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The Protective Value of Hesperidin in Mitigating the Biochemical Perturbations and Trace Element alterations induced by Acrylonitrile in Rats.

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ABSTRACT

Objective: Acrylonitrile (a chemical pollutant) has been reported to induce harmful effects in humans. Therefore, this study was designed to evaluate the protective effects of hesperidin, a natural bioflavonoid, against the toxicity induced by acrylonitrile (AN) in rats. **Material&Methods**: This study includes determination of serum total scavenger capacity "TSC", liver enzymes (aspartate transaminase "ASAT", alanine transaminase "ALAT" and alkaline phosphatase "ALP"), total proteins, albumin, glucose, creatinine, urea and lipid profile. Moreover, liver and kidney homogenate glutathione content "GSH", catalase, superoxide dismutase "SOD", glutathione peroxidase "GPx", malondialdehyde "MDA" and some minerals were estimated.

Results: revealed that administration of AN (orally 50mg/ kg b.wt.) induced alterations in TSC level as well as liver, kidney and lipid profiles. In addition, a decrease in GSH-content and catalase, SOD and GPx activities was observed with an increase in MDA levels in both liver and kidney. There was disturbance in certain minerals such as Cu, Zn, Fe, Se, Ca, Mg and Mn. **Conclusion:** particularly, Hesperidin administration (orally 200 mg/kg b.wt.) ameliorates the oxidative stress induced by AN, consistent with the reported antioxidant activity of hesperidin.
Key Words: Acrylonitrile, hesperidin, antioxidants and rats.

INTRODUCTION

Acrylonitrile (AN) is a colorless, liquid and synthetic chemical with a sharp, onion- or garlic-like odor. It can be dissolved in water and evaporates quickly. AN is used to make other chemicals such as plastics, synthetic rubber, and acrylic fibers **(1)**. The important use of AN in clinical practice is in the manufacture of high permeable dialysis tubings **(2)** and in the synthesis of artificial membrane to encapsulate Langerhans islet implants **(3)**. AN has been found in drinking water, occupational environments, food and cigarette smoke **(4)**. International Agency for Research on Cancer (IARC) reported that acrylonitrile is possibly carcinogenic to humans based on sufficient evidence of carcinogenicity in experimental animals and inadequate evidence for carcinogenicity in humans **(5)**.

Citrus fruits are well known for providing ample amounts of vitamin C. But they are also supply bioflavonoids, substances that may improve health. The major bioflavonoids found in citrus fruits are hesperidin, diosmin, rutin, naringin, tangcretin, diosmetin, narirutin; neahesperidin, nobiltin, and quercetin **(6)**.They exhibit higher biological activities even though they occur in low concentration.

Hesperidin is a flavanone glycoside abundantly found in sweet orange and lemon and is an inexpensive by – product of citrus cultivation **(7)**. A deficiency of this substance in the diet has been linked with abnormal capillary leukiness as well as pain in the extremities causing aches, weakness and night leg cramps. No signs of toxicity have been observed with the normal intake of hesperidin or related compounds. Both hesperidin and its aglycone hesperitin have been reported to possess a wide range of pharmacological properties **(8)**.

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The objective of this study is to evaluate the potential protective effect of hesperidin in mitigating the biochemical perturbations as well as the oxidative stress induced by acrylonitrile in rats.

MATERIAL AND METHODS

Experimental Animals

Male albino rats were used, weighing 130 ± 150 g and obtained from Egyptian National Authority for Drug Research and Control, Ministry of Health. The animals were kept for 15 days for laboratory acclimatization and fed commercial pellets and provided with tap water, *ad-lipitum* .

Treatment

Chemical pollutant, Acrylonitrile (AN) was supplied to the animals at a dose of 50 mg /kg b.wt. diluted in distilled water **(9).** Antioxidant, Hesperidin was supplied to certain groups of animals at a dose of 200 mg / kg b.wt. **(10)** dissolved in distilled water. Both AN and hesperidin were purchased from Sigma-Aldrich Co, ST. Louis. Mo, USA.

Experimental Design

After adaptation period of one week, animals were divided into four groups, each of ten rats, Group 1, control, non-irradiated rats. Group 2, rats received 200 mg hesperidin / kg body weight (three times per week) for 4 weeks orally. Group 3, rats received 50mg acrylonitrile / kg body weight orally for 4 weeks (twice per week). Group 4, rats received 200mg Hes/ kg body weight (three times per week) and 50mg AN / kg body weight (twice per week) orally for 4 weeks.

