

## Effects of Inulin as Pollutant Elements Removal and Enhancer for Calcium and Magnesium Absorption

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### ABSTRACT

Functional foods are a unique opportunity to improve the quality, safety and nutritive value. Inulin is a natural product as functional food ingredient. Wheat has the highest cadmium content among the grains as one of pollutant elements which produces free radicals initiating various pathological, and at the same time wheat is the most important ingredient for bread making. Evaluation the effect of inulin on pollutant elements was the aim of this work. Therefore, inulin was extracted from Jerusalem artichoke tubers. Wheat Giza 61 from Assiut was used for bread making, inulin was added to flour for bread making. Rheological and sensorial properties of dough and bread were evaluated to determine the proper percent of inulin addition to the flour for bread making. Diets for biological evaluation by animal experiment were prepared according to the chosen wheat bran bread (WBB) with the proper percent of inulin which produced highest quality of dough as indicated by rheological properties and highly accepted sensorial properties. Inulin and cadmium content in bread after backing were determined to calculate the amount of inulin or cadmium addition to diets for the biological evaluation. The prepared diets were fed to rats for 30 days. Body weight gain, feed conversion, feed efficiency were determined. Tibia, femur, femur width, feces characteristics and minerals content of feces, hematological parameters, blood biochemical, and histological examination of tissue specimens of the experimental rats were also carried out. Results of this study clearly revealed that body weight gain % was the lowest in groups fed on diets containing Cd Cl<sub>2</sub> while it increased in groups fed diets containing inulin + Cd Cl<sub>2</sub>. Tibia of rats fed on diet contained Cd Cl<sub>2</sub> had lower content of calcium and magnesium comparing with that fed on diet containing inulin + Cd Cl<sub>2</sub>. At the same time calcium and magnesium decreased in rats feces fed on diets with inulin addition. Removal of cadmium content in feces of group fed on diets containing Cd Cl<sub>2</sub> + inulin was increased. The histological examination of tissue specimens of studied rats cleared that Cd Cl<sub>2</sub> alone led to an extensive nephrotoxic as well as hepatotoxic alteration that mostly resulted clinically into failure of liver and kidney functions in the affected animals. Also, it was concluded that the inulin led to some antagonistic or protective effect against cadmium. Likewise the absorption of calcium and magnesium was enhanced.

## INTRODUCTION

The development of using functional foods is a unique opportunity to improve the quality, safety and nutritive value which will be reflected on consumer health. In that context, inulin and oligofructose are natural products that may become classified in the future as functional food ingredients for which validated health claims may become authorized (Roberfroid, 1999; Roberfroid, 2005). Inulin and oligofructose have attracted much attention recently as non absorbable carbohydrates with in-colonic prebiotic properties. When inulin and oligofructose were added to controlled diet, significant increases were noted in colonic bifidobacterial populations, and it has been proposed that these changes promote both colonic and systemic health through modification of the intestinal microflora. Inulin and oligofructose are rapidly and completely fermented by the colonic microflora with the production of acetate and other short-chain fatty acids (Gibson & Roberfroid, 1995; Gibson, 1998; Jenkins *et al.*, 1999; Gibson *et al.*, 2004; Roberfroid, 2005).

Inulin and oligofructose are mixtures of  $\beta$  (2  $\longrightarrow$  1) linked fructans, in most chains the terminal sugar is glucose. The degree of polymerization ranges between 3-60 and between 2 and 7 for inulin and oligofructose, respectively (Roberfroid *et al.*, 1998). Inulin and oligofructose are natural food ingredients commonly found in varying percentages in dietary foods. They are present in > 36.000 plant species (Carpita *et al.*, 1989). Jerusalem artichoke tubers (*Helianthus tuberosus*) is considered one of the most interesting potential source of Inulin (Roggen, 1976). The bread is considered as main element in Egyptian diet (Ahmed, 2001). Demand for bread and wheat flour consumption has been increased as the result of increasing the high rate of population. Wheat generally has the highest Cd concentration among the grains most commonly consumed and, of the different fractions of wheat grains (Anderson, 1989 Anderson and Siman, 1991; Abd El-Lateef *et al.*, 2001 ) Also, heavy metals like cadmium have been demonstrated to produce enormous amounts of free radicals, resulting in oxidative deterioration of lipids, proteins and DNA, initiating various pathological conditions in humans (Moore and Ramamoorthy, 1984; Hussain *et al.*, 1997; Zhang *et al.*, 2004). Therefore the aim of this study was to evaluate the effects of adding inulin to wheat bran bread (WBB) on pollutant elements removal and enhancing calcium and magnesium absorption.

## **MATERIALS AND METHODS**

### **Materials**

Jerusalem artichoke tubers (*Helianthus tuberosus*) harvested in October 2004 was obtained from the Experimental Station, Agricultural Research Center, El-Sabaheia, Alexandria, Egypt.

Wheat grains Giza 61 were obtained from three different localities in Egypt (Assiut, Demiatta and El-Behira) of the same degree of quality.

Cadmium chloride ( $Cd Cl_2$  99% pure) was purchased from Aldrich Chemical Co. in Alexandria.

### **Methods**

#### **Determination of minerals**

Calcium, Iron, Cadmium and magnesium were determined using Perkin-Elmer 23865 atomic absorption (Spectrophotometer, Germany) according to the method described in AOAC (1995). Phosphorus was determined according to the methods described by Herbet *et al.* (1971).

#### **Extraction of inulin**

Inulin was extracted from the tuber of Jerusalem artichoke (*Helianthus tuberosus*) according to methods described by Zeitoun *et al.* (2003). Inulin was determined in Jerusalem artichoke extract as well as in the produced wheat bran bread (WBB) with inulin addition to determine the retained inulin in the bread after backing according to methods described by Prosky and Hoebregs (1999). The chemical composition of extracted product of Jerusalem artichoke tubers as inulin was 5.99% moisture, 90.31% inulin, 3.25% crude protein, 1.50% ether extract, 2.01% crude fiber and 2.93% ash.

