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Protective Effect of *Bidens Pilosa* L. Leaves Against Carbon Tetrachlorid-Indced Liver Injured Rats

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ABSTRACT

This study aimed to investigate the protective effect of *Bidens Pilosa* (*B.Pilosa)* leaves against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats and to determine its phytochemical constituents. Forty eight adult males albino rats were distributed into six groups (n=8). Group (1) fed on basal diet B.D and used as a control negative group, (2) fed on B.D and injected with carbon tetrachloride CCl4 (2mlL/kg) twice a week for two consecutive weeks to induce hepatotoxicity in rats and used as control positive group, Groups (3), (4), (5) and (6) injected groups were fed on B.D and treated daily with 75, 150, 300 and 450mg/kg of *B.pilosa* extract, respectively. At the end of experiment blood samples were collected from each rat on fasting state for biochemical analysis, the blood samples were examined for liver enzymes activities (ALT and AST), lipid profile including total cholesterol, HDL, LDL and VLDL, and kidney function including (uric acid & urea nitrogen). The result showed that dried powder of *B.pilosa* leaves contains carbohydrates, glycosides, sterols, triterpenoids, flavonoids, tannins, saponins and alkaloids. *B.pilosa* extract increased body weight gain, liver enzyme ALT, AST, LDL,VLDL , uric acid and urea nitrogen on the other hand HDL was increased. Therefore *B.pilosa* may be beneficial for patient suffering from liver toxicity.

Kewords: *Bidens* pilosa- Hepatotoxicity- Phytochemical analysis- Biochemical parameters- Histology

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INTRODUCTION

Liver disease is the major cause of death every year. Approximately 29 million people suffer from a chronic liver condition ***(*Blachier, *et al, 2013)*** and more than 30 million Americans have liver diseases **(American liver foundation, 2017).** Liver diseases are the fifth big killer in England after cancer, stroke and respiratory disease. The most common causes of liver disease worldwide are chronic hepatitis B and C, alcohol and non-alcoholic steatohepatitis associated with obesity and metabolic syndrome ***(Hepamap, 2007).***

Egypt recorded the highest prevalence of hepatitis C in the world, and this epidemic is expected to reach its peak soon. According to a 2010 study, an estimated more than half a million people contract the virus for the first time each year. While the Egyptian Ministry of Health and Population estimates that the number of cases of HIV infection annually reaches 100,000 people. Studies have shown that the rate of infection with hepatitis C virus, "C" in Egypt, is the highest in the world, as it is equivalent to ten times that of Europe and America. ***(Sivakrishnan, 2019)***

Bidens pilosa L. belongs to the Asteraceae family originated from South America, is popularly known in Brazil as picão, picão preto, marcela-do-campo and carrapicho de agulha and in the USA as black-jack, beggar-ticks, cobbler's pegs and Spanish needle, and grows spontaneously in agricultural crops in all regions of Brazil. **(Lorenz and Matos2008; Alonso, 2016).**

In traditional medicines of many countries of the world, different parts of *B. pilosa* in form of juice, powder, decoction or taken orally have been reportedly used to treat hepatitis, stomach disorders, hypertension, inflammation and digestive disorders (**Silva *et al.*, 2011 and Bartolome *et al.*, 2013**). 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 antiulcerogenic **(Alvarez *et al.*, 1999)**, anti-leukemic **(Chang *et al.*, 2001)**, anti-inflammatory **(Pereira, *et al*., 1999 and Horiuchi and Seyama, 2008),** antihypertensive **(Leandre *et al*., 2008**), antitumor **(Kviecinski *et al.*, 2008),** hepatoprotective **(Yuan, *et al.*, 2008 and Kviecinski et al., 2011)**, antidiabetic (**Chien *et al*. 2009**), immunosuppressive**,** antioxidant and antibacterial **(Lawal *et al*., 2015**)

B. pilosa is an extraordinary source of phytochemicals and 201 compounds have so far been identified from this plant, including 70 aliphatics (36 polyynes), 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds **(Silva *et al*., 2011).** Phytochemical studies of *B. pilosa* L. leaf extract have revealed the

presence of many flavonoids, polyacetylenes and glycosides **(Hoffmann and Hölzl, 1989),** essential oils and terpenes **(Zollo *et al.*, 1995)** with anti-microbial and anti-inflammatory properties **(Wong-Leung, 1988).**