Biochemical Analysis

Serum total scavenger capacity was measured according to the method of **Koracevic *et al.* (11)**. Serum ASAT & ALAT were determined according to the method **Reitman and Frankel, (12)** and serum ALP was determined according to the method **Belfield and Goldberg**, **(13)**. Total Proteins and albumin were measured according to the method of **Doumas *et al.*, (14, 15).**Urea and creatinine were measured according to the method of **Fawcett and Soctt. (16)** and **Bartles *et al.*, (17),** respectively. Cholesterol, Triacylglyceroles and HDL- c were measured according to **Tietz (18)**. LDL- c and glucose were measured according to the method of **Wieland and Seidel (19)** and **Trinder (20),** respectively. The lipid peroxidation products were estimated in the liver & kidney according to **Yoshika *et al.* (21).** Glutathione peroxidase, catalase, superoxide dismutase and GSH-content were estimated according to **Gross *et al.* (22)**, ***Sinha* (23)**, **Minami and Yoshikawa, (24)** and **Ellman, (25)** respectively. Copper, zinc, selenium, iron, manganese, calcium and magnesium were estimated by atomic absorption spectrophotometer after digestion in conc. HNO_3 & H_2O_2 in 5:1 ratio by using Milestone MLS-1200 Mega, High Performance Microwave Digestor Unit, Italy **IAEA, (26)**.

Statistical Analysis of Data:

Differences between groups were analyzed using analysis of variance (ANOVA) followed by Tukey's multiple comparisons test by statistical Graph- Pad software (Prism program version 4). All data points are presented as mean ± standard error of the mean (S.E.). Statistical significance was determained at $P<0.05$.

Results:

A- Effect of acrylonitrile and hesperidin on serum hepatic and renal biomarkers (Table 1).

Results of the present study revealed that acrylonitrile administration alone recorded elevation in ASAT, ALAT, ALP, glucose, urea and creatinine levels with percentage change from control. While a sharp drop in albumin & total proteins represented with percentage change -26.73& -44.90 respectively. On the other hand, the prolonged oral administration of hesperidin induced non significant changes in these parameters.

Supplementation of rats with hesperidin ameliorate the alterations in

ASAT, ALAT, ALP, albumin, total proteins, glucose, urea and creatinine induced by AN.

B- Effect of acrylonitrile and hesperidin on lipid profile (Table 2).

Treatment with hesperidin alone induced non significant effect on cholesterol, triacylglycerols, HDL-c and LDL-c concentrations compared to control. While acrylonitrile group recorded an increase in cholesterol, triacylglycerols and LDL-c and a decrease in HDL-c levels.

Supplementation of rats with acrylonitrile and hesperidin restored the lipid profile towards the control.

C- Effect of acrylonitrile and hesperidin on serum TSC and liver oxidative stress biomarkers [MDA, catalase, GPx, SOD and GSH] (Table 3).

Acrylonitrile treated rats had a highly significant decrease in TSC, catalase, GPx, SOD and GSH while a significant increase in MDA concentration compared to control value was observed. The percentage change from control were as follow: for TSC: -19.33, for MDA: 128.04, for catalase: -13.76, for GPx: -54.43, for SOD: -34.17 & for GSH: -25.19. While, the prolonged oral administration of hesperidin recorded non significant changes in the above mentioned parameters.

On the other hand, administration of hesperidin to AN treated rats compensated the decline in TSC, catalase, GPx, SOD & GSH, the percentage change from control became -4.24, -7.97, 0.42, 0.733 and -8.20 respectively. Moreover it minimized the MDA propagation from 128.04 to -4.13% compared to acrylonitrile group.

D- Effect of acrylonitrile and hesperidin on kidney oxidative stress biomarkers [MDA, catalase, GPx, SOD and GSH] (Table 4). Hesperidin group recorded non significant changes in MDA, catalase, GPx, SOD and GSH compared to control , but acrylonitrile group had an increase in MDA level and a decrease in other parameters.

Administration of hesperidin to AN treated rats induced amelioration in these parameters.

E- Effect of acrylonitrile and hesperidin on hepatic trace element contents (Fig.1).

Fig.1. showed the changes in Cu, Zn, Se, Fe, Mn, Ca and Mg levels. Administration of hesperidin alone recorded a marked increase in Mg level ($P<0.001$) and non significant changes in the other trace elements compared to the control. While acrylonitrile group had a significant decrease in Se, Mn & Mg, an increase in Zn and Fe levels and no changes in Cu & Ca levels.

Administration of hesperidin to AN treated rats minimized the changes in the above mentioned parameters.

F- Effect of acrylonitrile and hesperidin on renal trace element contents (Fig.2).