#### **Preparation of wheat flour and bread making**

Wheat grains (Giza 61) obtained from Assiut (the highest  $Cd Cl_2$  content) were cleaned, moisted to 14% moisture for 24 hours and then milled by roller mill in Food Technology Research, Agriculture Research Center Giza Egypt. The whole wheat flour was sieved through 0.5 m.m sieve to get flour with similar characteristics of flour (72% extraction rate). The obtained wheat flour (72% extraction) was mixed with wheat bran at a ratio of 1:1 w/w to obtain wheat bran flour mixture for making Wheat Bran Bread (WBB). As inulin was extracted from Jerusalem artichoke tuber, it was dried and prepared to be added at different levels (3.5, 7.0, 10.5 and 14.0%) to the prepared flour for bread making. Some of the important parameters during backing processing were evaluated to

determine the effect of inulin addition during the stages of making dough by extensograph and farinograph. Quality and sensorial properties of bread were determined also. Regarding to the obtained results for rheological properties of dough and sensorial properties of bread the amount of inulin to be added into flour for producing bread of the best quality and acceptability for human consumption were 10.5% for WBB (72% flour mixed with bran 1:1). According to the obtained data of WBB contained 10.5 % inulin was chosen to calculate inulin and Cd Cl<sub>2</sub> content in diets prepared for biological evaluation.

### **Biological experiments**

**Animal experiments:** Twenty weaning male albino rats weighting 50-75 g each were used in this experiment. Animals were housed in cages under normal health laboratory conditions house at Food Technology Research Institute, Giza, Egypt.

**Diet Composition:** Rats were fed on a basal diet prepared according to the methods described in AOAC (1995) for two weeks for adaptation in the experimental animal cages.

**Experimental design:** After the adaptation period, the animals (20 rats) were divided into 4 groups; each group consisted of 5 rats. The following factors were highly considered in diets formulation:

1. Diets formulation were prepared depending on the inulin and CdCl<sub>2</sub> content retained in the chosen WBB of 10.5 % inulin after baking.
2. Since the maximum tolerance of CdCl<sub>2</sub> for male rates was 0.05 mg/kg diet (Kedwany, 2003) therefore, amount of WBB bread containing 0.05 mg /kg diet was found in 694.44 g of WBB. The inulin content in this amount of 694.44 g of bread was determined to be 50 g inulin. Therefore the diet formulated to contain 50 g/kg diet of inulin as one of the treatment and the other treatment contained 0.05 mg CdCl<sub>2</sub>/kg diet. The animals were fed, *ad libitum*, on experimental diets as the following:

Composition of the basal diet was formulated according to Abd-El-Hady (2003) as following:

**Group 1:** The rats were fed on a basal diet

**Group 2:** The rats were fed a basal diet + 50 g inulin powder/kg diet.

**Group 3:** The rats were fed on a basal diet + 0.05 mg Cd Cl<sub>2</sub>/kg diet (the the maximum tolerance of cadmium as reported by the method described by Kedwany, 2003).

**Group 4:** The rats were fed on a basal diet + 0.05 mg Cd Cl<sub>2</sub>/kg diet + 50 g inulin/kg diet

**Composition of the experimental diet (g/kg)**

Ingredient	Group 1	Group 2	Group 3	Group 4
	Basal diet control	Basal diet + 50 g I*/kg diet	Basal diet + CdCl <sub>2</sub>	Basal diet + CdCl <sub>2</sub> +50 g I/kg diet
Casein	100	100	100	100
Starch	700	650	700	650
Fat (Corn oil)	100	100	100	100
Mineral mixture	40	40	40	40
Vitamin mixture	10.00	10.00	10.00	10.00
Cellulose	50	50	50	50
Cadmium			0.05	0.05
Extracted inulin	22	50		50

\* I = inulin

Total body weight of the animals was recorded at the beginning and during the experimental period. All animals were fed daily, the rats had free access to diets and tap water. The animals were fed on experimental diets for 30 days. The actual weight of food intake was calculated. The rats were weighed weekly to determine body weight gain, feed conversion ratio and feed efficiency calculated (feed conversion = feed consumption total / body weight), (feed efficiency = weight gain x 100/feed intake). At the end of experiment, the animals were killed by decapitation.

**Measurement of Tibia, femur and head femur width**

Right and left tibia, femur and head femur were thawed and cleaned of all soft tissues, then the width of each were measured using Caliper to the nearest 0.01 mm.

**Feces characteristics**

During the experiment all feces were polled from day 27 to day 30 of the experimental period and subjected to the following measurements.

**Feces weight**

The weights of feces were recorded using (Sartorius, GMBH Gottingen, Germany) balances.

**Specific volume of feces**

Volume was determined using rapeseed. Specific volume was calculated by the following equation:

Specific volume = volume (cm<sup>3</sup>) / weight (gm).

### **Crude protein of feces**

Crude protein was determined by Microkjeldahl according to A.O.A.C. method (1990).

### **Minerals content in feces**

Feces dried (100°C over night), weighed, ashed, dissolved and then subjected to atomic absorption to determine calcium, iron, cadmium and phosphorus.

### **Histological examination**

Different group's tissue specimens were collected from the liver and kidney of Group 1, 2, 3 and 4 and put directly in the fixative solution of the 10% neutral buffered formalin. After fixation, specimens were routinely processed in the paraffin embedding technique after which paraffin blocks were done. Paraffin sections of 3-5 microns thick were prepared from the paraffin blocks by microtomy followed by routine staining with hematoxylin and eosin (Bancroft and Stevens, 1990). Stained slides were then subjected for the light microscopy for histological examination, finally photomicrographs were taken.

### **Hematological parameters of experimental animals**

Blood samples were collected from the killed animals at the end of each experiment. Blood samples were obtained and placed immediately on ice. Heparin was used as an anticoagulant. Plasma was obtained by centrifugation of samples at 860 g for 20 min, and was stored at -60°C until used for analyses. Non coagulated blood was tested, shortly after collection, for hemoglobin (Hb), total erythrocyte count (TEC) and total leukocyte counts (TLC). Blood Hb concentration was determined by the cyanomethemoglobin procedure (Wintrobe, 1965). Erythrocytes were counted on hemocytometer using a light microscope at 40 x 10 magnification. Blood samples were diluted to 200 times by physiological saline (0.9% sodium chloride solution) before counting. Leukocytes were counted on AO Bright line hemocytometer using a light microscope at 10 x 10 magnification after diluting blood samples to 20 times with a diluting fluid (1% acetic acid solution and little of Leishman's stain) before counting (Hepler, 1966). All the parameters were calculated as mean of five readings.

