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RESEARCH ARTICLE

El-Shafey A. A. Mohamed
Seddek M. Nour El-Din
El-Ezaby M. Mohamed
Seliem M. M. Ezaat
Abd El-Maksoud M. A. Elewa

PROTECTIVE EFFECTS OF GARLIC *"ALLIUM SATIVUM"* AND KARKADA *"HIBISCUS SABDARRIFA"* ON ACRYLAMIDE TREATED MALE ALBINO RATS

ABSTRACT:

Recent studies showed that foods contain acrylamide (ACR) may cause increases in the oxidative stress in animals and human. Garlic "*Allium sativum*" is widely uses in our life as food, while karkada *"Hibiscus sabdarrifa";* is a local soft drink. The present study is conducted for estimating the protective effects of the two herbs as antioxidants. Forty two adult male albino rats weighting 130-150 g were divided randomly into six groups (7 rats each) as follows: the 1st group (control group; standard diet only), the 2nd group (ACR group; daily 50 mg/kg BW for consecutive 5 days), the 3rd group (garlic group; daily 54 mg/kg BW for 4 weeks), the 4th group (karkada group; daily 81 mg/kg BW for 4 weeks), the 5th group (garlic then ACR group) and the 6th group (karkada then ACR group) at the same dose levels applied. All treatments were administrated orally. At the end of the experiment blood was collected from all groups, serum was separated and stored at -20°C until used. The obtained results revealed significant increases in the serum levels of total cholesterol "TC", triglycerides "TG", low density lipoprotein cholesterol "LDL-C" and very low density lipoprotein cholesterol "VLDL-C". A significant reduction in serum level of high density lipoprotein cholesterol "HDL-C" in ACR treated rats group was noticed. However, serum antioxidant enzyme (superoxide dismutase "SOD", gluthathione peroxidase "GPx" and catalse "CAT") activities were significantly decreased in ACR treated group compared to control one. Garlic and karkada administration as natural antioxidants before ACR administration help to keep the most measured parameters near the control values. Both garlic and karkada possessed protective effects against oxidative stress induced by ACR.

KEY WORDS:

Acrylamide, Garlic, Karkada, Lipid Profile, Antioxidant enzymes, Maillard reaction

CORRESPONDENCE:

Aziza A.M El-Shafey

Zoology Department, Faculty of Science, Benha University, Benha, Egypt

E-mail: elshafeya@yahoo.com

Mohamed N. Seddek
Magda M. El-Ezaby
Moshira M.E. Seliem
Marwa A.E. Abd El-Maksoud

Zoology Department, Faculty of Science, Benha University, Benha, Egypt

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INTRODUCTION:

Acrylamide "ACR" is formed in many types of prepared or cooked food at high temperatures; higher than 200°C (Gertz and Klostermann, 2002). Baked and fried starchy foods have higher contents of ACR but ACR has not been detected in unheated or boiled foods (Tareke *et al*., 2002). Twaddle *et al*. (2004) mentioned that ACR formation is connected to the Maillard-reaction during food preparation above 120 ̊ C. This reaction occurs between reducing sugars and amino acids such as asparagines, the major amino acid found in starchy foods as potatoes and cereals.

Rats showed significant increases in serum total cholesterol "TC" and triglycerides "TG" levels after treatment with ACR (Abd El-Aziem *et al.,* 2004). ACR treatment induced significant increases in serum total lipid of female rats and significant increases and decreases in serum TC and TG levels respectively in both male and female rats. Treatment with ACR did not induce any significant differences in serum levels of HDL-C and LDL-C in male and female rats (Rawi *et al.,* 2012).

Rats fed with garlic showed significant decreases in plasma levels of TC and increases in plasma high density lipoprotein cholesterol “HDL-C” level. Plasma levels of TG as well as low density lipoprotein cholesterol “LDL-C” and very low density lipoprotein cholesterol VLDL-C not changed. Also, diabetic rats treated with garlic oil showed significant decreases in plasma levels of TG and TC (Anwar and Meki, 2003).

Zahid *et al.* (2005) mentioned that garlic decreased serum TC, LDL-C and TG levels and increased HDL-C through inhibiting the activity of hydroxyl-3-methylglutaril- CoA reductase "HMG-CoA"; the rate determining enzyme in cholesterol biosynthesis and inhibition of LDL-C oxidation.