Administration of hesperidin alone showed non significant changes in all elements estimated except Fe level which recorded highly significant decrease and Mg level which recorded highly significant increase compared to control. In addition, acrylonitrile group showed the same results as hesperidin group.

The administration of hesperidin to AN treated rats recorded a highly significant increase in Mg level and restoration in Fe level.

Discussion

In the current study, the treatment with acrylonitrile led to an increase in liver enzymes. The toxicity of acrylonitrile may be due to the metabolic release of cyanide, which inhibits numerous enzymes, including cytochrome oxidase, resulting in cellular asphyxiation. While toxicity which might not related to cyanide formation is due to the formation of reactive vinyl groups and epoxide which can deplete glutathione stores and cause liver damage **(27)**. Also, AN increased glucose level and this is in agreement with **Gut *et al.*, (28)** who found that the elevation of blood glucose proved to be the most sensitive and dose-related indicator of AN exposure and this is in accordance with the present study.

In addition it increased cholesterol, triacylglycerols and LDL-c levels and decreased HDL-c levels. This is in agreement with **Gut *et al.*, (28, 29)** who found that exposure to AN induced depletion of glutathione and affected carbohydrate and lipid metabolism. These effects would be expected considering the

affinity of acrylonitrile for sulfhydryl groups on proteins and the involvement of glutathione in the metabolism of acrylonitrile.

AN exposure led to significant increase in urea and creatinine. This is supported by results of **Rouisse *et al.*, (30),** who found that AN could produce acute nephrotoxic insult preferably at the proximal tubular region of the rat kidney.

AN exposure led to significant decrease in SOD, CAT, and GPx, an increase in MDA and a non significant decrease in GSH. This is consistent with those reported by **Guangwei *et al.* (31)** who found an increase in the levels of lipid peroxidation end-products (MDA) and decrease in antioxidative enzyme activities and non-enzymatic antioxidants contents both in the liver and brain of AN-treated rats. The major pathway of elimination is via conjugation with GSH. Accordingly, GSH depletion may decreasing antioxidant capacity and the redox status, resulting in increased intercellular ROS production and oxidative damage **(32)**. Metabolism of AN also results in the production of cyanide **(33)**. Cyanide has been shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice and in cell lines by inhibiting the mitochondrial respiratory chain, CAT and glutathione peroxidase activities **(34)**. Therefore, oxidative stress likely plays a causal role in the toxicity of AN. Binding of AN to GSH results in the induction of oxidative stress and impaired regeneration of other antioxidants **(35)**.

The increase in selenium following acrylonitrile treatment in the present work disagreed with the results reported by **Rongzhu *et al.*, (36)**, but the results of Zn and Cu of the present study were in agreement with **Rongzhu *et al.*, (36)**. Since, zinc is a key element of rhodanese, which is required for detoxification of cyanide while copper plays a key role in the activity of the enzymatic antioxidant, Cu-Zn superoxide dismutase (SOD) also, is required for the optimal function of cytochrome c oxidase. On the other hand, the treatment with hesperidin ameliorated the alterations in liver function parameters, which was confirmed by **Pradeep *et al.*, (37)** who implied that hesperidin tends to prevent liver damage, suppresses the leakage of enzymes through cellular membrane, preserves the integrity of the plasma membranes and hence restores these enzyme levels.

Also, it improved the increase in blood glucose level. **Ibrahim, (38)** suggested its role in the progression of hyperglycemia, partly by increasing hepatic glycolysis and glycogen synthesis and / or by lowering hepatic gluconeogenesis. In addition, it restored the lipid metabolism via inhibition of both hepatic hydroxyl methyl glutaryl reductase (HMG-CoA reductase) and acetoacetyl- CoA transferase (ACAT) activities, while increasing fecal cholesterol and LDL receptor mRNA levels in animals and cells **(39)**.

Hesperidin administration ameliorated the alteration in kidney function in the present study and results are in agreement with **Sahu *et al.*, (40)** who reported that hesperidin treatment significantly attenuated the cisplatin - induced oxidative stress/ lipid peroxidation, inflammation, apoptosis/ necrosis as well as increased the expression of nitric oxide in the kidney and improved renal function.

Hesperidin increased the antioxidant system in the present study. This is supported by **Tirkey *et al.*, (41)** who mentioned that, only the higher dose of hesperidin (200 mg/kg) was able to show improvement in the levels of endogenous antioxidant enzymes (SOD and catalase) and GSH in liver. It has been reported to act as a powerful consumer of superoxide, singlet oxygen and hydroxyl radicals **(42)**, thereby contributing significantly to the intracellular antioxidant defense system. Furthermore, **Hosseinimehr and Nemati,(43)** have reported that hesperidin possesses a strong anticlastogenic activity against γ-irradiation induced mouse bone marrow damage. The hepatoprotective effects of hesperidin observed in this study might be due to its ability to enhance glutathione production by

providing more substrate for reactive intermediates that promote the detoxification mechanisms. This might be the reason for the restoration of other antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase.