### **Blood biochemical measurements**

Stored plasma samples were analyzed for total protein (TP) by the Biuret method according to Gornall *et al.* (1949). Albumin (A) concentration were determined by the method of Doumas *et al.* (1971).

Globulin (G) concentrations were determined by difference (Total protein-albumin) and A/G ratio was calculated. Concentration of urea was determined by the method of Fawcett and Scott (1960). Plasma total bilirubin was measured using the method of Walter and Gerade (1970). Plasma calcium was determined by the method of Gindler and King (1972).

The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Alkaline phosphatase (AIP) activity was measured in plasma by the method of Belfield and Goldberg (1971).

#### Statistical analysis

Data were analyzed according to Steel Torrie (1981). Statistical significance of the difference in values of control and treated animals was calculated by (F) test with 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS, 1986).

## **RESULTS AND DISCUSSION**

### **Chemical characteristics of Giza 61 wheat grains collected from three different locality**

Chemical constituents of Giza 61 wheat grains collected from different places (on dry weight basis) are given in Table (1). Data showed that, phytic acid contents ranged from 0.32 to 0.44%. Total carbohydrate, crude protein, ether extract, crude fiber and ash contents were ranged from 78.32 to 81.19, 9.54 to 11.37, 3.15 to 3.77, 3.75 to 4.17 and 2.01 to 2.73%, respectively. The contents of minerals are also shown in Table (1). Cadmium contents ranged from 0.067 to 0.091 mg/kg. Calcium, phosphorus and iron contents were ranged from 0.15524 to 0.25423 g/kg, 2.38 to 3.28 g/kg, 57.28 to 104.68 mg/kg respectively. Wheat grains collected from Assiut had the highest content of minerals.

### **Effect of different diets on Growth Performance of male rats**

#### **Effect of different diets on body weight (gain)**

The changes in body weight (gain) and feed intake of male rats fed on diets containing basal diet (group 1), basal diet + 50 g inulin powder/kg (group2), basal diet + 0.05 mg CdCl<sub>2</sub>/kg diet (group3), basal diet + 0.05 mg CdCl<sub>2</sub> + 50 g inulin/kg diet (group4), for 30 days are presented in Table (2). Results revealed that body weight gain was the lowest in case

of rats fed on diets containing CdCl<sub>2</sub> (groups 3,). While it increased in case of groups of rats fed on diets containing inulin or inulin + CdCl<sub>2</sub> (groups 2 and 4).

**Table (1):** Chemical composition of wheat grains (Giza 61) collected from three different locality in Egypt (dry weight basis)

<b>Constituents (%)</b>	<b>EI-Beheira</b>	<b>Demiatte</b>	<b>Assiut</b>
Moisture	13.31	14.01	12.98
Crude protein	11.370	9.610	9.540
Ether extract	3.770	3.540	3.150
Crude fiber	4.170	3.650	3.750
Phytic acid	0.320	0.330	0.440
Ash	2.370	2.010	2.730
Total carbohydrate	78.320	81.190	80.830
<b>Mineral contents</b>			
Calcium (g/kg)	0.155.240	0.184.730	0.254.230
Phosphorous (g/kg)	2.380	2.500	3.280
Iron (mg/kg)	57.280	72.490	104.680
Cadmium (mg/kg)	0.069	0.067	0.091

Body weight gain in case of rats fed on diets containing CdCl<sub>2</sub> (group3) was 3.4 g while it increased to 6.05 g when rats fed on diets containing Cd Cl<sub>2</sub> + inulin (group4).



**Table (2):** Effect of different diets (G<sub>1</sub>-G<sub>4</sub>) on the growth performance of rats

Ingredient	Group 1	Group 2	Group 3	Group 4
	Basal diet control	Basal diet 50 g EI/kg diet	Basal diet+CdCl <sub>2</sub>	Basal diet + CdCl <sub>2</sub> + 50gEI/kg diet
Initial body weight (gm)	64.20	64.60	64.40	64.20
Final body weight (gm)	80.50	80.00	67.80	70.25
Body weight gain (gm)	16.30	15.40	3.40	6.05
Body weight gain %	25.39	23.84	5.28	9.42
Food intake for 30 days (gm)	330	342	279.9	303.3
Food intake (g/day)	11.00	11.40	9.33	10.11
Feed conversion ratio	20.25	22.21	82.32	50.13
Feed efficiency	4.94	4.50	1.41	1.99

EI: extracted inulin powder

### **Effect of different diets contained inulin on width (mm) of tibia, femur and head femur**

Changes in width (mm) of right and left (tibia, femur and head femur) of male rats fed on different diets for 30 days are shown in Table (3). It was clear that, the width (mm) of right and left (tibia, femur and head femur) were higher in case of group of rats fed on diet contained inulin compared with that group of rats fed on basal diet. It was 2.05 mm for right tibia, 1.73 mm for left tibia, 2.62 mm for right femure, 2.25 mm for left femure, 5.86 mm for right head femur and 5.72 mm for left head femure in case of rats fed on diet contained 50 g inulin/kg diet (group2). While it was (1.65, 1.57), (2.3, 2.01) and (5.8, 5.6) mm for right and left of

tibia, femure and head femure respectively in case of rats fed on diet contained basal diet (control).

