (2 to 4) were fed on basal diet for the prepared extract daily at doses of 3, 6, and 9 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 had 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 **(KodaKimble 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 Analysis:

Results will be presented as Mean ± SE. Data will be compared by oneway analysis of variance (ANOVA), followed by appropriate post hoc test, to determine the statistical significance of the difference using Statistical Package for the Social Sciences (SPSS), version 22. The values of $p < 0.05$ were regarded as statistically significant. (**SAS, 2006)**

Results:

Chemical Composition:

Data present in table (1) showed that, the leaves of *B. Pilosa* powder contains protein (28.91%), fats (1.22%), carbohydrate (32.22%), crude fibers (7.82%), moisture (11.87%) and ash (17.96%).

Table (1): The chemical composition of *B. Pilosa* leaves powder

Chemical composition	Value (%)
Moisture	11.87
Protein	28.91
Fat	1.22
Ash	17.96
Crude fibers	7.82
Total carbohydrate	32.22

Hemoglobin and Hematocrit of rats which received different levels of *Bidens Pilosa* extract Table (2) illustrates the effect of BP on hemoglobin and hematocrit 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 4 (rats received 9ml BP) by the per cent of (26.05 and 38.74 respectively) compared with the control.

Table (2) Hemoglobin and Hematocrit of rats which received different levels of *Bidens Pilosa* extract

Groups	HB(g/dl) Mean ± SD	% change of control	HCT (%) Mean ± SD	% change of control
Control	10.4 ± 0.11 d	----------	27.33 ± 0. 1 d	----------
BPE (3ml)	10.71 ± 0.15 c	2.98	29.23 ± 0.33 c	06.95
BPE (6ml)	12. 24 ± 0.75 b	17.69	34.25 ± 0.07 b	25.32
BPE (9ml)	13.11 ± 0.51 a	26.05	37.92 ± 0.16 a	38.74
LSD (p ≤ 0.05)	0.161	----------	0.327	----------

Means with different litters in the same column are significantly (p ≤ 0.05) different (Hemoglobin(HB) and Hematocrit (HCT)

Platelet (PLT), White Blood Cells (WBCs) and Red Blood Cells (RBCs) (cm) of rats which received different dosages of Bidens Pilosa extract.

Table (3): The effect of different dosages of *Bidens Pilosa* extract on Platelet (PLT), Wight Blood Cells (WBC) and Red Blood Cells (RBC) (cm)

Groups	PLT (103 cm) Mean ± SD	% change of control	WBC (103 cm) Mean ± SD	% change of control	RBC(106 cml) Mean ± SD	% change of control
Control	563.33 ±1.66 d	-----	11.35 ± 0.08 d	-------	3.12 ± 0.71 d	------
BPE (3ml)	671.33 ± 3.15 c	19.17	10.58 ± 0.03 c	- 9.32	3.96 ± 0.22 c	26.92
BPE (6ml)	697.33 ± 3.74 b	23.78	9.77 ± 0.11 b	- 8.60	4.48 ± 0.06 b	43.58
BPE (9ml)	742.33 ± 3.09 a	31.77	7.95 ± 0.17 a	- 7.00	5.15 a ± 0.33 a	65.06
LSD (p ≤ 0.05)	6.055	-----	0.163	-----	0.497	------

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

Table (3) 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 4 (rats received 9cm BPE) by the percent of the increase **(**31.77 and 65.06**%** For PLT and RBC, 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 3 (rats received 6ml BPE) compared with the control.

Table (3) showed the effect of BPE on SOD and CAT of healthy rats .Superoxide Dismutase (SOD u /ml) and Catalase (CAT ng/ml) of rats that received different dosages of *Bidens Pilosa* extract . For (SOD) data showed increases in mean values of all treatments compared with control without any significant differences except for group (4) which was increased by the percent of 11.49**%)** the lowest value was for the group (2) which was increased by the percent of 0.30%. For CAT, there were significantly higher means of CAT of all treated groups as compared with the control. The highest value was for the group (4) by the percent of the increase (50.35%) as compared with the control, and the lowest value was for the group (2) by the percent (13.82%).