The treatment with hesperidin led to restoration of the changed elements after acrylonitrile administration in the liver and kidney, this may be due to the antioxidant effects of hesperidin. While hesperidin led to an increase in Mg level. The reason wasn't unknown, further studies may confirm these data.

Based on the results obtained in the present study, it appears that, hesperidin has protective effects against the oxidative stress induced by acrylonitrile toxicity in the liver and kidney of rats, mainly because of its free radical scavenging and antioxidant properties.

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Table (1): Effect of acrylonitrile and hesperidin on liver & kidney functions in different animal groups.

Parameters	Control	Hesperidin	Acrylonitrile	Acrylonitrile+Hesperidin
ASAT (U/ml)	39.03±0.70	43.68±2.66	60.33±1.51**	52.95±1.05**#
%change	----	11.91	54.57	35.66
ALAT (U/ml)	19.88±1.008	19.0±1.15	35.50±0.387**	29.66±2.10*
%change	----	-4.42	78.57	34.49
ALP (IU/L)	129.60±1.51	133.60±8.91	184.40±7.69**	148.40±4.75##
%change	----	3.08	42.28	14.50
Albumin (g/dl)	3.03±0.07	2.73±0.17	2.22±0.134**	2.86±0.19
%change	----	-9.90	-26.73	-5.61
Total proteins (g/dl)	6.34±0.20	6.04±0.21	3.49±0.32**	5.87±0.20##
%change	----	-4.68	-44.90	-7.39
Glucose (mg/dl)	41.01±0.67	42.08±2.03	63.28±2.39**	48.79±1.45#
%change	----	2.60	54.30	18.97
Urea (mg/dl)	27.23±1.06	21.33±1.20	50.43±4.28**	36.50±0.2##
%change	----	-21.66	85.20	25.39
Creatinine (mg/dl)	0.764±0.05	0.758±0.05	1.14±0.06**	0.590±0.009##
%change	----	0.785	49.21	-22.77

Each value represents the mean± SE. * Significant difference compared to the values of control rats (*: p<0.05, **: P<0.01 &P<0.001). # Significant difference compared to the values of acrylonitrile- treated rats (#: p<0.05, ##: P<0.01 &P<0.001). The % change from control.

Table (2): Effect of acrylonitrile and hesperidin on lipid profile in different animal groups.

Parametrs	Control	Hesperidin	Acrylonitrile	Acrylonitrile+Hesperidin
Cholesterol (mg/dl)	54.96±4.73	51.85±3.50	75.70±4.29*	53.61±2.99#
%change	----	-5.19	38.41	-1.97
Triacylglycerols (mg/dl)	53.52±2.24	52.85±3.63	77.40±3.11**	44.32±1.45##
%change	----	-1.25	44.61	-17.18
HDL-c (mg/dl)	43.07±3.02	44.98±3.40	27.27±1.73**	40.72±4.09##
%change	----	4.43	-36.68	-5.45
LDL-c (mg/dl)	23.42±0.48	24.88±1.99	36.26±3.34**	28.14±2.20
%change	----	6.23	54.82	20.15

Legends are as in Table (1).

Table (3): Effect of acrylonitrile and hesperidin on serum TSC and liver oxidative stress biomarkers in different animal groups.

Parameters	Control	Hesperidin	Acrylonitrile	Acrylonitrile+ Hesperidin
TSC (mM/L)	2.12± 0.008	2.122± 0.008	1.71±0.055**	2.03±0.051##
% change	----	0.09	-19.33	-4.24
MDA (µM/g tissue)	73.10± 4.85	71.76± 3.24	166.70±12.71**	70.08±4.31##
% change	----	-1.83	128.04	-4.13
Catalase (µMole/min)	1.38± 0.02	1.33± 0.002	1.19±0.01**	1.27±0.03
% change	----	-3.62	-13.76	-7.97
GPx (mg/min/g)	2.37± 0.14	2.25± 0.05	1.08±0.09**	2.38±0.06##
% change	----	-5.06	-54.43	0.42
SOD (U/g tissue)	57.23± 0.48	59.59± 1.76	37.67±1.72**	57.65±1.81##
% change	----	4.12	-34.17	0.733
GSH (mg/g tissue)	29.01± 0.12	30.17± 1.68	21.70±0.02**	26.63±1.70
% change	----	3.99	-25.19	-8.20

Legends are as in Table (1).

