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The Potential Effects of Chicory (*Cichorium intybus*) on Kidney Diseases Using Rat Animal Model

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Abstract:

This work aimed to study the effect of Chicory (*Cichorium intybus*; *C. intybus* roots on kidney functions in male albino rats. Forty adult male Sprague Dawley rats were divided into two main groups; the first was the control negative (C-; n=8), and the rest were injected with gentamicin for kidney failure induction. All the second group was divided randomly into four sub-groups (Control positive C+) and three more groups (G3:G5) fed on a basal diet supplemented with powdered chicory roots (2.5, 5, and 7.5%, respectively). At the end of the experiment, body weight gains (BWG) and collected blood serum samples were analyzed for serum glucose levels and lipid profile (cholesterol, triglyceride, HDL, and LDL) in addition to kidney functions (Creatinine, Creatinine clearance Urea, and Uric acid). The collected data showed that gentamicin injection caused significant decreases in BWG, FI, FER, and HDL, while significant decreases were recorded in Urea, Uric acid, cholesterol, triglyceride, LDL & VLDL after the consumption of the studied root, especially at 5% supplementations. In conclusion, rats treated with supplemented chicory roots at 7.5% showed an improvement in kidney function and lipid profiles; therefore, such roots are highly recommended for helping with kidney disease; however, many more human studies are needed.

Key words: Gentamicin- Kidney function, Lipid profile and body weight gain.

1. Introduction:

Chronic kidney disease (CKD) affects between 8% and 16% of the population worldwide and is often under recognized by patients and clinicians. Defined by a glomerular

filtration rate (GFR) of less than 60 mL/min/1.73 m², albuminuria of at least 30 mg per 24 hours, or markers of kidney damage (e.g., hematuria or structural abnormalities such as polycystic or dysplastic kidneys) persisting for more than 3 months, CKD is more prevalent in low- and middle-income than in high-income countries [1]. Globally, CKD is most commonly attributed to diabetes and/or hypertension, but other causes such as glomerulonephritis, infection, and environmental exposures (such as air pollution, herbal remedies, and pesticides) are common in Asia, sub-Saharan Africa, and many developing countries. Genetic risk factors may also contribute to CKD risk. For example, sickle cell trait and the presence of 2 APOL1 risk alleles, both common in people of African ancestry but not European ancestry, may double the risk of CKD [2].

Chicory (*Cichorium intybus* L.) is an indigenous vegetable widely cultivated in Europe, America and Asia. In ancient times, the leaves, flowers, seeds, and roots have been used as a wealth of health benefits including its tonic effects, the ability to ease digestive problems and to detoxify liver. In Indian traditional therapy, chicory was known to possess antidiabetic effect. In the traditional medicine of Bulgaria and Italy, chicory was used as hypoglycemic decoctions [3]. The genus *Cichorium* (Asteraceae) is made up of six species with major geographical presence in Europe and Asia. *Cichorium intybus*, commonly known as chicory, is well known as a coffee substitute but is also widely used medicinally to treat various ailments ranging from wounds to diabetes. Although this plant has a rich history of use in folklore, many of its constituents have not been explored for their pharmacological potential [4].

Previous study shown that chicory could decrease serum uric acid levels by inhibiting the activity of xanthine oxidase (XOD); the enzyme that catalyses the generation of uric acid from hypoxanthine and xanthine, and by promoting uric acid excretion by upregulating the mRNA expression of OAT3 in hyper-uricaemic rats [5]. Further studies also showed chicory to have multi-channel and multi-target effects on anti-hyper-uricemia. In terms of urate generation, chicory can inhibit the activities of xanthine oxidase and adenine deaminase, two key enzymes involved in purine metabolism [6]. In terms of urate excretion, chicory can increase intestinal efflux of urate by regulating ATP-binding cassette super-family G member (ABCG) 2, a urate transporter in the intestine. Simultaneously, chicory can increase the clearance rate of urate (CRUA) from the kidney, but the mechanisms of chicory-mediated uricosuric action in the kidney have not been elucidated. Clarification of the mechanism of the renaluricosuric effect of chicory could help its development as an anti-hyperuricemia agent [7]. Therefore, the aim of the current study is to evaluate the effect of using Chicory roots between animal models with kidney failure.

Materials and Methods:

2.1. Materials :

Chicory (*Chicorium intybus*) roots obtained from Ministry of Agriculture farms, Giza, Egypt. All materials were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to Russo [8].

