Effect of water hyacinth (Eichhornia crassipes) roots on some heavy metals disorders in rats

By

Mohamed Sabry Abdelbaky

Nutrition and Food Science Dept.,
Faculty of Home Economics,
Helwan University

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Effect of water hyacinth (Eichhornia crassipes) roots on some heavy metals disorders in rats

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Abstract

The present study was performed to evaluate the effect of wheat germ and fermented wheat germ on some Immunoglobulin's productions , lipids profile and liver functions in cirrhotic rats by Ccl4 . Rats were divided into eight groups , control groups (1&2) negative and positive were fed on basal diet without supplementation. All treated cirrhotic groups (3:8) were fed on experimental diets with raw or fermented wheat germ by different levels (5, 10 &15%) . Results clearly revealed that fermented wheat germ recorded the highest values in Vitamin (A), (E) and (C), comparing with raw wheat germ . The best treatment were fermented wheat germ (15%) and raw wheat germ (15%) which had lowest values of total lipid , triglycerides , total cholesterol LDL , VLDL , AST , ALT and had the highest values of HDL , respectively. While , all groups administrated fermented wheat germ by different levels (5, 10,15%) had the highest values of serum level of interferon gamma (INF-y) and interleukin -10 (IL-10) respectively . It could be concluded that wheat germ and fermented wheat germ improve some Immunoglobulin's production , lipids profile and liver functions especially fermented wheat germ 10 and 15% which has a best significant protective effect against acute hepatotoxicity induced by CCl4 in rats.

Key Word: fermented wheat germ – cirrhosis – CCl4 – liver oxidative stress - immune .

[*] **Nutrition and Food Science Dept., Faculty of Home Economics, Helwan University**

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INTRODUCTION :

Studies confirm that heavy metals can directly influence behavior by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic body processes. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular, detoxification pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary (Kellas and Dworkin, 1996). Lakes exposed to many pollutants including untreated sewag agricultural and industrial wastes which increase the concentration of heavy metals (lead, cadmium and mercury, fish is one of the aquatic organisms affected by heavy metals and compromise the health state of the human (Rashed,2001). Cadmium is an environmental and industrial pollutants that effects the male reproductive system of humans and animals.Cadmium administration caused an increase in reactive oxygen species (ROS) by elevating testicular malondialdehyde and decreasing the activities of testicular enzymes such as glutathion peroxidase and super oxide dismutase (Sen Gupta, et al.,2004). Lead induced oxidative stress contributes to the pathogenesis of lead poisoning for disrupting the delicate prooxidant / antioxidant balance that exists within mammalian cells, production of (ROS) is increased after lead treatment in vitro studies (Hsu and Guo,2002). The toxic metals have been documented to be reproductive and developmental toxins, causing birth defects and damaging fetal development, as well as neurological effects, developmental delays, learning disabilities, depression, and behavioral abnormalities in many otherwise normal appearing children .(Pfeiffer,2001 and William et al .,2000).

Several aquatic macrophytes have been used for the removal of heavy metals from the waste water. The use of these plants in biomonitoring of metals (Cardwell et al., 2002) or as biofilters for polluted water (Dunbabin and Bowmer, 1992), and the aspects of removal (Miretzky et al., 2004,

[*] **Nutrition and Food Science Dept., Faculty of Home Economics, Helwan University**

Hassan et al., 2007) besides the toxicity of these metals for the plants (Drost et al., 2007) were studied. In recent years much attention has been given to wastewater treatment using the aquatic plants and recycling of the treated water. After treatment, these aquatic plants can be used for biogas production, as fiber, compost production for solid waste amendments (Haque and Sharma, 1986). Among all, the aquatic macrophytes Eichhornia crassipes, Lemna minor and Spirodela polyrhhiza have a very high growth rate and heavy metal accumulation capacity (Cardwell et al., 2002, Miretzky et al., 2004, Hassan et al., 2007). The whole plant of water hyacinth (Eichhornia crassipes) 500-600 g in 10 liters of water removes arsenic 400 µg/l of arsenic completely within 3-6 hr following placement in a bucket, fibrous roots removed 81% of the arsenic; the leaves and leaf stalks removed none of the arsenic. The mechanism by which water hyacinth removed arsenic from water was by adsorption (Misbahuddin et al., 2002). More than 93% of arsenite and 95% of arsenate was removed from a solution containing arsenic 200 µg/l within 60 minutes of exposure to the powder of non-living dried root of water hyacinth. The concentration of arsenic remaining in solution was less than WHO guideline value of 10 µg/l (Rmalli et al., 2005).