Table (4): Effect of acrylonitrile and hesperidin on kidney oxidative stress biomarkers in different animal groups.

Parameters	Control	Hesperidin	Acrylonitrile	Acrylonitrile+Hesperidin
MDA (µM/g tissue)	121.10± 4.72	119.5± 1.80	173.5±10.41*	152.4±4.86
% change	----	-1.32	43.27	25.84
Catalase(µMole/min)	2.09± 0.05	2.03± 0.01	1.89±0.01**	2.00±0.02
% change	----	-2.95	-9.56	-4.30
GPx (mg/min/g)	2.19± 0.08	1.86± 0.001	1.23±0.06**	2.16±0.19##
% change	----	-15.06	-43.83	-1.36
SOD (U/g tissue)	44.21± 2.10	42.58± 2.41	29.02±0.81**	33.09±1.85**
% change	----	-3.68	-34.35	-25.15
GSH (mg/g tissue)	25.29± 0.60	30.17±1.68	16.45±0.16**	25.70±0.74##
% change	----	3.99	-34.95	1.62

Legends are as in Table (1).

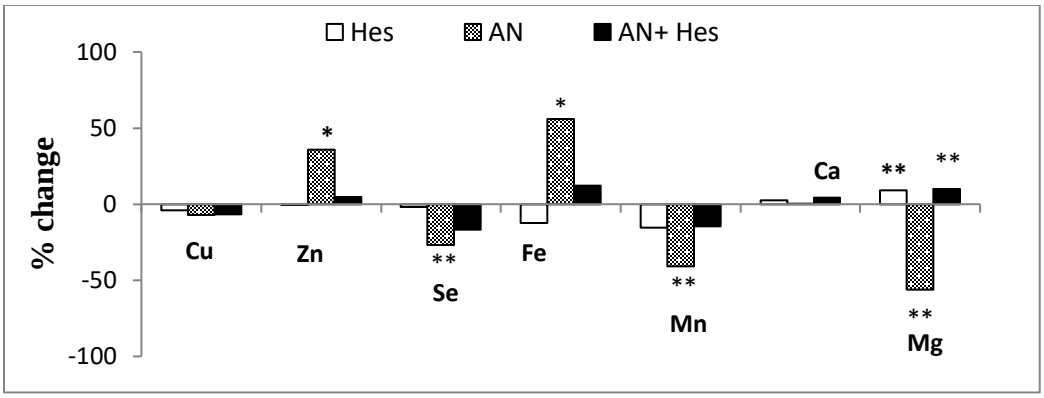

Fig. (1): Effect of acrylonitrile and hesperidin on hepatic trace elements contents.
* Significant difference compared to the values of control rats (*: p<0.05, **: P<0.01 &P<0.001).
The % change from control.

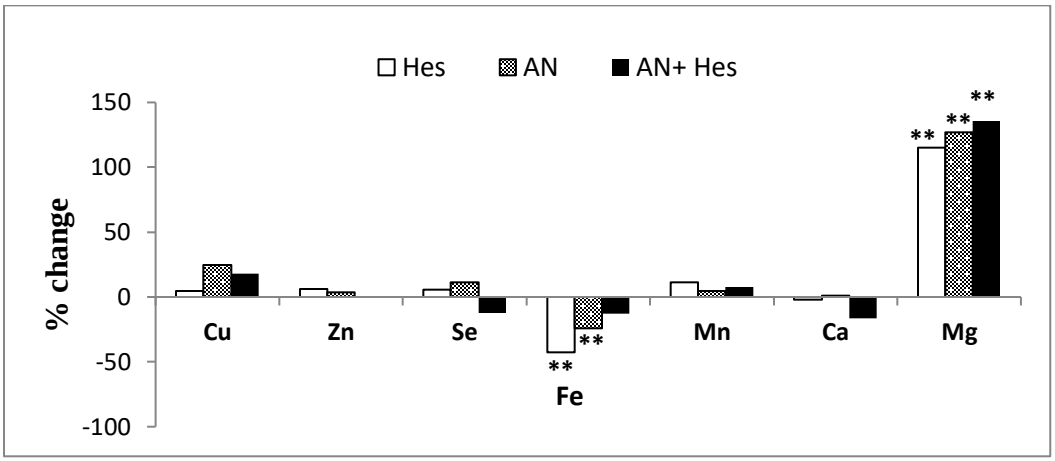

Fig. (2): Effect of acrylonitrile and hesperidin on renal trace elements contents.
Legends are as in Fig. (1).