Bidens pilosa: The main medicinal use of this plant is to reduce blood sugar levels, that is, to cure diabetes. The decoction of the whole plant is used to treat diseases of the liver, rebound of bile, indigestion, and diarrhea. The leaves prepared in infusion are used for the treatment of dysentery, diarrhea, flu, stomach pain, canker sores, angina pectoris, cough, fevers, diabetes, edema, hepatitis, hypertension, and gastroduodenal ulcers. It has also been used against inflammation and as a diuretic. **(García Barriga 1975;**

Pérez Arbeláez 1996; Ministerio de Protección Social 2008; Fonnegra-Gómez and Villa-Londoño 2011 and Fonnegra Gómez *et al*. 2012).

The whole fresh plant is used to treat liver, infections, and diarrhea. The bath with the infusion of the flowers is used to treat skin allergies **(Béjar *et al.* 2002; Bussmann and Sharon 2007, 2008).**

The whole plant, fresh or dried, is used to treat gallbladder, kidney inflammation, inflammation (general), kidneys, prostate, hair loss, diabetes, liver, blood, and heart **(Bussmann and Sharon 2007, 2008, 2015; Monigatti *et al*. 2013).** The plants are often found in local markets.

Feeding rats on basal diet supplemented with different levels from Bidens pilosa L. powder improved all parameters, reduced serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), and liver enzymes, while there was increase in serum high density lipoprotein cholesterol (HDL-c), and food intake especially when the high level 10% BPP is used. From the obtained results it can be concluded that, supplementation of bakery product with Bidens pilosa L. leaves powder exerts a positive impact on the liver functions, lipid profile and other biochemical parameters.

(Dimo *et al.*, 2001 and Dimo *et al* 2002)

plant extract elevates the serum concentrations of free fatty acid by lowering the activity of hepatic carnitine palmitoyltransferase, the rate-limiting enzyme of fatty acid oxidation, leading to hyperlipidemia. The elevation in serum triglyceride and cholesterol might be due to reduced fatty acid oxidation **(Khadara *et al.*, 1996).**

Aim of study

This study aimed to investigate:-The effect of *Bidens.pilosa* extract on liver functions, The effect of *Bidens. Pilosa* extract on lipid profile. And the effect of *Bidens. Pilosa* extract on kidney functions.

MATERIALS AND METHODS

Materials:

Fresh leaves of *B. pilosa* L. collected from the fields at El-Blakos Village, Kom Hamada City, El-Beheira Governorate. Leaves identified by Flora &Phytotaxonomy Researchers Department, belonging to Horticultural Research Institute, Agricultural Research Center.

Rats:

Forty eight adult male albino rats (Sprague Dawley strain) weighting about 120±10 gm. were purchased from the Animal House of National Research center, Dokki, Egypt.

Chemicals

Casein, cellulose, sucrose, choline chloride, D-L methionine vitamins and minerals constituents was purchased from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt.

Methods

Preparation of plant material

Leaves of *B. pilosa* washed with running tap water and air dried. The air dried leaves was grounded into fine powder and kept in tightly closed containers at room temperature for further use.

Preparation of plant extract

The aqueous ethanolic extract prepared by soaking 500 g of powdered *B. pilosa* leaves in 1 liter of a solvent composed of 700 ml ethanol 95% and 300 ml distilled water at room temperature for 24 h with stirring. The infusion filtered by a piece of double layer gauze and fresh solvent will then be added to the plant materials. The combined filtrates evaporated using a rotary evaporator at 40℃ under vacuum according to **(Muralidharan and Srikanth, 2009).**

Experiment Animals:

Forty eight adult male albino rats (Sprague Dawley strain) weighting about 120±10 gm. were purchased from the Animal House of National Research center, Dokki, Egypt.

