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Abstract: The present study aimed to examine the relation between consumption of freeze-dried tomato and carrot and alterations in kidney, spleen as well as antioxidant enzymes and serum analysis of albino mice. The protective effects of tomato and carrot were assessed against acrylamide that administered to Swiss adult albino mice. The experiment included twenty eight mice. The first group (7 mice) was considered as negative normal. The remaining mice were subjected to daily dose (50 µg acrylamide /kg body weight for 10 weeks. The treated groups were divided into three groups; each seven mice. One of these groups (group 2) was assigned as positive control. Both negative and positive groups were fed on basal diet. The other two groups (Group 3 and group 4) were given diets with formulas with 20% of freeze dried tomato and carrot, respectively. At the end of the experiment, kidney and spleen histopathologically examined; serum Glutathione (GSH), were Glutathione-S-transferase (GST), liver functions, kidney functions and lipid profile were estimated. The diets with vegetables demonstrated significant biochemical protection against acrylamide associated with significant reductions in the activity of (GSH), (GST) serum, liver functions, kidney functions and lipid profile compared to those fed on basal diet (positive control). Histopathological examinations revealed that kidney and spleen tissues were disturbed when mice were fed on acrylamide diet. While mice fed on diets with either tomatoes or carrots exerted protective effects and reversed histopathological changes, all in a dose dependent manner. The obtained results assured that the consumption of tomatoes and carrots ameliorated the harmful effect of acrylamide on both kidney and spleen.

Keywords : Vegetables, oxidative stress, antioxidant enzymes, histopathological alterations, Acrylamide, kidney, spleen, tomatoes, carrots.

Introduction

Vegetable juices have beneficial nutrients such as vitamins, antioxidants, dietary fibers, and minerals (Yoonet al., 2004). In general, vegetables do not contain any dairy allergens that prevent usage by certain groups of population (Luckow and Delahunty, 2004). The primary route of acrylamide exposure for people was observed through both drinking water and food consumption. It has been formed in carbohydrate rich foods cooked at high temperatures (Tarekeet al., 2002). Acrylamide forms used to conjugate with reduced glutathione (GSH), accumulate in higher levels in the blood than any other tissues following exposure via oral ingestion, inhalation, or via the dermis (Shipp et al., 2006). Acrylamide caused a disruption of hematological parameters, a decrease in erythrocyte membrane resistance and retarded synthesis or destruction of Hb (Ali et al., 2014). The imbalance between the production of reactive oxygen species (ROS) and antioxidant capacity increased oxidative stress, playing a critical role in the toxicity induced by acrylamide through rise of lipid peroxidation and impairment of endogenous antioxidant biomarkers (Aydın, 2017).

As oxidative stress inhibition considered beingan important therapeutic approach, efforts have been made to identify potential antioxidant compounds from medicinal plants (Stancu, et al., 2014). To counteract the oxidative stress, the body used to produce several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione *S*-transferase, etc. (Schafer andBuettner, 2005).

Fortunately, food has a range of antioxidants that could successfully compensate prooxidants and maintain healthy gut. Yellow, orange and green vegetables provide a range of carotenoids; the major provitamin A. Biological functions of these natural pigments in relation to animals or humans have not been well defined but their antioxidant properties seemed to be of major importance (Surai, et al., 2004).Recently, epidemiologic studies suggesting that those with greater intakes of carotenoid-containing foods have reduced risks for several chronic diseases, stimulating a greater interest in carotenoids (Canene-Adams et al., 2005 and Engelmann et al., 2011).). Because tomatoes have been viewed as the primary dietary source of phytoene and phytofluene, research on tomatoes and disease risk would serve as a

good starting point for evaluating the potential role of these carotenoids, either alone or in combination with lycopene (Engelmann, et al., 2011). Carrots appeared to be a good source of α -carotene (Landrum, et al., 2002). In vitro, several animal and human experiments have demonstrated the antioxidant properties of carotenoids such as βcarotene and lycopene (Elliott, 2005).Chlorogenic acid; the phenolic compound has been widely distributed in vegetables, including, carrot tomatoes(Upadhyay, & Mohan Rao, 2013). and Besides hepatoprotective effects, several studies also demonstrated that Chlorogenic acid has significant beneficial kidney protection effect in drug-induced nephropathy (Domitrović, et al., 2014).