El-Mahdi *et al.* (2008) concluded that fresh crushed garlic was effective in lowering total cholesterol level and plays an important role in preventing atherosclerosis. The authors found that garlic caused significant decreases in plasma levels of TC and LDL-C in rats and significant increase in plasma level of HDL-C and non significant difference in TG level compared to hypercholesterolemic rats. Abd El-Rahman *et al*. (2009) studied the effect of garlic on biochemical factors of hyperlipidemic rats. The authors found that fresh garlic is effective in protection from heart diseases.

Karkada extract have a potency to inhibit the production of oxidized LDL-C and the ratio of LDL-C to HDL-C was reduced in rats (Chen *et al.,* 2004).Treatment of rats with karkada extract induced significant decreases in serum levels of TC, TG, LDL-C and VLDL-C and caused increase in serum HDL-C level. The authors concluded that karkada extract has antihyperlipidemic activity (Ochani and D´Mello, 2009).

Also, rabbits treated with karkada extract showed decreases in serum and tissue levels of LDL-C, TC, and TG and increases in serum level of HDL-C (Ekor *et al.,* 2010 and Peng *et al*., 2011). In human, the treatment with anthocyanins; the red pigments in the calyces of karkada caused reductions in serum TG and TC levels (Hernández-Pérez and Herrera-Arellano, 2011). Other authors reported that there were no significant changes in TC and TG levels with karkada extract treatment (Mohagheghi *et al.,* 2011).

The activities of glutathione peroxidase “GPx” and superoxide dismutase “SOD” were significantly increased in liver of rats treated with ACR (Yousef and El- Demerdash, 2006). Other authors concluded that ACR induced significant decreases in catalase “CAT” activity in human erythrocytes (Catalgol *et al.,* 2009). Allam *et al.* (2010) reported that ACR treatments in rats showed significant increases in lipid peroxidation and activity of reduced glutathione but showed significant inhibition in activity of SOD. Teodor *et al*. (2011) concluded that ACR administration was associated with significant increases in oxidative stress. Garlic co-administration to alcoholic rats help in return reduced glutathione and SOD activities toward normal values (Hussein *et al*., 2007).

Garlic extract administration to normal rats enhanced the activities of GPx, SOD and CAT in testis (Ola-Mudathri *et al*., 2008). Oral administration of garlic to rats showed a significant decrease in GSH activity; however SOD and CAT colon activities showed non significant decreases compared to control ones (Harisa *et al*., 2008). Hassan *et al*. (2009) found that rats treated with garlic oil showed non significant changes in liver and kidney GPx and CAT activities. The authors concluded that garlic has an excellent antioxidant properties and highly nutritional values. Aqueous garlic extract administration to mice caused significant increases in levels of antioxidant enzymes (Flora *et al*., 2009). In contrast, Shaarawy *et al*. (2009) found that aqueous garlic extract administration to rats showed non significant changes in hepatic total glutathione content, glutathione reductase and SOD activities. The total antioxidant status significantly improved after treatment with garlic extract. Alcoholic extract of karkada leaves caused significant increases in levels of CAT, SOD, GPx, and GSH in brain tissues of rats (Essa and Subramanian, 2006).

Karkada extract attenuated the decreases in the activities of SOD, CAT and glutathione in rats which caused by alloxan (Farombi and Ige, 2007). Rats treated with karkada showed a significant increase in hepatic SOD activity (Adaramoye *et al*., 2008). The authors concluded that karkada increased antioxidant defense system and decreased lipid peroxidation. Treatment of mouse with karkada induced significant increases in CAT activity and glutathione level and also decreased lipid peroxidation. Also, karkada prevented the depletion in SOD and CAT activities in cholesterol fed rabbits (Ekor *et al.,* 2010). Karkada anthocyanin extract attenuated the CCL4-mediated decreases in antioxidant enzymes “CAT and SOD”. Karkada anthocyanin extract has drug detoxification potential (Ajiboye *et al.,* 2011). Karkada extract caused significant increases in activities of catalase and SOD in rats (Samuel *et al.,* 2012). Lee *et al.* (2012) concluded that karkada caused increases in glutathione and CAT levels and decreased lipid peroxidation level in mice.

The present study aimed to investigate the protective effects of garlic and karkada against the toxicity of acrylamide in male albino rats "*Rattus norvegicus*".