After the period of adaptation on basal diet, the rats was divided into two main groups, the 1st group was fed on basal diet as a negative control (8 rats), the 2nd main group (n=40) rats were intoxicated by subcutaneous injection with CCl4 in paraffin oil (1:1 v/v; 2 ml/kg) twice a week for two consecutive weeks to induce. hepatic toxicity (Jayasekhar et al., 1997). The 2nd main group was divided into five subgroups as follow, the first

subgroup fed on basal diet as a (positive control), the subgroups 2, 3, 4 and 5 fed on basal diet and treated with 75, 150, 300 and 450mg of *B.P.E* respectively.

Experimental Design.

Animals were housed in well-aerated cages under hygienic condition. They were fed on basal diet according to (Reeves, et al. 1993), given tap water ad libitum and left to accommodate Two weeks before experimental use. After the period of adaptation, animals were divided into six groups (6 animals each) as follows:

Group 1: Negative control group, was fed on basal diet during the experimental period.

Subgroup1: (positive control group), rats was fed on basal diet

Supgroup2: Rats fed on basal diet and treated with 75mg *B.P.E /* kg

Subgroup3: Rats fed on basal diet and treated with 150mg/kg *B.P.E./* kg

Supgroup4: Rats fed on basal diet and treated with 300mg/kg *B.P.E./* kg

Subgroup5: Rats fed on basal diet and treated with 450mg/kg *B.P.E* /kg

Biological Evaluation

Feed Intake was recorded daily and animals were weighted at the beginning and twice a week throughout the experimental period. Body weight gain% and feed efficiency ratio was calculated at the end of the experimental period, the blood Collection and Serum Separation. Blood Sample was withdrawn from each animal, on fasting state collected blood samples were centrifuged to obtain the serum for biochemical analysis from National Research Center, Dokki, Egypt. Biological-experiment was done in biological laps at Faculty of home Economics Helwan University, for a period of (6 weeks).

Biochemical Analysis

1-Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined in the serum according to the method describe by **(Reitman and Frankel, 1975),** Determination of Blood Cholesterol, was determined in the serum according to the method describe by **(Lopes-Virella *et al* 1977),** High Density Lipoprotein (HDL) was determined in the serum according to the method described by **(Lopes – Virella *et al*., 1977),** Low Density Lipoprotein (LDL) was calculated according to **(Fiedwald *et al*., 1972),** Very Low Density Lipoprotein (VLDL) was calculated according to **(Fiedwald *et al*., 1972), Uric acid** was determined in serum according to method describe by **(Fossati and *et al* 1980).** And **Urea nitrogen** was determined in serum according to the method describe by **(Patton and Crouch, 1977).**

Statistical Analysis:

The obtained result was presented as mean ± SD. Data was subjected to one way analysis of variance (ANOVA) using the SPSS statistic computer program. The mean difference was significant at the ($p<0.05$) level according to **(Armitage, and Berry, G. 1987).**

RESULTS AND DISCUSSION

Data in table (1) showed that all levels of *B. pilosa* alcoholic extracts caused a significantly increase in feed intake and BWG % while decreased FER in rats as compared to the control positive group. This result was similar to those of **(Fonnegra Gomez and Villa-Londoño, 2011)** and **(Sequeda-Castañeda *et al.* 2015)** who reported that *B. pilosa* extract increased BWG % and FER, because it act as treatment for digestive disorder

Table(2): shows that the best result in effect of the *B.P.E* on mean the values of ALT was recorded in the group treated with *B.P.E* 450mg with a significant decrease comparing with the control positive group and other treated groups. At the same time group *B.P.E*450mg recorded the lowest score in mean value of AST in all protective groups with a significant decrease comparing with the control positive group. This results were du to contains *B.P.E* of polyacetylenes, polyacetylenic glycosides, aurons, auron glycosides, p-coumeric acid, flavonoids and flavonoid glycosides. This phytochemicals are play an important role in protect the liver against hepatotoxicity and inflammatory by reduce the liver enzymes. This result was reported by **(LI-Wha. 2004, Zhao, 2004, Farah, 2007 and Fonnegra Gomez *et al* 2012).**

Table (3) showed the effect of B.P.E on T-cholesterol, HDL, LDL and VLDL in hepatotoxicity rats. The result in this table revealed that all treated groups recorded significant decrease $p\leq0.05$ in the mean value of T-cholesterol, LDL-c, and VLDL-c, while HDL-c increased as comparing to the positive control group. The best results in these parameters recorded in the group which treated with 450mg *B.P.E,* followed by the group treated with 300mg *B.P.E* respectively. This result proved that the *B.P.E* have a clear healthy effect in lipid profile, which agreement with **(Khadara *et al.*, 1996, Dimo *et al.*, 2001) and Dimo *et al* 2002). The** previous studies reported that *B.P.E* was used to treat hyperlipidemia.