The human body has several mechanisms for defense against free radicals and other reactive oxygen species. The various defenses includedcomplementary to one another because they tended toact on different ROS or in different cellular compartments. These defenses couldbe grouped into enzymatic and non-enzymatic antioxidant, including glutathione peroxidases, superoxide dismutase and catalase, which decrease the concentration of the most harmful ROS. The best way to ensure an adequate intake of phytonutrients would beeating a diet rich in a wide variety of fresh vegetables(Ozougwu, 2016).Glutathione, an important water-soluble antioxidant, synthesized from the amino acids glycine, glutamate, and cysteineused directlyto bequenches ROS such as lipid peroxides and also playing a major role in xenobiotic metabolism (Sirisha, et al., 2010).

The main objective of the present study was to assess the effects of acrylamide on the histopathological alterations and some hematological indices in mice using tomatoes and carrots.

Materials and Methods

Materials

Materials used included carrots (*Caucus carota*) and tomatoes (*Solanumlycopersicum*) were purchased from the local market in Giza, Egypt.

Preparation of the raw materials

Carrot and tomatoes were separately washed with tap water, chopped into small pieces and blanched with water vapor, then frozen and put into a vacuum drying chamber (freeze drying) as described by Hui (1992). The dried materials were separately reduced into powder form as far as possible and stored in the refrigerator at 4°C until use.

Chemicals

Casein, cellulose, starch Minerals and vitamins were obtained from El-Gomhoria Company for chemical and medical equipment, Cairo, Egypt. Kits and Acrylamide were purchased from Gama Trade Company for chemicals, Cairo, Egypt.

Biological Experiment

Animals

Male albino mice $(25\pm2 \text{ g})$ were obtained from Agricultural Research Center, Giza, Egypt. Animals were individually housed in stainless steel wire-bottom cages, climate-controlled with 12-h day and night cycles and were fed on a standard laboratory diet (basal diet) and water *ad libitum*. After treatment, animals were sacrificed. Spleen and kidney were separated and fixed in 10% formalin for histological examination.

Experimental design

One week after acclimatization to laboratory conditions, mice were randomly divided into four groups of seven each. The first group was considered as negative normal. The remaining mice were subjected to daily dose (50 μ g acrylamide /kg body weight for 10 weeks). The treated groups were divided into three groups; each seven mice. One of these groups (group 2) was assigned as positive control. Both negative and positive groups were fed on basal diet. The other two groups (Group 3 and group 4) were given diets with formulas with 20% of freeze dried tomato and carrot, respectively.

Methods

Serum analysis

The blood was collected; serum from non-heparinized blood was used for kidney function tests (urea, creatinine and liver function (ALT, AST) according to Henry et al. (1974) and Reitman and Frankel, (1957) in respective order. Lipid profile; total cholesterol,triglycerides (TG), and high density lipoprotein-cholesterol (HDL-c) were determined as described by Thomas (1992), Fossati and Principle (1982), and Albers et al. (1983), respectively. Low density lipoprotein-cholesterol (LDL-c) and Very low density lipoprotein-cholesterol (VLDL-c) were calculated as mentionedby Lee and Nieman (1996)antioxidant enzymes tests; (Glutathione (GSH)wasassessed by Moron et al (1979),andGlutathione-S-transferase (GST) byHabig et al (1974).

Histopathologicalexamination

The spleen and kidneys were excised and fixed in neutral buffered formalin 10%; the organs were routinely processed and sectioned at 4-5 mm thickness. The obtained tissue sections were mounted on glass slides, deparaffinized and stained with Hematoxylin and Eosin stain. The sections were then examined and observed under a light microscope at magnifications X100 and X400.