**Table (3):** Changes in width (mm) of right and left of tibia, femure and head femure of male rats as affected by different diets

Parameters	Group (1)	Group (2)	Group (3)	Group (4)	LSD <sub>0.05</sub>
	Basal Diet (control)	Basal diet+50g inulin/kg diet	Basal Diet + CdCl <sub>2</sub>	Basal diet + CdCl <sub>2</sub> + EI	
Width of right tibia (mm)	1.65 cd	2.05 a	1.62 d	1.93 ab	0.187
Width of left tibia (mm)	1.57 a	1.73 a	1.48 a	1.60 a	0.270
Width of right femure (mm)	2.30 b	2.62 a	2.11 c	2.25 bc	0.144
Width of left femure (mm)	2.01 b	2.25 a	1.97 b	2.17 ab	0.207
Width of right head femur (mm)	5.80 bc	5.86 b	5.50 d	5.65 cd	0.184
Width of left head femur (mm)	5.60 b	5.72 b	5.42 c	5.60 b	0.164

\* Means in the same raw with different letters are significantly different ( $P \leq 0.05$ )  
EI: extracted inulin powder

Also, these data revealed that rats fed on diet containing CdCl<sub>2</sub> (group 3) had the lowest width of tibia, femure and head femure (right and left compared with rats fed on diet containing CdCl<sub>2</sub> + inulin (group4). From the above results, it could be concluded that the presence of CdCl<sub>2</sub> had decreased the width of tibia, femure and head femure (right and left), while the addition of inulin to diets improved these parameters. These results are in agreement with those reported by Roberfroid *et al.*, (2002) who found that inulin is a natural linear fructan that is not digested in the upper part of the gastrointestinal tract but is fermented in the cecocolon. It enhances calcium absorption in rats and improves femur and tibia

mineral. Also, cadmium induced bone damage (Alfen *et al.*, 2000), but inulin is decreasing the risk of osteoporosis by increasing the minerals contents (Kaur *et al.*, 2002).

**Effect of different diets containing inulin on tibia calcium and magnesium content**

The changes of calcium and magnesium contents in tibia (mg/g dw) of male rats fed on different diets for 30 days are shown in Table (4). It was clear that basal diet + 50 g inulin /kg diet (group 2) had the highest contents of calcium and magnesium. It was, 262 (mg/g) for calcium and 5.13 (mg/g) for magnesium, respectively which is clearly reflect the effect of inulin on calcium and magnesium in tibia of male rats as it was increased with about 10% during one month of feeding the experimental diets. On the other hand, tibia of group of rats fed on diet contained basal diet + CdCl<sub>2</sub> (group3) had lower contents of calcium and magnesium (238 and 4.41 mg/g) comparing with the group of rats fed on diets contained basal diet + CdCl<sub>2</sub> + inulin (group4) which had 245 and 4.52 mg/g of calcium and magnesium respectively. Alfven *et al.* (2000), Nordberg *et al.* (2002) and Kuriwaki *et al.* (2005) found that cadmium exposure inhibits calcium uptake.

**Table (4):** The changes of calcium and magnesium in Tibia (mg/g dry weight) of male rats as affected by different diets.

Group of rats		Calcium (mg/g dry weigh)	Magnesium (mg/g dry weigh)
Basal diet (control :	<b>group (1)</b>	243 ab	4.90 b
Basal diet + 50 g inulin /kg diet:	<b>group (2)</b>	262 a	5.13 a
Basal diet + CdCl <sub>2</sub> :	<b>group (3)</b>	238 ab	4.41 cd
Basal diet + CdCl <sub>2</sub> + inulin:	<b>group (4)</b>	245 ab	4.52 c
LSD <sub>0.05</sub>		23.46	0.17

Means in the same column with different letters are significantly different P ≤ 0.05.

Inulin is not hydrolyzed by enzymes in the small intestine of monogastrics, it reaches the colon intact. Inulin is fully metabolized by colonic microflora. End products of carbohydrate fermentation are gases, lactate, and short chain fatty acids such as acetate, propionate and butyrate. The high concentration of organic acid in the cecum leads to a

decrease of cecal pH that raises the concentration of soluble Ca. In parallel, in rats fed fermentable carbohydrate diets, hypertrophy of the cecum is observed (increase of cecal wall weight, crypt column height, and cell number per crypt), leading to a greater exchange surface area. Thus, the enlargement of cecum and the elevation of Ca solubility allow a better cecal absorption of Ca in rats adapted to inulin diets, it is also possible that short chain fatty acids can directly stimulate Ca absorption in the rat colon (Lutz and Scharrer, 1991) and Ca could pass through the cell membrane more readily in the form of a less charged complex (Ca acetate) by a passive pathway (Trinidad *et al.*, 1993).

### Effects of different diets fed to male rats on the physical characteristics of their feces

Physical characteristics male rats feces fed on different diets for 30 days are shown in Table (5). It could be observed that the reduction rate in specific volume of feces of rats fed on diet containing CdCl<sub>2</sub> (group3) was 18.75 comparing with that rats fed on basal diet (control) (group 1), while this reduction decreased to 7.0 % when the rats fed on diets containing CdCl<sub>2</sub> + EI (group4). Inulin will affect the volume and bulk of small intestine contents. An increase in dry weight is directly related to the fact that, with consumption of inulin, whereas the increase in volume

**Table (5):** Effect of different diets on the physical characteristics of male rats feces.

Characteristics	Volume* cm <sup>3</sup>	Specific volume (Sp) cm <sup>3</sup> /gm	Increasing rate in (Sp) %	Reduction rate in (Sp) %
<b>Group (diets)</b>				
(Group 1) Basal diet (control)	6.50	1.28	0.00	0.00
(Group2) Basal diet + 50g EI/kg diet	8.50	1.68	40.00	--
(Group3) Basal diet + CdCl <sub>2</sub>	5.25	1.04	--	18.75
(Group4) Basal diet + CdCl <sub>2</sub> + EI	6.00	1.19	--	7.03

\* Volume of feces was determined for a constant weight of 5.00 g for each treatment.

of the small-intestinal contents is related to the water-holding capacity and viscosity of inulin, inulin with high water-holding capacity tended to be more readily degraded or fermented by microbes in the large bowel (Ebihara and Schneeman, 1989).

### Effects of different diets fed to male rats on protein content of their feces

Changes in protein contents of feces of male rats fed on different diets for 30 days are presented in Table (6). The results revealed that, feces protein content decreased in case of rats fed diets containing inulin, since the highest decrease was in fecal protein content in rats fed on diet containing 50 g inulin/kg diet (group2) (7%), while the fecal protein content of rats fed on diet of (group3 and (group4) were 11.61 and 14.60%, respectively. Cadmium may produce histological changes in gastrointestinal tract and pancreas (Kido *et al.*, 1989). This may affect absorption of all amino acids, also cadmium may cause desquamation of the enlarged intestinal mucosa. Cadmium may have effect on metabolism of protein. Cadmium causes functional lesion in kidney and Cd nephrotoxicity that causes increase in urea and  $\text{NH}_3$  of blood (Gibson and Roberfrid, 1995).