The present study was carried out to illustrate the effect of water hyacinth (Eichhornia crassipes) roots and it's extract on some heavy metals disorders in rats fed on contaminated fish.

Materials and Methods :

Materials:

- Boulti fish (Tilopia Nilotica) was obtained from El-Manzala lake in Damiatte governorate . Each fish weighted from 400 to 450 gm .
- Male albino rats of Sprague Dawelly strain (100 ± 5 g B.W.) were obtained from Helwan laboratory for Animals and Colonies, Ministry of Health and Population, Cairo, Egypt.
- Casein and Cellulose were purchased from Morgan Company for chemicals, Cairo, Egypt.
- Kits were purchased from Gamatrade Company, Cairo, Egypt.
- water hyacinth (Eichhornia crassipes) roots were collected from the Nile river

Methods:

Preparation of Fresh Fish:

Fresh Boulti fish (Tilopia Nilotica) was cleaned and washed perfectly from external immediately after purchased , then bones, thorns and viscera were separated . After that fish was cut into similar slices of flesh fish , and dried in oven at 500c for 12 hours . Then the dried fish meat was grind to become powdered.

Chemical Analysis of Powdered Fish :

Crude protein and fat were determined in 100gm of dried fish according to the methods outlined in A.O.A.C.(1990), the results indicated that 100 gm dried fish contain 60 gm protein and 7.4 gm fat .

Heavy Metals Determination :

Both treated and non treated dried fish samples were analyzed for determination heavy metals (lead , cadmium and nickel) according to the method described in A.O.A.C.(1995).

One gram of homogenized sample was digested in 25 ml sulphoric acid (Conc.) when the color become clear two drops of perchloric acid have been added , complete digestion was indicated by discoloration of liquid . The flask was allowed to cool and dilution was made by added distilled water to made 50 ml and then filtered . The filtered solution was stored at room temperature until determined by used Atomic absorption spectrophotometer (A. Analyst 100 Apparatus) . The metal concentration were expressed as ppm metals per one gram fish tissue based on dry weight basis .

Preparation of water hyacinth (Eichhornia crassipes) roots powder :

water hyacinth (Eichhornia crassipes) roots were collected from the Nile river , washed with water and cut into small pieces and dried in oven 50°C until fully dried and ground by using a grinder.

Preparation of diet .

The basal diet consisted of protein (13%) , fat (4%) , salt mixture (3.5%) , vitamin mixture (1%) , choline (0.2%) , cellulose (5%) and the remainder was starch (Reeves ,et al.,1993).

Experimental Diet :

was prepared by replacement the casein in the basal diet by powdered fish as a source of protein.

Experimental design:

A total of twenty five male healthy rats , weighing between (100+5g) were divided into five groups; Each group containing 5 rats . Negative control group (1) were fed on basal diet without supplementation . Positive control group (2), fed on experimental diet which contained contaminated fish as a source of protein . Groups (3,4&5) fed on experimental diet which contained contaminated fish as a source of protein and supplemented with water hyacinth roots powder (WHRP) by (2.5 , 5 &10%) respectively .

Blood sampling.

At the end of the experimental period (4weeks), rats were starved for 12 hr. , then sacrificed under ether anesthesia . Blood samples were collected from the aortic vein into clean dry centrifuge tubes and were stored at room temperature for 15 minutes , put into a refrigerator for 2 hour , then centrifuged for 10 minutes at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wasser –man tubes by using a Pasteur pipette and kept frozen at (-20c) till analysis .