Statistical analysis

All the values were represented as means \pm standard deviation and were analyzed by ANOVA. Difference was considered significant when P \leq 0.05.

Results and Discussion

Serum analysis

From the results presented in Table (1)it could be noticed that AST, ALT, creatinine and urea were significantly higher in the serum of positive control mice receiving acrylamide than those in the negative control group.Whereas, these levels in mice receiving acrylamide together with tomatoes or carrots were significantly lower than those in mice that were subjected to acrylamide treatment alone. Administration of vegetables exhibited a protective effect against acrylamide-induced some blood parameters elevation.

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Groups	AST(GOT)	ALT(GPT)	Creatinine	Urea nitrogen	
	(IU /L)				
Negative Control	38.00±3.61 ^c	67.33±6.03 ^b	0.28 ± 0.04^{b}	37.00±6.24 ^b	
Positive Control	132.00±3.61 ^a	81.33 ± 2.52^{a}	0.52 ± 0.03^{a}	72.00 ± 3.00^{a}	
Diet 1	116.00 ± 3.60^{b}	$52.00 \pm 3.61^{\circ}$	0.26 ± 0.04^{b}	37.00 ± 4.00^{b}	
Diet 2	119.67 ± 6.03^{b}	66.66 ± 6.51^{b}	0.25 ± 0.06^{b}	21.33±3.21 ^c	
LSD =0.05	8.17	9.32	0.08	8.12	

Table (1): AST, ALT, creatinineand urea in mice of the different experimental groups after 10 wk.

In this respect Mahmood*et al.*, (2015) who noted no significant changes in serum urea levels between the low dose acrylamide group and the control group; was indicating that the kidney can perform, to some extent, even with a low dose of acrylamide. They also added that creatinine levels showed a non-significant difference between the low dose acrylamide group and the control group, and a significant decrease in both mild and high dose acrylamide groups compared to the control group. In contrast, Ghorbel*et al.*, (2017) observed abnormalities in some blood cell parameters of acrylamide treated rats.

With regard to lipid profile (Table 2), post-treatment with vegetables in mice exhibited lower levels of serum cholesterol, TG, LDL, and VLDL compared to those in untreated (group 1) and acrylamide treated mice (group 2, positive control). Meanwhile, higher levels of HDL were recorded for mice receiving diet 1 and diet 2 than its parallel values of acrylamide treated mice fed on basal diet.

Groups	TC	TG	LDL	HDL	VLDL
			(mg/dl)		
Negative Control	142.67 ± 4.16^{b}	174.00 ± 5.00^{b}	54.33 ± 5.51^{b}	63.67 ± 4.04^{a}	30.93 ± 4.90^{b}
Positive Control	173.33 ± 4.51^{a}	190.33 ± 1.53^{a}	89.33 ± 9.50^{a}	30.00 ± 2.00^{d}	44.13 ± 5.35^{a}
Diet 1	99.00±3.61 ^d	$165.67 \pm 4.52^{\circ}$	$40.33 \pm 9.02^{\circ}$	38.33±3.06 ^c	29.33±4.51 ^b
Diet 2	$133.33 \pm 4.58^{\circ}$	137.00 ± 3.00^{d}	57.00 ± 2.65^{b}	49.00 ± 6.56^{b}	27.67 ± 2.52^{b}
LSD = 0.05	7.97	7.09	13.61	8.03	8.39

Table (2): Lipid profile in mice of the different experimental groups after 10 wk.

Lee *et al.*, (2015) declared that the tomato juices contained the hypolipidemic activity.da Silva Dias (2014) indicated that carrot

possessed the property of lowering cholesterol due to the presence of phytoconstituents.