**Table (6):** Effects of different diets fed to male rats on protein content of their feces

Protein content of feces	Group 1	Group 2	Group 3	Group 4
	Basal diet control	Basal diet + 50 g EI/kg diet	Basal diet + $\text{CdCl}_2$	Basal diet + $\text{CdCl}_2$ +50 gEI/kg diet
%	8.01	7.00	11.61	14.60

EI: extracted inulin powder.

### Effects of different diets fed to male rats on mineral contents in their feces

Changes of mineral contents (Ca, Fe, Cd and P) in feces of male rats fed on different diets for 30 days are presented in Table (7). It was observed that the mineral contents (Ca, P and Fe) removal in feces decreased in case of rats fed on diets containing inulin group 2, and 4 ).

In case of group of rats fed on diet containing basal diet + CdCl<sub>2</sub> the lost of minerals (Ca, P, Fe and Cd) were higher (2.70 g/kg Ca, 8.61 g/kg P, 0.32 g/kg Fe and 40.54 mg/kg Cd, respectively). While this lost decreased in case of rats fed on diet containing basal diet CdCl<sub>2</sub> + inulin (group4), it was 8.34 g/kg for phosphorus and 0.31 g/kg for iron. The removal of cadmium content in case of group of rats fed on diets containing CdCl<sub>2</sub> + inulin increased. It was 77.13 mg/kg compared with the group of rats fed on diet containing CdCl<sub>2</sub> only (40.50 mg/kg) These results are in agreement with those reported by Lopez *et al.* (2000) who found that inulin fermentation can stimulate mineral absorption in the distal part of the digestive tract through decreasing pH and increasing mucosal mass and bacterial hydrolysis of phytic acid. Thus, the lowering of mineral bioavailability due to phytic acid can be totally offset by inulin. Also inulin has effect on cadmium absorption. It has a suppressing effect on intestinal absorption of Pb and Cd present in drinking water (Choi *et al.*, 1999).

**Table (7): Effect of different diets fed to male rats on mineral content in their feces**

Minerals	Groups	1	2	3	4
		Basal diet control	Basal diet 50 g EI/kg diet	Basal diet CdCl <sub>2</sub>	Basal diet CdCl <sub>2</sub> 50 g EI/kg diet
Calcium (g/kg)		2.41	2.30	2.70	2.63
Phosphorous (g/kg)		6.98	5.19	8.61	8.34
Iron (g/kg)		0.284	0.28	0.32	0.31
Cadmium (mg/kg)		2.13	3.17	40.54	77.13

EI: extracted inulin powder

#### **Effects of different diets fed to male rats on hematological parameters**

Changes in blood hemoglobin (Hb mg/dl), total erythrocyte count (TEC, X 10<sup>6</sup>/mm<sup>3</sup>), total Leukocyte count (TLC, X 10<sup>3</sup>/mm<sup>3</sup>), and hematocrit of male rats fed on different diets for 30 days are shown in Table (8). It was clear observed that rats fed on diet containing cadmium chloride had a decline in hemoglobin (Hb), total erythrocytic count (TEC) and Hematocrit, while the total Leukocyte count (TLC) increased. Rats fed on diets containing inulin had increased in these parameters.

Blood hemoglobin (Hb) and erythrocyte count (TEC) of rats fed on diet containing CdCl<sub>2</sub> was 8.09 mg/dl and 3.03 x 10<sup>6</sup> mm<sup>3</sup>, respectively. While it was 9.93 mg/dl and 3.74 x 10<sup>6</sup> mm<sup>3</sup> in case of rats fed on diet containing CdCl<sub>2</sub> + inulin. This means that inulin in the diet containing cadmium caused a significant increase in hemoglobin and erythrocyte. Rucker *et al.* (1994) and Watzl *et al.* (2005) found that, inulin feeding increased iron absorption in rats fed a non-hem iron containing diet. In rats fed the hem diet, but anemia is secondary to the possible accelerated hemolysis, hemorrhage and/or reduced erythropoiesis, inflicted by cadmium, indicated that previous studies showed that cadmium chloride caused changes in the hematological indices of carps (Zikic *et al.*, 1997).

Table (8): Changes in blood hemoglobin (Hb), total erythrocyte count (TEC) total leukocyte count (TLC) and hematocrit of male rats as affected by different diets

Parameters	Groups of diets				LSD <sub>0.05</sub>
	(1) Basal Diet (control)	2 Basal diet+50g inulin/kg diet	3 Basal Diet + CdCl <sub>2</sub>	4 Basal diet + CdCl <sub>2</sub> + EI	
Hemoglobin (Hb, mg/dl)	11.60 cd	14.20 a	8.09 e	9.93 d	1.78
Total erythrocyte count (TEC, X10 <sup>6</sup> /mm <sup>3</sup> )	3.80 ab	4.85 a	3.03 b	3.74 ab	1.29
Total leukocyte count (TLC, X10 <sup>3</sup> mm <sup>3</sup> )	6.50 c	4.90 d	12.00 a	3.20 e	1.24
Hematocrite	35.96 bc	44.02 a	25.10 e	30.78 d	3.27

Means in the same raw with different letters are significantly different ( $P \leq 0.05$ ).  
EI: extracted inulin powder

### **Effects of different diets fed to male rats on plasma parameters**

The mean values of plasma total protein (TP), albumin (A), globulin (G), and A/g ratio as affected by rats fed on different diets for 30 are shown in Table (9). It is clear that groups of rats fed on diet containing 50 g inulin/kg diet had an increase of total protein content (g/dl), albumin (g/dl), globulin (g/dl) and plasma calcium (mg/dl) but that increase was not significant. While it had lower contents of bilirubin (mg/dl) and urea (mg/dl) than the group fed on basal diet (group 1). It can be observed also that rats fed on diet containing CdCl<sub>2</sub> had a lower contents of plasma total protein, albumin and globulin as it was 5.46, 3.17 and 2.29 g/dl compared with rats fed on diets containing CdCl<sub>2</sub> + EI, it was 6.21, 3.85 and 2.36 g/dl, respectively. The same group of rats had higher content of bilirubin (1.55 mg/dl), urea (172.63 mg/dl) and plasma calcium content (5.08 mg/dl) compared with 1.13, 158.06 and 5.91 mg/dl in rats fed on diets containing CdCl<sub>2</sub> + EI, respectively. The ratios of albumin (A)/globulin (G) of all groups fed on diets containing inulin were higher compared with that group fed on diet containing CdCl<sub>2</sub> without supplemented with extracted inulin. Kaur *et al.* (2002) reported that inulin is effective in lowering the blood urea and uric acid levels, thereby maintaining the nitrogen balance.