2.1.1. Chemicals and kidney failure/disorder induction:

Gentamicin obtained from El-Gomhoriya Company, Cairo, Egypt, and used by intra - peritoneal injection as a dose of 100 mg/kg of rat's body weight for 5 days between normal healthy male rats according to the method described by Morales et al., [9].

2.2. Methods:

2.2.1. Basal diet composition of tested rats:

The basal diet in the experiment consisted of casein (12%), corn oil (10%), mineral mixture (4%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (67.5%) according to AIN [10].

2.2.2. Experimental and animal models' design:

Forty adult male rats, weighting between 140 ± 10 g were used in the study. The animals were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan University, Egypt. The experimental was done in the Faculty of Medicine, Menoufia University, Shebin El-Kom, Egypt. Rats were housed in wire cages in a room temperature 25 Co and kept under normal healthy conditions. All the rats were divided into two main groups. One used as Group 1 that was healthy control group fed only the basal diet (C- ; n=8). The second was injected by Gentamicin for kidney disease as described early that has divided to four subgroups as following:

Group 2 (C+ve): injected by Gentamicin and used as positive control group.

Group (3): injected by Gentamicin and fed powdered chicory roots at 2.5%.

Group (4): injected by Gentamicin and fed powdered chicory roots at 5%.

Group (5): injected by Gentamicin and fed powdered chicory roots at 7.5%.

At the end of experiment, body weight gains (BWG) and collected blood serum samples were kept for further biochemical analyzes as following.

2.2.3. Biochemical blood analysis:

The rats firstly were scarified under ether anesthesia and blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. Blood samples were received into in clean dry centrifuge tubes, in which blood was left to clot at room temperature, and then centrifuged for 10 minutes at 3000 r.p.m to separate the serum.

Serum was carefully aspirated and transferred into clean cuvette tubes and stored frozen at -20°C for biochemical analysis as described by Schermer [11]. All samples were analyzed for determination the following parameters:

Urea levels that were determined according to the enzymatic method of Patton and Crouch [12] while creatinine and uric acid levels were measured according to kinetic method of Henery [13] and enzymatic colorimetric test of Schultz [14] respectively. Serum glucose determined according to Young, [15] and serum levels of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL.c) were determined by using the methods of Thomas [16] and Fossati and Principe [17] and Grodon & Amer [18] respectively. Also, the low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) determination were carried out as described by the methods of Lee and Nieman [19] as follows:

$$\text{VLDL-c (mg/dl)} = \text{Triglycerides}/5$$

$$\text{LDL-c (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-c.}$$

2.2.4. Statistical Analysis:

The data were statistically analyzed using a computerized SPSS Program by one way ANOVA when a significant mean effect was detected. The means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant and results are presented as mean \pm SD [20].

3. Results and Discussion:

Chicory is rich in polyphenols so the polyphenol content of used chicory roots was determined as total phenolic content (TPC), total flavonoids content (TFC) and total tannins content (TTC). The results showed that polyphenolic contents varied considerably in chicory (Table 1).

Table (1): Total phenolic, flavonoids and tannins content of used chicory roots

Phenolic Content	Chicory roots (n=3)
TPC (mg GAE/g)	0.44 \pm 0.04
TFC (mg QE/g)	0.07 \pm 0.01
TTC (mg CE/g)	0.84 \pm 0.03

Results are reported on a dry weight basis; the terms mg GAE/g, mg QE/g and mg CE/g for milligrams of gallic acid equivalents, milligrams of quercetin equivalents and milligrams of catechin equivalents, respectively.

It can be seen from table 1 that value of TPC was 0.44 \pm 0.04 mg GAE/g while TFC was 0.07 \pm 0.01 mg QE/g and TTC was 0.84 \pm 0.03 mg CE/g. so the biggest phenolic levels

were with the tannins total levels that was 0.84 ± 0.03 mg CE/g; the milligrams of catechin equivalents.

Table (2): Body weight gain (BWG), feed intake (FI) and food efficiency ratio (FER) in adult male albino rats subjected to different treatments

Groups	Parameters	FI (g/d)	BWG (g)	FER
G1: Control –ve		13 \pm 1.05 bc	0.55 \pm 0.013 ab	0.042 \pm 0.0001a
G2: Control +ve		10 \pm 0.07ef	0.21 \pm 0.132b	0.021 \pm 0.0002c
G3: fed Chicory roots 2.5%		12 \pm 0.5 cd	0.41 \pm 0.133ab	0.034 \pm 0.0007 b
G4: fed Chicory roots 5%		18 \pm 0.07 a	0.75 \pm 0.028 a	0.042 \pm 0.0007 a
G5: fed Chicory roots 7.5%		9 \pm 1.05 f	0.30 \pm 0.283 b	0.033 \pm 0.004 b

Each value is representing Mean \pm SD for each group (n=3). Data in the same column with different superscript letters are significantly difference ($P < 0.05$).