Figure(1): Showed the effect of *B.P.E* on the mean value uric acid and urea nitrogen. The best result of all parameters (uric acid and urea nitrogen) were recorded in the group treated with *B.P.E* 450mg this treatment showed a significant decrease ($p\leq0.05$) in the parameters comparing with control positive group. The result confirms that *B.P.E* has a protective effect via

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contains *B.P.E* of phytochemicals substances which act as anti-oxidative, Which agreement with **(Pereira *et al* ., 1999, Chang *et al*., 2001, Horiuchi; Seyama, 2008).**

Histopathological examination:

Histopathological examination of liver in rats with hepatotoxicity treated with some doses of *B.P.E*

Liver was removed from each rat, careful dissection, washed with saline solution, dried with filter paper. Liver was examined histopathologically, according to **(Sheehan and Hrapchak, 1980).**

Fig (2): Photomicrograph of rat liver of the different collected groups: (2): Negative control group showing normal histological structure of liver, (3) Positive control group showing heavy inflammatory cells in liver, (4) Group protected by *B.P.E* 75mg showing moderate focal area of inflammatory cells in rat liver, (5): group protected with *B.P.E*150mg showing few foci of inflammatory cells aggregation in the liver cells. (6): Group protected with *B.P.E* 300mg showing apparently normal histological architecture of liver. (7): Group of *B.P.E* 450mg, the figure showing the best result in all groups, it was almost similar the normal liver cells.

Figures and Tables.

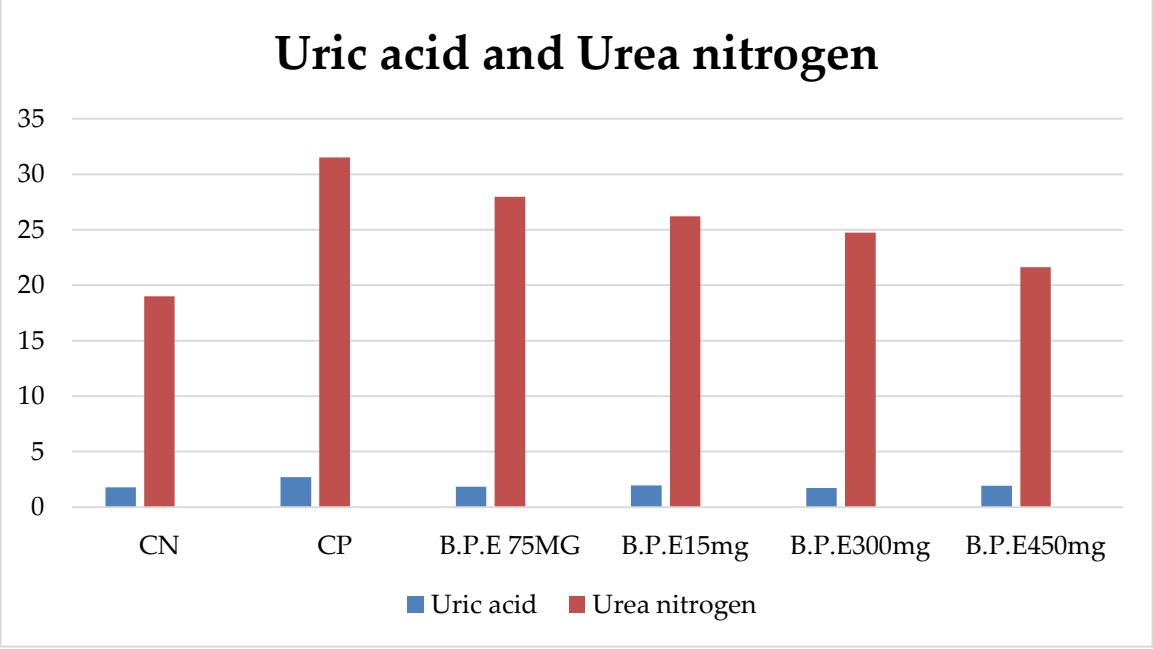
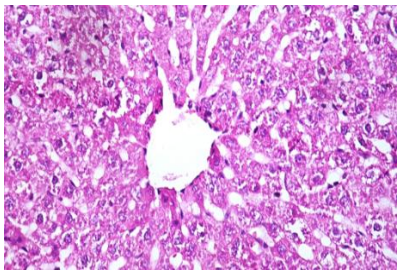