Table (4): The effect of different dosages of *Bidens Pilosa* extract on Superoxide Dismutase (SOD) and Catalase (CAT ng/ml)

Groups	SOD (u /ml) Mean ± SD	% change of control	CAT (ng/ml) Mean ± SD	% change of control
Control	141.6 ± 0.11 c	----------	2.82 ± 0.21 d	----------
BPE (3ml)	142.03 ± 0.52 c	0.30	3.21 ± 0.11 c	13.82
BPE (6ml)	143. 75 ± 0.89 b	1.01	3.57 ± 0.32 b	26.59
BPE (9ml)	157. 88 ± 2.65 a	11.49	4.24 ± 0.31 a	50.35
LSD (p ≤ 0.05)	25.13	----------	0.186	----------

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

Table (5) showed the effect of BPE diet on the GSH and GPx of healthy rats. Glutathione (GSH u /ml) and Glutathione Peroxidase (GPx ng/ml) of rats which received different dosages of Bidens Pilosa extract. 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 4 (rats received 9ml BPE) by the percent of the increase **(**14.54 **and** 15.53% respectively) compared with the control lowest value was for group 2 (rats received 2 ml BPE by the percent of 1.10 **and** 5.17**%** respectively) comparing with the control.

Table (5): The effect of different dosages of *Bidens Pilosa* extract on Glutathione (GSH) and Glutathione Peroxidase (GPx)

Groups	GSH (ng /ml) Mean ± SD	% change of control	GPx (ng /ml) Mean ± SD	% change of control
Control	118.03 ± 0.07 c	----------	121.07 ± 0. 16 d	----------
BPE (3ml)	119.33 ± 0.14 c	1.10	127.33 ± 1.31 c	5.17
BPE (6ml)	127.93 ± 0.41 b	8.38	133.15± 1.02 b	9.97
BPE (9ml)	135.2 ± 0.16 a	14.54	139.88 ± 1.52 a	15.53
LSD (p ≤ 0.05)	0.513	----------	3.264	----------

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

Discussion

In this study the current results was similar to that reported by **Deba et. al. (2008)** whom found the same chemical composition of ***B. Pilosa*** leaves powder. Supplemented rats with BPE for 28 days cussed some improvement in most haematological parameters. Significant increases for RBCs and platelets count were observed. 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 Bidens Pilosa for 3 weeks increased haemoglobin levels significantly. And this may be due to the amounts of iron and amino acids in different species of *B. pilosa* **(Mohammed Elhassan and Yagi, 2010)**

The antioxidant effect of Bidens pilosa extract could be attributed to the presence of its phytochemical constituents as polyacetylenes, polyacetylenic glycosides, aurons, aurons glycosides, flavonoids and flavonoid glycosides **(Ibrahim et al.,2023).** The antioxidant effect of B. pilosa extract in this study was similar to those obtained by **Repetto and Llesuy (2002); Deba et. al. (2008); Krishnaiah et. al. (2011) and Kviecinskiet et. al., (2011).**

Compounds with molecules capable of scavenging or reducing high ROS/RNS and free radicals would be of health benefit in managing illnesses such as cancer, diabetes, heart disease, neurodegenerative diseases, and ageing **(Saibabu et al., 2015).** *B. pilosa* could offer these benefits because of its

high polyphenols (an antioxidant) ranging from 15.66 to 20.19 mg GAE/g in the water extract and from 30.86 to 32.52 mg GAE/g for the ethanol extract, with a subclass of 54% and 42% phenolic acid, respectively (**Cortés-Rojas et al.,2013).**

In addendum, it was indicated that different dosages of *Bidens Pilosa* 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 mega karyocytes observed in rats that received an aqueous extract of *Bidens Pilosa* for one week **(Deutsch and Tomer, 2006).** Supporting with ethanolic extract (E.X) of *Bidens Pilosa* 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 *Bidens Pilosa* may be due to the antioxidant contents in the extract. Supplementation with *Bidens Pilosa* extract improved the levels of haemoglobin in rats. These obtained results support the main use of *Bidens Pilosa* ethanol extract fruits with the exception of anaemic **conditions (Al- Said et al., 2011).** Antioxidant compounds of different species of *Bidens Pilosa* are known to initiate the oxidative effects **(Ramesh et al., 2010).**

Conclusion

Bidens Pilosa plant extract examined 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, authors recommended more interest and consumption of *Bidens Pilosa* plant as an (9ml) extract in our diets.

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