Biological Determination :

Determination of food intake and body weight gain: Food Intake (FI) was calculated every other day, The biological value of the different diets was assessed by the determination of its effect on Body Weight Gain ratio (BWG%) and organs / body weight % at the end of the experimental period using the following formulas:

BWG = (Final body weight - Initial body weight)* 100

Organ/body weight % = (Organ weight/ Weight of rat in the end)* 100

Chemical methods:

Cadmium , lead and nickel were determined in serum of rat by using HPLC .

Biochemical Determination :

Determination of liver functions :

Serum activities of aspartate amino transferase AST, alanine amino transferase ALT Alkaline Phosphatase (ALP) activities were measured according to the method described by Reitman and Frankel (1957) .

Determination of kidney functions :

Serum urea nitrogen, uric acid, creatinine were determined according to the methods described by Patton and Crouch, (1977) (22) , Fossati et al., (1980) (23) and Husdan and Rapoport, (1968) (24) respectively.

Histopathological Examination:

Specimens from the liver and kidney were taken immediately after sacrificing the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, embedded in paraffin, sectioned at 4-6 micron thickness and stained with Hematoxylen and Eosin (Carleton, 1979) (37) and examined microscopically.

Statistical Analysis :

The obtained data were statistically analyzed according to SAS, 1996 (19).

Results and discussions :

Table (1): Heavy metals content (lead , nickel and cadmium) of fresh fish .

	Lead (Pb) ppm	Nickel (Ni) ppm	Cadmium (Cd) ppm
Fresh fish	10.9±3.55	13.4±3.37	0.19 ±0.04

Heavy metals concentration in fresh fish with was recorded in table (1), it could clearly revealed that fresh fish which obtained from El-Manzala lake in Damiatte governorate , Egypt, had high heavy metals contents by mean value (10.9±3.55) , (13.4±3.37) and (0.19 ±0.04) ppm of (lead , nickel and cadmium) respectively .

Table (2) : Effect of water hyacinth (Eichhornia crassipes) roots powder WHRP on food intake FI and body weight gain ration BWG% in rats fed on contaminated fish .

Parameters Groups	FI (gm/day)	BWG%
Control (-)	11.70	68.24±10.14 b
Control(+)	11.90	78.98±4.25 a
Contaminated fish with WHRP 2.5%	11.40	67.75±13.01 b
Contaminated fish with WHRP 5%	11.30	64.02±9.03 b
Contaminated fish with WHRP 10%	11.40	42.40±11.76 c

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

Table (3) : Effect of water hyacinth (Eichhornia crassipes) roots powder WHRP on organs / body weight ration in rats fed on contaminated fish .

Organs Groups	Liver	Spleen	Kidney	Heart	Brain
Control (-)	3.44±.0.09 b	1.01±0.05 b	1.24±.0.02b	0.84±0.1a	1.22±.0.04a
Control(+)	4.20±.0.04 a	1.98±0.04a	1.62±.0.09a	0.85±0.04a	1.27±.0.07a
Contaminated fish with WHRP 2.5%	3.32±.0.06 b	0.89±0.04b	1.18±.0.03b	0.85±0.01a	1.24±.0.04a
Contaminated fish with WHRP 5%	3.44±.0.05 b	0.98±0.04b	1.21±.0.05b	0.86±0.04a	1.33±.0.04a
Contaminated fish with WHRP 10%	3.35±.0.08 b	0.90±0.03b	1.23±.0.03b	0.88±0.02a	1.34±.0.07a

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

Table (4): Effect of water hyacinth (Eichhornia crassipes) roots powder WHRP on serum heavy metals in rats fed on contaminated fish .