Regarding the body weight gain (BWG) data collected within the running experimental, it can be observed in Table (2) again that body weight gain at the end of the experimental has been increased between all the animal groups. However, group consumed chicory roots at 5% shown the wide effect (improved within their body weight gain; 0.75 ± 0.028 g) comparing with both negative and positive control groups (0.55 ± 0.013 and 0.21 ± 0.0002 g respectively).

Also, it can be seen from table (2) that, feed intake (FI; g/d) mean values were at the highest levels on G4 that was feeding chicory roots 5% (18 ± 0.07 g/d) comparing to the positive gentamicin rats (10.10 ± 0.07 g/d), G3 (gentamicin group consuming 2.5% chicory roots) and G5 (gentamicin group consuming 7.5% chicory roots) that have various values of their feed intake (12 ± 0.5 and 9 ± 1.05 g/d respectively).

Regarding the feed efficiency ratio (FER), the negative control group and group (4; fed 5% plant roots) showed the highest value (0.042 g/d). According to Montagne et al., [21] a diet with high content of fiber like Chicory will result in body weight gain increases. The results of the current study demonstrated that feeding chicory improved growth performance by enhancing absorption [22].

It can be seen in table (3) that the lowest blood serum urea level was in (G1) control negative (2.4 ± 3.2 mg/dl) while the highest level was seen within G2; gentamycin group (93.1 ± 3.9 mg/dl). Additionally, G5 was the lowest group between groups fed chicory (fed 7.5% of roots; 36.5 ± 1.7 mg/dl).

For creatinine, data could be observed that the lowest blood creatinine level was in control negative (G1; 0.54 ± 0.03 mg/dl) while the highest level was seen within gentamycin group

(G2; 1.53 ± 0.11 mg/dl). Also, group 5 was the best group consumed chicory roots 7.5% (0.61 ± 0.04 mg/dl). Additionally, it could be revealed that the lowest blood creatinine clearance level was in control negative (G1; 0.13 ± 0.01) while the highest level was seen within gentamycin group (G2; 0.46 ± 0.02). Also, it could be illustrated that the highest uric acid level was in gentamycin group (G2; 5.44 ± 0.25) mg/dl while the lowest level was in control negative (G1; 3.09 ± 0.07 mg/dl) followed by G5 which had the lowest group between groups fed chicory 7.5% of roots (3.32 ± 0.07 mg/dl). All in total it could be seen from such kidney function tables; measured parameters that the best group was G5. Indeed, Helal et al., [23] found that the treatment with chicory ameliorated the urea, creatinine levels and improved the induced degenerative histopathological changes. The pretreatment with chicory before the induction of fatty liver gave some protection against factors that experimentally induced fatty liver. Also, another study by Li et al., [5] found that chicory could decrease serum uric acid levels by inhibiting the activity of xanthine oxidase (XOD); the enzyme that catalyzes the generation of uric acid from hypoxanthine and xanthine, and by promoting uric acid excretion by upregulating the mRNA expression of OAT3 in hyperuricaemic rats.

Table (3): kidney functions affected by chicory roots consumptions between used experimental animal model

Parameters	Kidney functions (mg/dl)			
	Urea	Creatinine	Creatinine clearance	Uric acid
Groups				
G1: Control –ve	$2.4 \pm 3.2e$	$0.54 \pm 0.03d$	$0.13 \pm 0.01d$	$3.09 \pm 0.07c$
G2: Control +ve	$93.1 \pm 3.9a$	$1.53 \pm 0.11a$	$0.46 \pm 0.02a$	$5.44 \pm 0.25a$
G3: Chicory roots 2.5%	$72.5 \pm 3.8b$	$0.97 \pm 0.06bc$	$0.38 \pm 0.01b$	$4.72 \pm 0.19b$
G4: Chicory roots 5%	$53.8 \pm 3.5c$	$0.89 \pm 0.02c$	$0.28 \pm 0.02c$	$3.87 \pm 0.12c$
G5: Chicory roots 7.5%	$36.5 \pm 1.7d$	$0.61 \pm 0.04d$	$0.22 \pm 0.01c$	$3.32 \pm 0.07c$

Each value is representing Mean \pm SD for each group. Data in the same column with different superscript letters are significantly difference (<0.05).