MATERIAL AND METHODS:

Experimental animals:

Forty two male albino rats (*Rattus norvegicus*) weighting 130-150g were used in the present study. The animals were obtained from Helwan Farm, Egyptian Organization for Vaccine and Biological Preparations. Rats were caged for 10 days before the beginning of the experiment in the laboratory, at 25±2 °C, 12 hr light/dark cycle and given food and water *ad libitum*.

Acrylamide:

Acrylamide "ACR" was obtained from Win Lab Company for Laboratory Chemical Reagents and Fine Chemicals. ACR is a white odorless crystalline at room temperature. It dissolves in distilled water and used according to Abd El-Aziem *et al*. (2004).

Garlic:

Used as Tomax® produced by ATOS Pharm. Comp., Cairo, Egypt.

Tomax® contains highly concentrated garlic powder prepared from organically grown garlic without using chemical fertilizers or pesticides. Each tablet contains 200 mg dry garlic powder. It dissolves in distilled water. Garlic used according to Paget and Barnes (1964).

Karkada:

Used as Hybisc® produced by Sigma pharm. Industries-Comp., Egypt. It contains concentrated karkada flowers extract. Each tablet contains 300 mg karkada flowers extract. It dissolves in distilled water and used according to Paget and Barnes (1964).

-Experimental Design:

Animals were divided randomly into six groups, supplied with standard diet and water *ad libitum.*

The first group: (control group).

Normal untreated rats.

The second group: (acrylamide"ACR" group).

Rats administered a daily oral dose of acrylamide (50 mg/kg BW dissolved in 0.5 ml H_2O) for consecutive 5 days.

The third group: (garlic group).

Rats administered a daily oral dose of garlic (54 mg/kg BW dissolved in 0.5 ml H_2O) for 4 weeks.

The fourth group: (karkada group).

Rats administered a daily oral dose of karkada (81 mg/kg BW dissolved in 0.5 ml H_2O) for 4 weeks.

The fifth group: (garlic then ACR group).

Rats administered a daily oral dose of garlic for 4 weeks then ACR for consecutive 5 days at the same dose levels applied for groups 2&3.

The sixth group: (karkada then ACR group).

Rats administered a daily oral dose of karkada for 4 weeks then acrylamide for consecutive 5 days at the same dose levels applied for groups 2&4.

All administrations were given by gastric tube.

At the end of the experimental period rats were sacrificed, blood samples were collected from the heart in dry centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. Then sera were separated and stored at -20˚C until biochemical analysis.

Determination of serum lipid profile

Serum triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol (mg/dL) were determined spectrophotometerically according to the methods of Shephard and Whiting (1990), Röschlau *et al.* (1974), Matsuzaki *et al.* (1996) and Rifia *et al*. (1992) respectively using Roche Diagnostics kit.

Very low density lipoprotein cholesterol (VLDL-C) concentration was calculated according to Matos *et al.* (2005) by the following equation: VLDL-cholesterol = TG/5

Determination of serum antioxidant enzymes

Activities of serum SOD "u/mL", GPx "u/L" and CAT "u/L" were determined according to Goldstein and Czapski (1991), Paglia and Valentine (1967) and Ponting and Joslyn (1948), using SOD assay Kit-WST, OXLtek total glutathione peroxidase assay kit and CAT assay Kit-WST, respectively..

Statistical analysis

The values of the measured and calculated parameters were expressed as the mean of 7 individuals ± standard deviation "SD". Statistical analysis were carried out using one way analysis of variance (ANOVA) and Duncan test by using SPSS (version 20) statistical program.

RESULTS:

All parameters of lipid profile showed significant differences between groups at $P<0.001$ (Table 1). In ACR-treated group there were significant increases in serum levels of TG, TC, LDL-C and VLDL-C, but and significant decrease in HDL-C level compared with control and other treated groups. Garlic and karkada treatments caused a significant reduction in serum levels of TG, TC, LDL-C and VLDL-C and induced significant elevation in HDL-C level as compared with control and ACR treated groups. Treatments with garlic and karkada before ACR treatment caused that most lipid profile parameter values to be in control ranges.