### **Effects of different diets fed to male rats on enzymatic activities**

Table (10) showed the mean values of plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AIP) of rats fed on different diets for 30 days. The results showed that the group of rats fed on diet containing basal diet (group 1) had higher contents of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) than that of group of rats fed on diets containing 50 g inulin/kg diet. The enzymes of AST and ALT in case of rats fed on diets containing CdCl<sub>2</sub> (group 3) were higher than that in rats fed on diets containing CdCl<sub>2</sub> + EI (group 4). It was 143 and 82.67 IU/L in case of rats fed on diets containing CdCl<sub>2</sub> (group 3), while it had lower content of AIP 309.62 IU/L compared with 140, 74 and 321.18 IU/L for AST, ALT and AIP in case of rats fed on diets containing inulin + CdCl<sub>2</sub> (group 4), respectively. The increment of the activities of AST and ALT in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al.*, 1993), which gives an indication on the hepatotoxic effect of cadmium chloride. Rana *et al.* (1996) revealed that cadmium chloride *in vitro* caused a significant inhibition of AIP activity.



**Table (9):** Changes in plasma total protein (TP), albumin (A), globulin (G), A/G ratio, bilirubin, urea and calcium of male rats as affected by different diets.

Parameters	Group of diets				LSD <sub>0.05</sub>
	(1) Basal Diet (control)	2 Basal diet+50g inulin/kg diet	3 Basal Diet + CdCl <sub>2</sub>	4 Basal diet + CdCl <sub>2</sub> + EI	
Total protein (TP, d1)	6.58 <sup>a</sup>	6.85 <sup>a</sup>	5.46 <sup>a</sup>	6.21 <sup>a</sup>	1.13
Albumin (A, g/dl)	4.35 <sup>ab</sup>	4.50 <sup>a</sup>	3.17 <sup>c</sup>	3.85 <sup>abc</sup>	0.91
Globulin (G, g/dl)	2.23 <sup>a</sup>	2.35 <sup>a</sup>	2.29 <sup>a</sup>	2.36 <sup>a</sup>	0.67
A/G Ratio	1.95 <sup>abc</sup>	1.91 <sup>a</sup>	1.38 <sup>cd</sup>	1.63 <sup>abcd</sup>	0.52
Bilirubin (B, mg/dl)	1.10 <sup>bc</sup>	0.87 <sup>bc</sup>	1.55 <sup>a</sup>	1.13 <sup>bc</sup>	0.37
Urea (U, mg/dl)	45.11 <sup>b</sup>	32.55 <sup>b</sup>	172.63 <sup>a</sup>	158.06 <sup>a</sup>	18.31
Calcium (Ca, mg/dl)	7.67 <sup>a</sup>	9.14 <sup>a</sup>	5.08 <sup>b</sup>	5.91 <sup>b</sup>	1.55

Means in the same row with different letters are significantly different ( $P \leq 0.05$ )  
EI: extracted inulin powder

The reason for the decrease in AIP activity in plasma of rats exposed to cadmium chloride may be due to a consequence of changes in the permeability of plasma membrane in addition to changes in the balance between synthesis and degradation of enzyme protein.

### Effect of different diets fed to male rats on histological criteria of liver and kidney

Kidney and liver of rats fed on diet containing basal diet (G1), basal diet + inulin (G2), basal diet + cadmium chloride (G3) and basal diet + cadmium chloride + inulin (G4) were evaluated by histological studies.

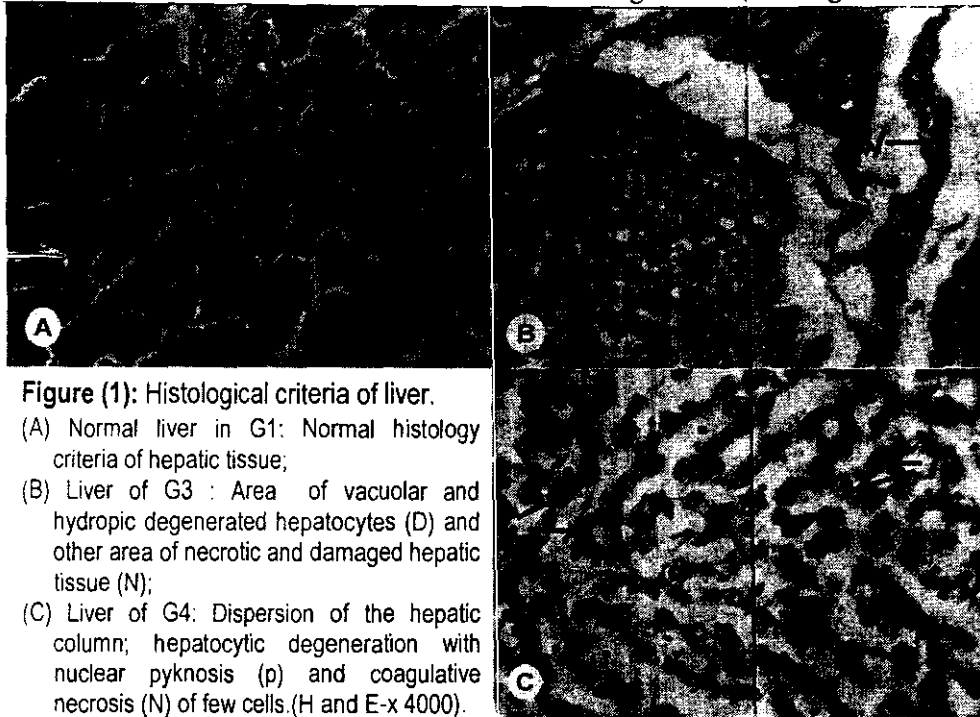
**Table (10):** Changes in plasma aspartate aminotransferase (AST) alanine aminotransferase (ALT) alkaline phosphatase (AIP) of male rats as affected by different diets.