Cichorium intybus L. herb caused significant decrease in uric acid levels according to Khodadadi et al., [24]. *Cichorium intybus* L extract and vitamin C in chicken under heat stress induced improvement within kidney activity. They also explained that the effects of chicory on kidney functions may be due to its antioxidant compounds with their potential properties as a source of phelanoeid compounds. Again, Jin et al., [25] indicated that chicory extract clearly reduced serum uric acid levels in rats with hyperuricemia (HUA) induced by 10% fructose. Such study was the first to observe the effect of chicory on serum uric acid levels and renal function in rats with HUA and renal injury.

Table (4): Serum glucose levels between adult male rats affected by used different chicory roots treatments

Groups	Parameters	Final glucose (mg/dl)	Differences from G2
G1: Control –ve		132.5±5.5e	-250.5
G2: Control +ve		383.0±15.6a	0
G3: Chicory roots 2.5%		252.6±13.6b	-130.4
G4: Chicory roots 5%		216.1±7.8c	-166.9
G5: Chicory roots 7.5%		168.7±4.3d	-214.3

Each value is representing Mean \pm SD for each group. Data in the same column with different superscript letters are significantly difference ($P < 0.05$).

It can be seen in table (4) that the lowest blood glucose level was in (G1) control negative (132.5±5.5 mg/dl) while the highest level was seen within (G2) group with gentamycin 5% of roots and that group was the nearest to the control negative group (168.7±4.3 mg/dl). Indeed, Ghamarian et al., [26] reported that after one to three weeks' treatment of streptozotocin (STZ) diabetic rats with the methanol extract of *C. intybus* resulted in significant decrease of blood glucose levels between treated animals. Additionally, Abdolreza et al., [27] revealed that chicory appeared to have short-term (about 2 hours, as far as glucose tolerance test (GTT) is concerned) and long-term (28 days) effects on diabetes. Chicory may be useful as a natural dietary supplement for slowing down the pace of diabetes progress, and delaying the development of its complications. Again such effects confirmed by Samarghandian et al., [28] who suggested that the *Cichorium intybus* extract has antioxidant properties and prevent diabetes complications by modulation of oxidative stress system. Additionally, Nishimura et al., [29], suggested that chicory root extract could delay or prevent the early onset of diabetes mellitus and improve bowel movements.

It can be seen in table (5) that the lowest blood measured total cholesterol (TC) level was in G5 (fed chicory in 7.5% roots) in the lowest obtained levels (80.71± 0.007 mg/dl) that was the closest to the levels obtained in G1; control negative group while the highest level was seen within gentamycin group (positive group; 175.93±1.25 mg/dl).

Furthermore, the TG, data could be observed that the lowest blood TG level was in control negative (G1; 62.90±1.15 mg/dl) while the highest level was seen within gentamycin group (G2; 148.6±1.82 mg/dl). Also, group 5 was the best group fed chicory roots (7.5%; 71.47± 1.38 mg/dl).

Data presented in the same table showed that the lowest blood HDL level was in gentamycin positive group (G2; 47.56±0.006 mg/dl) while the highest level was seen

within G4 and G5 that fed 5 and 7.5 % chicory roots by 50.42 ± 0.002 and 51.14 ± 0.001 respectively comparing to the negative control group (51.9 ± 0.08 mg/dl).

Table (5): Serum lipids profile in adult male albino rats subjected to different treatments

Parameter Groups	Lipid profile (mg/dl)				
	TC	TG	HDL	LDL	VLDL
G1: Control -ve	$81.61 \pm 0.01d$	$62.90 \pm 1.15e$	$51.9 \pm 0.08a$	$17.13 \pm 0.009e$	$12.58 \pm 0.005e$
G2: Control +ve	$175.93 \pm 1.25a$	$148.6 \pm 1.82a$	$47.56 \pm 0.006c$	$98.65 \pm 0.003a$	$29.78 \pm 0.008a$
G3: Chicory roots 2.5%	$132.4 \pm 1.69b$	$124.2 \pm 1.42b$	$48.8 \pm 0.009c$	$58.7 \pm 0.007b$	$24.9 \pm 0.009b$
G4: Chicory roots 5%	$96.60 \pm 1.76c$	$87.84 \pm 1.59c$	$50.42 \pm 0.002b$	$28.61 \pm 0.004c$	$17.57 \pm 0.003c$
G5: Chicory roots 7.5%	$80.71 \pm 0.007d$	$71.47 \pm 1.38d$	$51.14 \pm 0.001a$	$19.27 \pm 0.005d$	$14.29 \pm 0.006d$

Each value is representing Means \pm SD for each group. Data in the same column with different superscript letters are significantly difference ($P < 0.05$).