Table 1. Protective effects of antioxidants (garlic "54 mg/kg BW" and karkada "81 mg/kg BW" daily for 4 weeks) on serum lipid profile of acrylamide treated rats "50 mg/kg BW" daily for 5 consecutive days.

Parameter \ Groups	Control	Acrylamide	Garlic	Karkada	Garlic then acrylamide	Karkada then acrylamide	P-value
Triglycerides TG (mg/dl)	63.66 ± 05.86^{c}	177.00 ± 10.53^{a}	64.66 ± 04.16^{c}	64.00 ± 07.93^{c}	76.33 ± 02.08^{b}	77.00 ± 04.35^{b}	***
Total cholesterol TC (mg/dl)	57.00 ± 07.00^{b}	148.00 ± 05.68^{a}	45.66 ± 03.78^{c}	48.33 ± 03.05^{bc}	55.32 ± 07.57^{bc}	50.66 ± 02.08^{bc}	***
High density lipoprotein cholesterol HDL-C (mg/dl)	27.33 ± 03.51^{c}	13.33 ± 04.16^{d}	40.66 ± 01.15^{a}	42.00 ± 01.00^{a}	37.33 ± 07.50^{ab}	32.00 ± 02.64^{bc}	***
Low density lipoprotein cholesterol LDL-C (mg/dl)	25.33 ± 04.50^{b}	42.00 ± 02.00^{a}	11.50 ± 00.50^{d}	16.50 ± 2.50c^{d}	25.50 ± 04.50^{b}	18.00 ± 03.00^{c}	***
Very low density lipoprotein cholesterol VLDL-C (mg/dl)	11.33 ± 00.50^{b}	35.83 ± 02.46^{a}	11.66 ± 01.53^{b}	12.33 ± 02.08^{b}	11.33 ± 02.06^{b}	12.00 ± 01.73^{b}	***

Number of animals in each group = 7; Data were represented as mean ±SD; ***=significantly different at P<0.001

In the same row: The same letters mean non significant difference; The different letters mean significant difference.

Table 2 indicates significant differences at P<0.001 for SOD and GPx activities and significant difference at P<0.05 for CAT activity between all treated groups and control one. ACR induced significant decreases in activities of antioxidant enzymes compared to control and other treated groups. Garlic and karkada treatments caused significant increases in activities of SOD and GPx compared to control and ACR-treated groups. Garlic treatment induced a significant increase in the activity of CAT compared to control and ACR-treated groups, while karkada treatment caused a significant increase in CAT activity compared to ACR-group. Treatments with garlic and karkada before ACR induced significant increases in serum activities of SOD and GPx compared to ACR-treated group. Treatment with garlic before ACR or karkada before ACR caused significant and non significant increases in CAT activity, respectively.

Table 2. Protective effects of antioxidants (garlic "54 mg/kg BW" and karkada "81 mg/kg BW " daily for 4 weeks") on serum antioxidant enzymes of acrylamide treated rats "50 mg/kg BW" daily for 5 consecutive days .

parameter \ Groups	Control	Acrylamide	Garlic	Karkada	Garlic then acrylamide	Karkada then acrylamide	P-value
Superoxide dismutase "SOD" (u/mL)	307.86 ± 06.70^{b}	281.06 ± 10.02^{c}	329.63 ± 8.86^{a}	337.70 ± 08.44^{a}	292.50 ± 07.24^{bc}	299.06 ±10.68^{b}	***
Glutathione peroxidase "GPx" (u/L)	370.44 ± 14.61^{c}	322.86 ± 13.96^{d}	398.90 ± 17.95^{ab}	408.53 ± 16.29^{a}	387.46 ± 14.52^{abc}	379.56 ±07.58^{bc}	***
Catalase “CAT” (u/L)	360.03 ± 10.30^{bc}	336.70 ± 08.74^{d}	384.46 ± 07.76^{a}	366.63 ± 07.21^{b}	362.16 ± 10.40^{bc}	359.21 ±17.80^{cd}	*

Number of animals in each group = 7; Data were represented as mean ± SD; ***=significantly different at P<0.001; *= significantly different at P<0.05

In the same row: The same letters mean no significant difference; The different letters mean significant difference.

DISCUSSION:

Acrylamide, one of the major environmental public health problems results from its increased accumulation in the process of cooking food materials. It is a small organic molecule with very high water solubility; these properties facilitate its rapid absorption and distribution through the body (Mannaa *et al.,* 2006).