Parameters Groups	Pb (ppm)	Ni(ppm)	Cd (ppm)
Control (-)	0.00±0.0 b	0.041±0.09 b	0.03±0.008 b
Control(+)	0.10±0.0 a	0.55±0.15 a	0.54±0.01 a
Contaminated fish with WHRP 2.5%	0.01±0.0 b	0.054±0.17 b	0.06±0.004 b
Contaminated fish with WHRP 5%	0.00±0.0 b	0.034±0.12 b	0.03±0.014 b
Contaminated fish with WHRP 10%	0.00±0.0 b	0.030±0.17 b	0.02±0.007 b

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

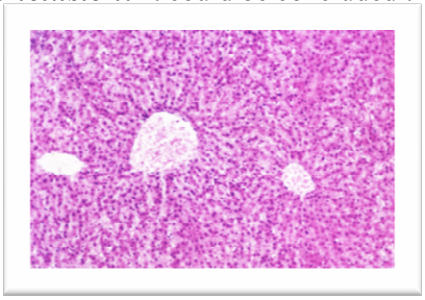
Table (5): Effect of water hyacinth (Eichhornia crassipes) roots powder WHRP on serum liver function AST & ALT in rats fed on contaminated fish .

Parameters Groups	AST(U/L)	ALT (U/L)
Control (-)	80.04 ± 1.5 b	9.93 ± 0.2 b
Control(+)	121.01 ± 1.3 a	21.40 ± 1.8 a
Contaminated fish with WHRP 2.5%	81.61 ± 2.5 b	7.53 ± 1.6 b
Contaminated fish with WHRP 5%	80.64 ± 3.6 b	7.92 ± 1.8 b
Contaminated fish with WHRP 10%	76.15 ± 2.1 b	8.42 ± 0.2 b

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

Parameters Groups	Urea (mg/dl)	Uric acid (mg/dl)	
Control (-)	9.09 ± 0.8 b	2.40 ± 0.2 b	0.56 ± 0.02 b
Control(+)	12.85 ± 1.7 a	3.97 ± 0.2 a	0.81 ±0.02 a
Untreated fish+ vit. E	5.30 ± 0.6 c	1.19 ± 0.3 b	0.64 ±0.02 b
Untreated fish+ vit.E+Selenium	6.27 ± 0.5 cb	2.50 ± 0.4 b	0.61 ±0.02b
Treated fish 10% EDTA+Vit C	8.12 ± 1.7 bc	2.45 ± 0.1 b	0.62 ± 0.02b

Conclusion: It could be concluded that



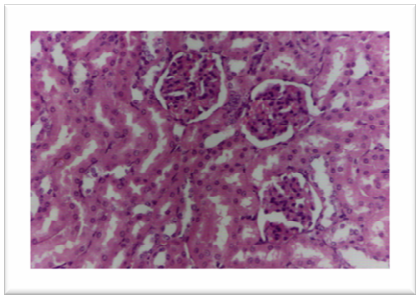
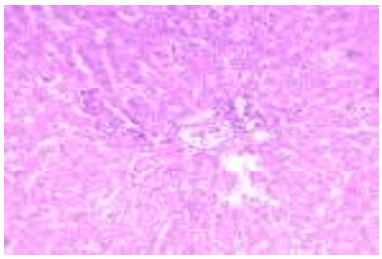


photo (1): Microscopicall examination of liver of rat in negative control group , showing the normal histological structure .	photo (2): Microscopicall examination of kidney of rat in negative control group , showing the normal histological structure



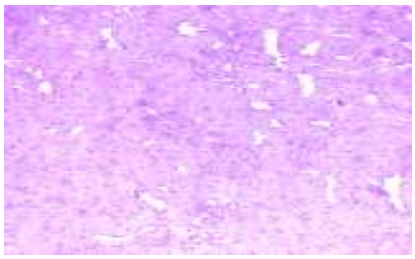
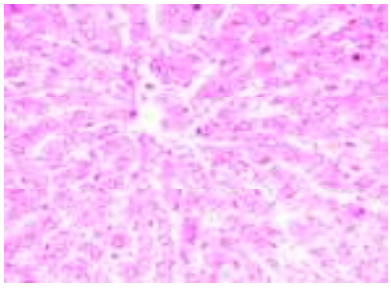


Photo (3): Microscopicall examination of liver of rats fed on contaminated fish without treatment WHRP showing marked hepatocytes hydropic degeneration and portal tract with chronic inflammatory cells.	Photo (4): Microscopicall examination of kidney of rats fed on contaminated fish without treatment WHRP showing mild inflammatory reaction and cloudy swelling of the epithelial lining of the collecting tubules leading to different grades of their lumen obliteration.