For LDL, data could be observed by their lowest level in control negative (G1; 17.13 ± 0.009 mg/dl) while the highest level was seen within gentamycin positive group (G2; 98.65 ± 0.003 mg/dl). Group 5 was the best group that fed in chicory roots 7.5% (19.27 ± 0.005 mg/dl).

Moreover, It could be revealed that the lowest blood VLDL level was in G1 (control negative; 12.58 ± 0.005 mg/dl) while the highest level was seen within G2 (gentamycin group; 29.78 ± 0.008 mg/dl). G5 was the best group fed chicory in 7.5% of roots (14.29 ± 0.006 mg/dl). Such all collected lipid profile measurements are in great agreements within previous similar research by Nishimura et al., [29] who showed that chicory root extract decrease TC, TG, LDL & VLDL and increase HDL level in 47 healthy adult participants in a randomized, double-blind, placebo-controlled study. Additionally, Abdel-Rahim et al., [30] found that administration of chicory leaves or psyllium seeds to diabetic rats produced a significant reduction in cholesterol, triglyceride and low density lipoprotein cholesterol (LDL-c) levels.

4. Conclusion:

To conclude, chicory roots improved the kidney functions especially the 5% supplementations a day between used animal models. Also, such used animal models have been enhanced with their measured lipid profiles between animal models with

kidney failure. Therefore, chicory roots could be recommended to such patients; however, much more human studies are needed.

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التأثيرات المحتملة للهندباء على أمراض الكلى باستخدام حيوانات التجارب

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¹ قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر، ² قسم الفسيولوجي، كلية الطب، جامعة المنوفية، شبين الكوم، مصر

الملخص العربي:

كان الهدف من هذا البحث دراسة تأثير جذور نبات الهندباء على وظائف الكلى بين ذكور الفئران البيضاء. تم تقسيم أربعين من ذكور الفئران من سلالة سبراج داوولي إلى مجموعتين رئيسيتين؛ الأولى هي المجموعة الضابطة السالبة (-C عدد 8) والثانية تم حقنها بالجنتاميسين لإحداث الفشل الكلوي، وقسمت المجموعة الثانية عشوائياً إلى أربع مجموعات فرعية المجموعة الضابطة الموجبة (+C) وثلاث مجموعات أخرى (G3:G5) تم تغذيتها على نظام غذائي أساسي مكمل بمسحوق جذور الهندباء. بنسب (2.5 و 5 و 7.5٪ على التوالي)، وفي نهاية التجربة تم تقدير الجسم المكتسب وعينات مصل الدم المجمع لمستويات الجلوكوز في الدم ودهون الدم (الكوليسترول والجليسيريدات الثلاثية و الليبوبروتينات مرتفعة الكثافة والليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جداً) بالإضافة إلى وظائف الكلى (الكرياتينين، تصفية الكرياتينين واليوريا وحمض اليوريك). أظهرت البيانات التي تم جمعها أن حقن الجنتاميسين تسبب في انخفاض معنوي في وزن الجسم المكتسب و المأخوذ الغذائي و معدل الاستفادة من الغذاء والليبوبروتينات مرتفعة الكثافة بينما تم تسجيل انخفاضات كبيرة في اليوريا و الكوليسترول والجليسيريدات الثلاثية والليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جداً بعد أن شاركت الجذور المدروسة خاصة عند مكملات 5 ٪. والخلاصة انه أظهرت الفئران التي عولجت بجذور الهندباء المكمل بنسبة 5 ٪ تحسناً في وظائف الكلى وخصائص الدهون، لذلك يوصى بشدة بهذه الجذور للمساعدة في أمراض الكلى، ومع ذلك، هناك حاجة إلى مزيد من الدراسات علي البشر.

الكلمات المفتاحية: الجنتاميسين - وظائف الكلى - دهون الدم - وزن الجسم المكتسب.