In the present study, acrylamide induced significant increases in serum lipid profile parameters (except HDL-C that was decreased). This finding is in accidance with Abd El- Aziem *et al.* (2004) and Allam *et al.* (2010) who reported that ACR caused an elevation in TC in rats. This may be due to ACR induced an increase in the relative weight of liver indicating that the liver may be a target for ACR toxicity. This may lead to increased TC in blood, thus caused an increase in TG and LDL-C. The elevation in TG and TC in ACR-treated rats may be due to an increase in the synthesis of plasma lipoproteins and high mobilization of lipids from the liver. Rawi *et al.* (2012) indicated that changes in lipid profile may be attributed to liver disorders which caused by ACR.

Garlic treatment induced a significant reduction in serum TC and LDL-C levels. These reductions may be due to components of garlic as S-allyl-cysteine "SAC" which may caused a reduction in LDL-C release by liver and inhibit HMG-CoA reductase. These results are in line with those of Chowdhury *et al.* (2008). They concluded that garlic caused a decrease in TC via reduction in hepatic activities of lipogenic and cholesterogenic enzymes, which increase the excretion of TC via feces, while cholesterol decreased in the blood via an increase in HDL-C level.

Garlic also caused inhibition of HMG-CoA reductase and increased bile acid excretion. Reduction in LDL-C by garlic may be attributed to its content of allicin which reduced the production and release of LDL-C by the liver and promote LDL-receptor activity in the liver cells. This helps liver to clear the circulating LDL-C. The increase in HDL-C may be attributed to allicin which caused inhibition in apo D; cholesteryl ester transferase protein. This inhibition caused increase in HDL-C. The decrease in TG level may be due to garlic which caused inhibition of fatty acids synthesis (El-Mahdi *et al.* (2008).

Rats treated with karkada showed a significant reduction in serum TC, TG and LDL-C levels. These reductions may be attributed to that karkada activate secretion of adrenocortical hormones, which stimulate metabolic pathway of cholesterol by converting it into other compounds (Ali *et al.,* 2005). Karkada extract caused a significant decrease in LDL-C. This may be due to alternation in small intestine which increased hepatic LDL-receptor and decreased the conversion of VLDL to LDL-C. These results agreed with Olatunji *et al.* (2005). They reported that the decrease in TC may be attributed to the decrease in LDL-C level. The present results are in accordance with those of Gosain *et al.* (2010) who concluded that hibiscus acid could inhibit carbohydrate absorption and metabolism, thus hibiscus acid could inhibit lipogenesis.

Pectin molecule in karkada had cholesterol-lowering properties. Pectin in plants caused an increase in lipoprotein lipase in adipose tissues which responsible for the decreased triglycerides in serum and increased cholesterol catabolism (Hirunpanich *et al.,* 2006). Pectin decreased liver cholesterol and TG because it induced HMG-CoA redutase, decreased mitochondrial fatty acid oxidation and augmented liver cholesterogenesis and lipogenesis. Karkada caused an increase in HDL-C to help in return cholesterol from peripheral tissues to liver, thus cholesterol decreased in blood.

Acrylamide induced a significant decrease in antioxidant enzymes. These results are in agreement with those of Zhang *et al.* (2012). They reported that ACR caused damage to the oxidative defense system of cells and lead to the release of reactive oxygen species “ROS”. These results disagreed with those of Yousef and El-Demerdash (2006), who reported that ACR caused increases in activities of SOD and GST. The increase in activity of SOD may be combat free radical generation during ACR toxicity. Increased GST activity with the increase of ACR concentration could be due to increased formation of S-conjugates between acrylamide and GSH.

Both garlic and karkada act as natural antioxidants. Garlic and karkada induced significant increases in activities of antioxidant enzymes. These results are in line with those of Ola-Mudathir *et al.* (2008) and Hassan *et al.* (2009). They concluded that garlic contains several which posses’ antioxidant defense. The antioxidant properties of garlic may be attributed to polyphenols, a major class of bioactive phytochemicals in garlic which acting as a sacrificial antioxidant or as a chelator of transition metals. Polyphenols have the ability to up-regulate the expression of γ-glutamylcysteine synthase and the rate limiting enzyme in the biosynthesis of GSH. Photochemicals exert their antioxidant action by scavenging ROS, by enhancing the cellular antioxidant enzymes as SOD, CAT and GPx and increase glutathione in the cells which are the important defense mechanism in living cells (Zhang *et al.,* 2012). Garlic also contains Diallyl disulfide and Diallyl trisulfide which modulate the oxidative stress and detoxifying enzyme system. Garlic contains allicin which help to reduce oxidative stress by regulating the activities of antioxidant enzymes. Allicine also helps in blocking the epioxidation process of ACR by inhibiting P450 enzyme.