As LDL plays a vital role in the transportation of fat from the liver to periphery. The increased levels of LDL might lead to deposition of fat in artery and aorta, coronary heart diseases (Pedersen, 2001). The decreased levels of LDL levels were found in supplementation of probioticated tomato and carrot to high fat diet-fed rats (Seelam*et al.*, 2018).Dietary supplementation of lycopene decreased significantly LDL oxidation (Visioli*et al.*, 2003). Jiang*et al.*, (2016) assured that lycopene was able to inhibit the incremental changes in ALT and AST, while it would decrease the TG, TC and LDL-C levels, and the HDL-C level.

Antioxidant enzymes

Activities of Glutathione (GSH) and Glutathione-S-transferase (GST), both components of the antioxidant enzyme system were illustrated in Table (3).

Table (3): Serum Glutathione (GSH) and serum Glutathione-S-transferase(GST) in mice of the different experimental groups after 10 wk.

Groups	Glutathione	Glutathione-S-	
	(GSH)	Transferase(GST)	
	(IU/l)		
Negative Control	72.66±5.51 ^a	$68.66 {\pm} 7.09^{a}$	
Positive Control	$38.00 \pm 7.57^{\circ}$	43.67±6.11 ^c	
Diet 1	50.00 ± 2.65^{b}	55.00 ± 2.65^{b}	
Diet 2	55.00 ± 3.61^{b}	58.00 ± 4.36^{b}	
LSD = 0.05	10.02	10.04	

GSH and GST were significantly lower in serum of mice receiving acrylamide compared to the negative control group. Meanwhile, both Glutathione (GSH) and Glutathione-S-transferase (GST) activities were significantly higher in mice receiving tomatoes or carrots together with acrylamide compared to mice receiving acrylamide alone. Administration of tomatoes or carrots demonstrated a emending effect against a decrease in antioxidant enzymes caused by acrylamide.

Since a large reserve of Glutathione (GSH) is present in the hepatocytes, thus in the detoxification of acrylamide the utilization of

liver GSH would be more pronounced, and thus the detoxification mechanism could be the cause of the decreased concentration of GSH(Hashimoto *et al.*, 1995). Jahan*et al.*,(2009) suggested enhancing GSH and reducing the LPO level during chemical induced skin carcinogenesis in mice as confirmed the antioxidative activity of various active constituents.

The potential role of lycopene in human health has been recognized, and the most important health benefits are hypothesized to occur through their ability to protect against oxidative damage (Gerster, 1997). Jiang *et al.*, (2016) noted that oral administration of lycopene increased the activities of antioxidant enzymes (GSH, SOD).

Histopathologyexamination:

The histopathologicalalterations in spleen and kidney were illustrated in Figures (1-8) and Table (4). The mice in negative control group showed a normal histological appearance in spleen and kidney. In group 2 (positive control) exhibited severe changes, spleen of mice showed extramedullary megakaryocytosis and kidney showed vacuolation of endothelial lining glomerular tuft and epithelial lining renal tubules. Examined spleen from the other treated groups 3 and 4 fed on diet with tomatoes and carrots, respectively, demonstrated moderate changes. Examined kidney from groups 3 and 4 showed mild changes.

Table (4): The severity of the reaction in different organs according to histopathological alterations

		Experimental groups			
Organs	1	2	3	4	
Spleen	-	+ + +	++	++	
Kidney	-	+ + +	+	+	
a	36.1 3611	3 711			

+++ Severe, + + Moderate, + Mild, - Nil

1: negative control, 2: positive control, 3: basal diet with 20% dried tomatoes, and 4: basal diet with 20% dried carrots.