Group of diets	(1)	2	3	4	LSD <sub>0.05</sub>
	Basal Diet (control)	Basal diet+50g inulin/kg diet	Basal Diet + CdCl <sub>2</sub>	Basal diet + CdCl <sub>2</sub> + EI	
Parameters					
Aspartate aminotransferase (AST, IU/L)	83.78 bc	69.83 c	143.00 a	140.0 a	14.21
Alanine aminotransferase (AIT, IU/L)	72.00 ab	44.67 c	82.67 a	74.00 a	22.71
Alkaline phosphatase (AIP, IU/L)	349.43 ab	355.91 a	309.62 d	321.18 cd	16.42

\* Means in the same row with different letters are significantly different ( $P \leq 0.05$ )  
EI: extracted inulin powder

On regard to the microscopic examination for the liver in present work, the more extensive lesions were seen in case of rats fed on diet containing cadmium chloride (group 3) in comparison to those normal histologic criteria of the hepatic tissue of both group of rats fed on basal diet (group 1) (Fig. 1-A) and rats fed on diets containing inulin (group 2). These extensive lesions were manifested by hepatocytic vacuolar and hydropic degradation in some areas, in addition to necrosis and completely damaged hepatic tissue in other areas (Fig. 1-B). The liver of rats fed on diets containing cadmium chloride + inulin (group 4) showed less alteration where some lymphocytic infiltrations were seen in addition to an excess of edema led to dispersion and wide separation of the hepatic columns. In these areas hepatocytic degeneration, nuclear pyknosis as well as coagulative necrosis were seen in some cells (Fig. 1-c). The presence of these degenerative as well as inflammatory reactions are known indicator for the lesions of hepatotoxicosis due to the effect of the used cadmium chloride. On the other hand, the liver of rats fed on diet containing inulin (group 2) only administration for inulin no obvious hangs could be seen and they appeared nearly similar to those rats fed on diet containing basal diet (group 1) except for presence of some mild vascular changes of congestion in some areas.

On regard to the comparative microscopic studies for the kidneys in present work, it was noticeable that the more extensive renal alterations were also seen in kidneys of rats fed on diets containing cadmium chloride (group 3) in comparison to those normal histologic criteria of the renal tissue of rats fed on diets containing basal diet (group 1) (Fig. 2-A). These detected lesions were those of nephrotoxicosis where several changes of damaged glomeruli, hydropic degeneration, necrosis and desquamation in the lining epithelium of some renal tubules (Fig. 2-B) were seen. In some other areas the renal tissue appeared completely damaged with a spongy appearance (Fig. 2-C).



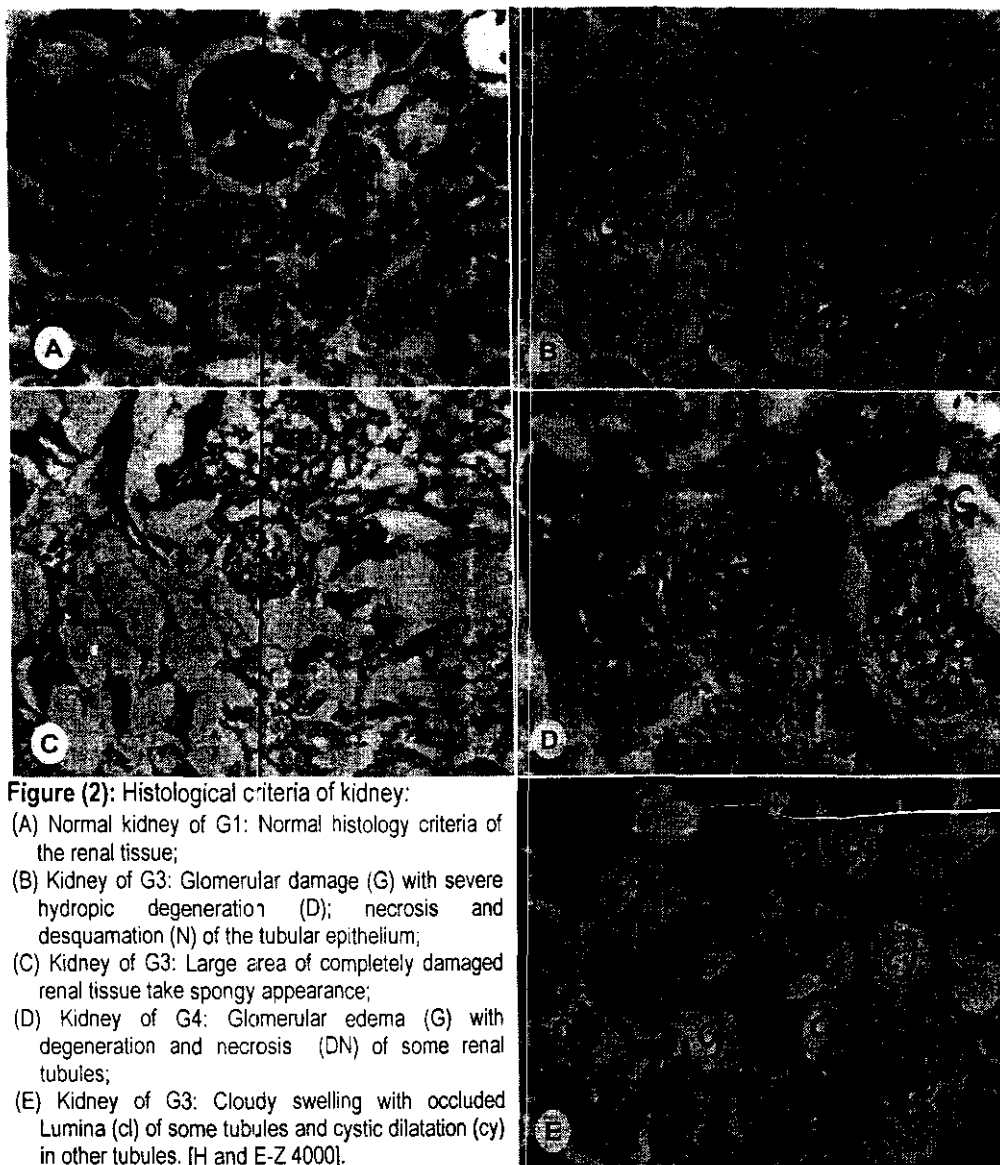
**Figure (1):** Histological criteria of liver.