Usoh *et al.* (2005) reported that karkada may suppress oxidative stress through different mechanisms including free radical quenching, electron transfer and radical recombination. Also karkada had a high content of ascorbic acid and anthocyanin that significantly reduced oxidative stress by maintaining the activities of the antioxidant enzymes as SOD and CAT in rats (Agoreyo *et al.,* 2008).

In conclusion, garlic and karkada act as natural antioxidants and effective in prevention of ACR-induced oxidative stress and abnormalities in lipid profile parameters. Garlic and karkada would be used as dietary supplements for a beneficial application in protection against ACR toxicity and hyperlipidemia.

REFERENCES:

Abd El-Aziem SH, Abd El-Wahab MA, Mohmoud AM, Hassan AM, Mahrous K.F. 2004. Chemoprevention of Barley and Sage against acrylamide-induced genotoxic, biochemical and histological alternations in rats. Egypt. J. Hosp. Med., 15: 40-56.

Abd El-Rahman MK, Mahmoud EM, Abd El-Moomin AR, Rafaat OGA. 2009. Re-evaluation of individual and combined garlic and flaxseed diets on hyperlipidemic rats. Pakistan J. Nutri., 8(1): 1-8.

Adaramoye O, Ogungbenro B, Anyaegbu O, Fafunso M. 2008. Protective effect of extracts of *Vernonia amygdalina, Hibiscus sabdariffa* and vitamine C against radiation induced liver damage in rats. J. Radiat. Res., 49(2): 123-131.

Agoreyo FO, Agoreyo BO, Onuorah MN. 2008. Effect of aqueous extracts of *Hibiscus sadbariffa* and *Zingiber Officinale* on blood cholesterol and glucose levels of rats. Afr. J. biotechnol., 7: 3949-3951.

Ajiboye TO, Salawu NA, Yakubu MT, Oladiji AT, Akanji MA, Okogun JI. 2011. Antioxidant and drug detoxification potentials of *Hibiscus sabdariffa* anthocyanin extract. Drug Chem. Toxicol., 34(2): 109-115.

Ali BH, Al Wabel N, Blunden G. 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa L.* a Review, Phytother. Res., 19(5): 369-375.

Allam AA, El-Ghareeb AW, Abdul-Hamid M, Bakery AE, Gad M, Sabri M. 2010. Effect of prenatal and perinatal acrylamide on the biochemical and morphological changes in liver of developing albino rat. Arch. Toxicol., 84(2): 129-141.

Anwar MM, Meki AR. 2003. Oxidative stress in streptozotocin induced diabetic rats: effects of garlic oil and melatonin. Comp. Biochem. Physiol. A, Mol. Integr. Physiol., 135(4): 539-547.

Catalgol B, Ozhan G, Alpertunga B. 2009. Acrylamide-induced oxidative stress in human erythrocytes. Hum. Exp. Toxicol., 28(10): 611-617.

Chen CC, Chou FP, Ho YC, Lin WL, Wang CP, Kao ES, Huang AC, Wang CJ. 2004. Inhibitory effects of *Hibiscus sabdariffa* L. extract on low-density lipoprotein oxidation and anti-hyperlipidemia in fructose-fed and cholesterol-fed rats. J. Sci. Food Agric., 84(15): 1989–1996.

Chowdhury MMR, Manuddin SM, Islam MK. 2008. Effects of turmeric and garlic on blood cholesterol level in guinea pig. Banglades J. Pharamacol., 3: 17-20.

Ekor M, Adesanoye OA, Udo IE, Adegoke OA, Raji J, Farombi EO. 2010. *Hibiscus sabdariffa* ethanolic extract protects against dyslipidemia and oxidative stress induced by chronic cholesterol administration in rabbits. Afr. J. Med. Med. Sci., 39(Suppl): 161-170.