Figure (1): Effect of *B.P.E* on mean value of uric acid and urea nitrogen in rats with hepatotoxicity

The histology of hepatotoxicity rats were:



Fig(2)

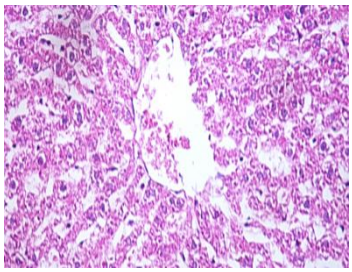


Fig 4

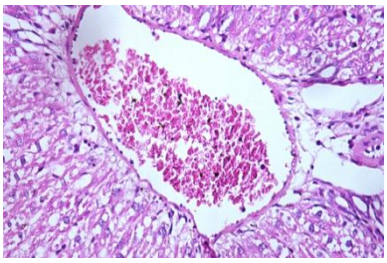
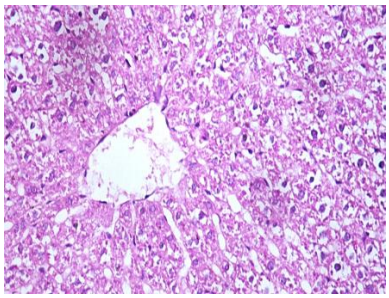
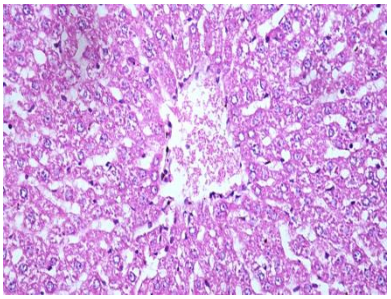


Fig 5



Fig(5)



Fig(6)

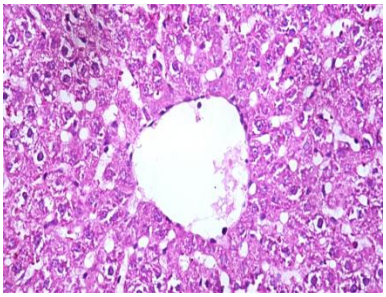


Fig 7

Table (1): Effect of *B.P.E* on (FI) food intake, (FER) Food Efficiency Ratio and (BWG) Body Wight Gain (%) in rats.

Parameters / Groups	FI (gm/ day)	BWG(%) Mean± SD	FER Mean± SD
CN	12.29±0.27[a]	55.43±0.93[a]	0.10±0.11[a]
CP	7.90±0.10[e]	33.01±1.31[c]	0.17±0.21[a]
B.P E 75mg	6.63±0.25[f]	38.01±1.23[d]	0.26±0.01[c]
B.P E 150mg	8.30±0.26[d]	47.35±0.98[c]	0.26±0.01[c]
B.P E 300mg	9.53±0.15[c]	51.0±0.69[b]	0.49±0.01[d]
B.P E 450mg	10.30±0.20[b]	52.18±1.13[b]	-0.26±1.01[c]

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

Table (2): Effect *B.P.E* on liver enzymes of rats with hepatotoxicity.