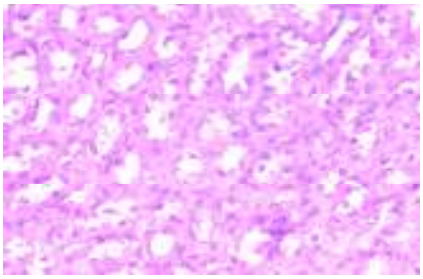
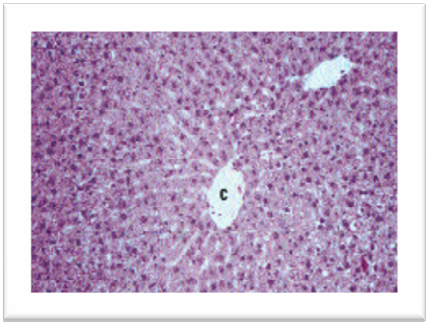


Photo (5) : Microscopicall examination of liver of rats fed on contaminated fish with WHRP (5%) showing mild histological alteration .	Photo (6) : Microscopicall examination of kidney of rats fed on contaminated fish with WHRP (5%) showing mild histological alteration with patent lumens.



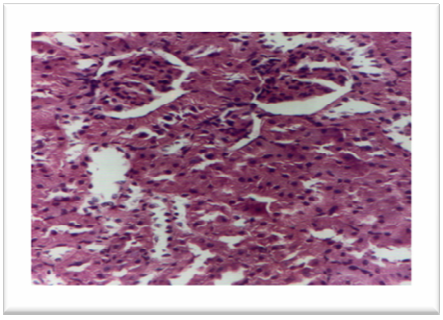
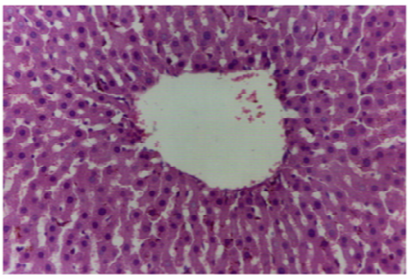


Photo (7): Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with BPME (2g / Kg / day) showing mild Degeneration alteration and mild swelling of hepatocytes .	Photo (8): Microscopicall examination of kidney of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with BPME (2g / Kg / day) showing mild histological alteration .



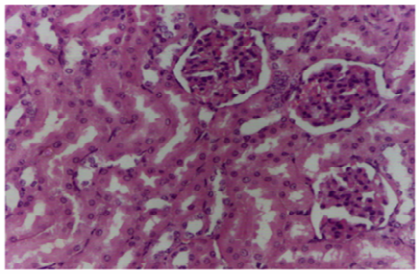
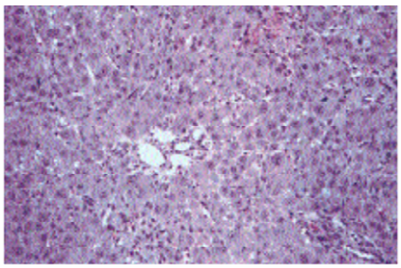


photo (9): Microscopicall examination of liver of rat fed on basal diet with Subcutaneous injection by Ccl4 and administrated with RBPME (2g / Kg / day) , showing apparent normal histological structure .	photo (10): Microscopicall examination of kidney of rat fed on basal diet with Subcutaneous injection by Ccl4 and administrated with RBPME (2g / Kg / day) , showing the normal histological structure .



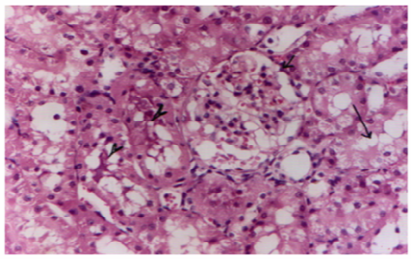


Photo (11): Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with PPME (2g / Kg / day) showing mild Degeneration alteration	Photo (12): Microscopicall examination of kidney of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with PPME (2g / Kg / day) showing hypertrophy of glomerular tuft.

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