El-Mahdi B, Khalil MM, Abulgasim AI. 2008. The effect of fresh crushed garlic bulbs *(Allium sativum)* on plasma lipids in hypercholesterolemic rats. Res. J. Anim. Vet. Sci., 3: 15-19.

Essa MM, Subramanian P. 2006. *Hibiscus sabdariffa* affects ammonium chloride-induced hyper-ammonemic rats. Advance Acess Publication, 4: 321-325.

Farombi EO, Ige OO. 2007. Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of *Hibiscus sadbariffa* in alloxan-induced diabetic rats. Fundam. Clin. Pharmacol., 21(6): 601-609.

Flora SJS, Mehta A, Gupta R. 2009. Prevention of arsenic-induced hepatic apoptosis by concomitant administration of garlic extracts in mice. Chem. Biol. Interac., 177(3): 277-233.

Gertz C, Klostermann S. 2002. Analysis of acrylamide and mechanisms of its formation in deep-fried products. Eur. J. Lipid Sci. Tech.., 104(11): 762-771.

Goldstein S, Czapski G. 1991. Comparison between different assays for superoxide dismutase-like activity. Free Rad. Res. Commun., 12-13 (Pt 1): 5-13.

Gosain S, Ircchiaya R, Sharma PC, Thareja S, Kalra A, Deep A, Bhardwaj TR. 2010. Hypolipidemic effect of ethanolic extract from *Hibiscus sabdariffa L.* in hyperlipidemic rats. Acta Pol. Pharm., 67(2): 179-184.

Harisa GEI, Abo-Salem OM, Taha EI, Abood AM. 2008. N-nitro-l-arginine methylester and garlic preserve paraoxonase-1 activity in acetic acid induced ulcerative colitis in rats. EJBM, 14: 79-95.

Hassan HA, El-Agmy SM, Gaur RL, Femando A, Raj MHG, Outhit A. 2009. *In vivo* evidence of hepato- and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. Int. J. Boil. Sci., 5(3): 249-255.

Hernández-Pérez F, Herrera-Arellano A. 2011. Therapeutic use *Hibiscus sabadariffa* extract in the treatment of hypercholesterolemia. A randomized clinical trial. Rev. Med. Inst. Mex. Seguro. Soc., 49(5): 469-480.

Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H, Herunsale A, Suthisisang C. 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. J. Ethnopharmacol., 103(2): 252-260.

Hussein JS, Oraby FS, El-Shafey N. 2007. Antihepatotoxic effect of garlic and onion oils on ethanol-induced liver injury in rats. J. App. Sci. Res., 3(11): 1527-1533.

Lee CH, Kuo CY, Wang CJ, Wang CP, Lee YR, Hung CN, Lee HJ. 2012. A polyphenol extract of *Hibiscus sabdariffa L.* ameliorates acetaminophen-induced hepatic steatosis by attenuating the mitochondrial dysfunction in vivo and in vitro. Biosci. Biotechnol. Biochem., 76(4): 646-651.

Mannaa F, Abd El-Wahhab MA, Ahmed HH, Park MH. 2006. Protective role of *Panax ginseng* extract standardized with ginsenoside Rg3 against acrylamide induced neurotoxicity in rats. J. Appl. Toxicol., 26(3): 198-206.

Matos SL, De Paula H, Pedrosa ML, Dos Santos RC, De Oliveira EL, Júnior D.A.C, Silva ME. 2005. Dietary models for inducing hypercholesterolemia in rats. Braz. Arch. Biol. Technol., 48(2): 203-209.

Matsuzaki Y, Kawaguchi E, Morita Y. 1996. Evaluation of two kinds of reagents for direct determination of HDL-cholesterol. J. Anal. Biosci., 19: 419-427.

Mohagheghi A, Maghsoud S, Khashayar P, Ghazi-Khansari A. 2011. The effect of *Hibiscus sabadariffa* on lipid profile, creatinine and serum electrolytes: A randomized clinical trial. ISRN Gastroenterol., 2011: 976019.

Ochani PC, D'Mello P. 2009. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa Linn.* Leaves and calyces extracts in rats. Indian J. Exp. Biol., 47(4): 276-282.

Ola-Mudathir KF, Suru SM, Fafunso MA, Obioha U.E, Faremi TY. 2008. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food and Chem. Toxicol., 46: 3604-3611.