- (A) Normal liver in G1: Normal histology criteria of hepatic tissue;
- (B) Liver of G3 : Area of vacuolar and hydropic degenerated hepatocytes (D) and other area of necrotic and damaged hepatic tissue (N);
- (C) Liver of G4: Dispersion of the hepatic column; hepatocytic degeneration with nuclear pyknosis (p) and coagulative necrosis (N) of few cells. (H and E-x 4000).

The moderately affected kidneys in the present study were in rats fed on diets containing those in cadmium chloride + inulin diet (group 4) of the concomitant administration for cadmium chloride with inulin. In these kidneys glomerular edema as well as tubular degeneration and necrosis (Fig. 2-D) were seen in some areas. The medley or nearly not affected kidneys were in case of rats fed on diet containing inulin those in the inulin diet group, where only some changes of cloudy swelling, narrowing or occlusioni-lumina of few tubules as well as slight cystic dilatation in some tubules (Fig. 2-E) were seen.

These last described lesions of tubular reactions in inulin diet group are indicative for some hyperactivities in the excretory function of the kidneys due to the effect of diet containing inulin.

The obtained results in the studies cleared that cadmium chloride alone led to an extensive nephrotoxic as well as hepatotoxic alteration that mostly resulted clinically into failure of liver and kidney functions in the affected animals. Also, it was concluded that the used inulin led to some but not complete antagonistic or protective effects against the used



**Figure (2):** Histological criteria of kidney:

- (A) Normal kidney of G1: Normal histology criteria of the renal tissue;
- (B) Kidney of G3: Glomerular damage (G) with severe hydropic degeneration (D); necrosis and desquamation (N) of the tubular epithelium;
- (C) Kidney of G3: Large area of completely damaged renal tissue take spongy appearance;
- (D) Kidney of G4: Glomerular edema (G) with degeneration and necrosis (DN) of some renal tubules;
- (E) Kidney of G3: Cloudy swelling with occluded Lumina (cl) of some tubules and cystic dilatation (cy) in other tubules. [H and E-Z 4000].

cadmium chloride, as a moderate changes of toxicosis were seen in the studied organs of these cases. Varga and Paksy (1991) found that cadmium strikingly alter the testes and ovaries of rat and mice. Moreover cadmium concentration at subtoxic levels in other tissues produces damage in the reproductive organs (Elinder, 1986). Oral administration of

cadmium to rodents results in necrosis, testicular atrophy, and sterility in males, whereas in females the astral cycle is altered (Paksy *et al.*, 1992).

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## الملخص العربى

### تأثيرات الأنبولين كمزيل للعناصر الملوثة ومحسن لإمتصاص الكالسيوم والماغنسيوم

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مصر.

تلعب الأغذية الوظيفية دوراً هاماً فى تحسين جودة وسلامة الأغذية بالإضافة إلى القيمة التغذوية ، ويعتبر الأنبولين من أهم المنتجات الطبيعية التى تعمل كأغذية وظيفية. كما أن القمح يعتبر من الحبوب ذات المحتوى العالى من عنصر الكاديوم والذى يعتبر أحد العناصر الملوثة للأغذية ذات تأثيرات مرضية خطيرة. وفى نفس الوقت يعتبر القمح هو أحد أهم مكونات صناعة الخبز. لذا كان الهدف من هذه الدراسة هو تقدير تأثير الأنبولين كمزيل للعناصر الملوثة فى الخبز ، حيث تم إستخلاص الأنبولين من درنات الطرطوفة ، وإستخدام صنف قمح جيزة ٦١ من محافظة أسيوط (الأعلى فى الكاديوم) فى صناعة الخبز. تم إضافة الأنبولين المستخلص للدقيق المستخدم فى صناعة الخبز، ثم تم تقييم الخواص الريولوجية للعجين والحسية للخبز وذلك لتديد أنسب كمية (%) من الأنبولين يتم إضافتها للدقيق فى صناعة الخبز. وتم تحضير الوجبات للدراسة البيولوجية على الفئران بناء على دقيق القمح المخلوط بالردة وبكمية الأنبولين المضافة التى أعطت أعلى جودة ريولوجية فى العجين وأعلى جودة فى الخواص الحسية للخبز. وقد تم تقدير الأنبولين والكاديوم المتبقى فى الخبز بعد الخبز لحساب الكميات من الكاديوم والأنبولين لمضافة للوجبات المعدة للتقييم الحيوى. وقد تم تغذية الفئران على هذه الوجبات المجهزة لمدة ٣٠ يوم ، وأجريت

الإختبارات البيولوجية على الفئران حيث تم تقدير الزيادة في وزن الجسم و تحويل الغذائى بالإضافة إلى تقدير مؤشرات عظمة الفخذ والساق وعرض الفخذ ، كذلك تم دراسة محتوى المعادن فى فضلات الفئران ، أيضاً إختبارات خصائص الدم البيوكيميائية وفحص الأنسجة التشريحية فى الفئران المختبرة. وقد أظهرت الدراسة أن النسبة المئوية للزيادة فى وزن الجسم كانت أقل فى مجموعات الفئران التى تم تغذيتها على وجبات تحتوى على الكاديوم ، بينما زادت هذه النسبة فى المجموعات التى تم تغذيتها على وجبات تحتوى على أنيولين بالإضافة إلى الكاديوم. وفى نفس الوقت إنخفضت نسبة الكالسيوم والماغنسيوم فى فضلات الفئران التى تم تغذيتها على الوجبات المضاف لها أنيولين. وقد إرتفعت نسبة التخلص من الكاديوم فى حالة الوجبات التى أضيف إليها أنيولين مع الكاديوم. وأظهرت الإختبارات التشريحية للفئران المختبرة أن كلوريد الكاديوم فى الوجبات أدى إلى حدوث سمية مرئية فى الكلى بالإضافة أيضاً إلى حدوث سمية فى الكبد مما نتج عنه حدوث فشل فى وظائف الكلى ولكبد فى الفئران المختبرة ، وخلصت الدراسة إلى أن إضافة الإنيولين للغذاء يؤدي إلى تأثير وقائى ضد الكاديوم ومحفز لإمتصاص الكالسيوم والماغنسيوم.