Journal of Home Economics, Volume 28, December (4), 2018



Fig 1: Spleen of mice from group 1 negative control untreated group showing normalnormal while pulp (H and EX400)

Fig 2: Spleen of mice from group 2 positive control showing extramedullarymegakaryocytosis (H and EX400)





Fig 6: Spleen of mice from group 3 showing slight lymphocytic depletion (H and EX400)

Fig 7: Spleen of mice from group 4 showing extramedullary mega karyocytosis (H and EX400)



Fig 5: Kidney of mice from group 1 negative control untreated group showing normal histopathologycal structure of renal parenchyma (H and EX400)

Fig 6: Kidney of mice from group 2 positive control group showing vacuolation of endothelial lining glomerular tuft and epithelial lining renal tubules (H and EX400)

Journal of Home Economics, Volume 28, December (4), 2018



Fig 7: Kidney of mice from group 6Fig 8: Kidney of mice from group 7showing vacuolation of endothelial lining
glomerular tuft (H and EX400)Fig 8: Kidney of mice from group 7

In animals, reduction of lipid peroxidation products (thiobarbituric acid reactive substances) and DNA damage markers were found in monkey kidney fibroblast and rat hepatocytes supplemented with lycopene (Srinivasan*et al.*, 2007).For human health,much evidence showed that the consumption of lycopene rich foods could help in preventing degenerativediseases, but very limited studies have found a beneficial role of the consumption of lycopene alone. The interaction of lycopene with other active compounds seemed to be crucial for obtaining its optimal function inhuman health (Kong *et al.*, 2010).

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الملخص العربي:

استهدف البحث دراسة العلاقة بين استهلاك الطماطم والجزر المجفدين والتغييرات الحادثة في الكلى والطحال وكذلك الأنزيمات المضادة للأكسدة وبعض مؤشرات الدم في الفئران. كما تم تقييم التأثير الوقائيللطماطم والجزر فى الفئران المعاملة بمادة الأكريلاميد (كمادة محدثة للإجهاد التأكسدى). وشملت التجربة ثمانية وعشرون فار اللبينوو أعتبرت المجموعة الأولى (٧الفئران) كنترول سلبي. تم معاملة الفئران المتبقية بجرعة يومية (٥٠ ميكروجرام من محموعات (سبعة فئران فى كل مجموعة). عينت المجموعة الثانية ككنترول موجب. تم تغذية ملموعات (سبعة فئران فى كل مجموعة). عينت المجموعة الثانية ككنترول موجب. تم تغذية المجموعتين الكنترول السالب والموجب على الوجبة الاساسية، وتم إعطاء المجموعتين الثالثة والرابعة على وجبات تحتوي على ٢٠٪ من الطماطم والجزر المجفدين على التوالي. في نهاية التجربة تم التقييم المستوباتولوجي للكلى والطحال. وكذلك تقدير انزيمات Olutathione أطهرت الوجبات الغذائية المدعمة بالخضروات حماية بيوكيميائية مهمة ضد الأكريلاميد والترب موض المعار الحمومية)، من الطماطم والجزر المجفدين على التوالي. في نهاية التجربة تم التقييم المستوباتولوجي للكلى والطحال. وكذلك تقدير انزيمات Olutathione أظهرت الوجبات الغذائية المدعمة بالخضروات حماية بيوكيميائية مهمة ضد الأكريلاميد عن الموري خفن نشاط انزيمات (GSH)، رحماي مالغين والطال، وخلك والكلى ودهون الدم.

بالمقارنة مع الكنترول الموجب. اظهرت التقييمات الهستوباثولوجية لأنسجة الكلى والطحال بعض التغييرات في الفئران التي تم معاملتها بالأكريلاميد. بينما وجد أن الفئران التي تغذت على وجبات مدعمة بكل من الطماطم والجزر لم تحدث لانسجة الطحال والكلى تغيرات هستوباثولوجية مثل المجموعة الموجبة. وأكدت النتائج المتحصل عليها أن استهلاك الطماطم والجزر أدى إلى تقليل التأثير الضار لمادة الأكريلاميد على كل من الكلى والطحال. الكلمات المفتاحية: الخضروات، الاجهادالتأكسدى، الانزيمات المضادة للأكسدة، التغيرات

الهستولوجية، الأكريلاميد، الكلي، الطحال، الطماطم، الجزر.