Parameters / Groups	AST	ALT
CN	101.19± 1.27 [e]	37.33± 1.14 [f]
CP	148.16± 1.10 [a]	94.76± 0.45 [a]
B.P.E75mg	137.59± 1.37 [b]	68.14± 0.68 [b]
B.P.E150mg	133.45± 0.80 [c]	54.27± 0.91 [c]
B.P.E300mg	126.27± 1.04 [d]	51.16± 1.68 [d]
B.P.E450mg	125.98± 2.12 [d]	48.83± 0.88 [e]

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

Table (3): Effect of *B.P.E* on lipid of rats with hepatotoxicity

Parameters / Groups	T- Cholesterol	HDL-c	LDL-c	VLDL-c
CN	90.67± 0.99 f	47.00± 1.76 a	29.43± 0.84 f	8.75± 0.28 d
CP	142.32± 0.62 a	28.29± 1.36 e	84.54± 0.90 a	17.85± 0.69 a
BPE75mg	135.25± 1.01 b	31.05± 0.17 d	78.53± 0.69 b	12.11± 0.19 b
BPE150mg	119.29± 0.88 c	34.38± 1.20 c	44.44± 1.30 c	11.58± 0.53 b
BPE300mg	116.33± 1.14 d	36.18± 0.84 c	45.98± 0.39 d	10.31± 0.52 c
BPE450mg	109.76± 0.92 e	41.16± 0.91 b	43.55± 0.97 e	9.84± 0.24 c

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

CONCLUSION

The present study proved that the *Bidens Pilosa* contains a various compounds of phytochemicals such as carbohydrates, glycosides, setrols, tritepenoids, flavonoids, tannins, saponins and alkaloids which made *Bidens Pilosa* have a strong effect in treat and protect liver against toxicity agents carbon tetrachloride (CCL4) induced hepatotoxicity, act as anti-inflammatory and stimulate the formation of free radicals. From biochemical analysis it could be condoled that *B.P.E* decreased body weight gain, liver enzymes ALT, AST, LDL, VLDL, uric acid and urea nitrogen, On other hand HDL was increased. Therfor B.P.E may be beneficial for patient suffering from liver toxicity

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التأثير الوقائي لأوراق نبات الحسيكة على اضطرابات الكبد المستحدث برابع كلوريد الكربون في الفئران

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المستخلص :

استهدفت هذه الدراسة التحقق من التأثير الوقائي لأوراق نبات الحسيكة ضد رابع كلوريد الكربون المحدث للتسمم الكبدي، وتقدير المركبات الكيميائية النشطة. تم استخدام 48 فأر ذكر بالغ من الألبينو و تقسيمهم الى 6 مجموعات. المجموعة (1) تم تغذيتها على الغذاء الأساسي واستخدامها كمجموعة ضابطة سالبة. المجموعة (2) تم تغذيتها على الغذاء الأساسي و حقنها برابع كلوريد الكربون واستخدامها كمجموعة ضابطة موجبة. المجموعات (3)، (4)، (5) و (6) تم حقنهم و تغذيتهم على الغذاء الأساسي وعولجت يوميا ب 75، 150، 300، و 450 ملجم/كجم من مستخلص الحسيكة على التوالي. في نهاية التجربة تم تجميع عينات الدم من كل فأر في حالة الصيام من اجل التحليلات البيوكيميائية. تم تحليل عينات الدم لقياس نشاط انزيمات الكبد وصورة دهون الدم وتشمل الكوليستيرول الكلي، الليبوبروتينات عالية الكثافة، الليبوبروتينات منخفضة الكثافة والليبوبروتينات شديدة انخفاض الكثافة، تقدير وظائف الكلى وتشمل حمض اليوريا ونيتروجين اليوريا. وقد أظهرت النتائج أن مسحوق الأوراق المجففة لنبات الحسيكة يحتوي على فلافونويد، تانين، سابونين و ألكالويد. قام مستخلص نبات الحسيكة بتقليل وزن الفئران، انزيمات الكبد، ودهون الدم (الليبوبروتينات منخفضة الكثافة و الليبوبروتينات شديدة انخفاض الكثافة)، حامض اليوريا ونيتروجين اليوريا، بينما قام برفع مستوى الليبوبروتينات العالية الكثافة. ولذلك فان نبات الحسيكة قد يكون فعال في علاج المرضى الذين يعانون من التسمم الكبدي.