Olatunji LA, Olatunji JO, Oguntoye OB, Olatunde NO, Olatunji VA, Soladoye AO. 2005. Effect of aqueous extracts of red and green *Hibiscus sabdariffa* on plasma lipid and heamatological variables in rats. Pharm. Biol., 43(5): 471-474.

Paget GE, Barnes JM. 1964. Toxicity tests. In: “Evaluation of drug activities: pharmacometrics. (Laurence DR, bachrach AL. Ed.)” Academic Press. London, New York. 1: 135-166.

Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70(1): 158-169.

Peng CH, Chyau CC, Chan KC, Chan TH, Wang CJ, Huang CN. 2011. *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. J. Agric. Food Chem., 59(18): 9901-9909.

Ponting JD, Joslyn MA. 1948. Ascorbic acid oxidation and browning in apple tissue extracts. Arch. Biochem., 19(1): 47-63.

Rawi SR, Marie MAS, Fahmy SR, El-Abied SA. 2012. Hazardous effects of acrylamide on immature male and female rats. Afr. J. Pharm. Pharamacol., 6(18): 1367-1386.

Rifia N, Warnick GR, Mcnamara JR, Belcher JD, Grinstead GF, Frantz JR. 1992. Measurement of low dinesity lipoprotein cholesterol in serum: a status report. Clin. Chem., 38(1): 150-160.

Röschlau P, Bernt E, Gruber W. 1974. Enzymatic determination of total cholesterol in serum. Z. Klin. Chem. Klin. Biochem., 12(9): 403-407.

Samuel AJ, Mohan S, Chellappan DK, Kalusalingam A, Ariamuthu S. 2012. *Hibiscus vitifolius* (Linn.) root extract shows potent protective action against anti-tubercular drug induced hepatotoxicity. J. Enthnopharmacol., 141(1): 396-402.

Shaarawy SM, Tohamy AA, Elgendy SM, Abd El-Mageed ZY, Bahnasy A, Mohamed, MS, Kandil E, Matrougui K. 2009. Protective effects of garlic and silymarin on NDEA-induced rats hepato-toxicity. Int. J. Boil. Sci., 5(6): 549-557.

Shephard MDS, Whiting MJ. 1990. Falsely low estimation of triglycerides in lipidemic plasma by enzymatic triglyceride methods with modified Trinder´s chromogen. Clin. Chem., 36(2): 325-329.

Tareke E, Ryberg P, Karlsson P, Eriksson S, Törnqvist M. 2002. Analysis of acrylamide, a carcinogen formed in heated food stuffs. J. Agric. Food Chem., 50(17): 4998-5006.

Teodor V, Cuciureanu M, Fillip C, Zamosteanu N, Cuciureanu R. 2011. Protective effects of selenium on acrylamide toxicity in the liver of the rat. Effect on the oxidative stress. Rev. Med. Chir. Soc. Med. Nat. Lasi., 115(2): 612-618.

Twaddle NC, Mcdaniel LP, Costa GG, Churchwell MI, Beland FA, Doerge DR. 2004. Determination of acrylamide and glycidamide serum toxicokinetics in B6C3F1 mice using LC–ES/MS/MS. Cancer Lett., 207(1): 9-17.

Uosh IF, Akpan EJ, Etim EO, Farombi EO. 2005. Antioxidant actions of dried flower extracts of *Hibiscus sadbariffa L.* on sodium arsenite induced oxidative stress in rats. Pakistan J. Nutr., 4(3): 135-141.

Yousef MI, El-Demerdash FM 2006. Acrylamide-induced oxidative stress and biochemical perturbation in rats. Toxicology, 219(1-3): 133-141.

Zahid AM, Hussain ME, Fahim M. 2005. Antiatherosclerotic effects of dietary supplementions of garlic and turmeric: restoration of endothelial function in rats. Life Sci., 77(8): 837-857.

Zhang L, Zhang H, Miao Y, Wu S, Ye H, Yuan Y. 2012. Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo. Food Chem. Toxicol., 50(9): 3306-3312.

التأثير الوقائي للثوم والكركديه على ذكور الجرذان البيضاء المعامله بالاكريلاميد

عزيزه عبد الصمد محمد الشافعي، محمد نورالدين صديق، ماجدة محمد العزبي، مشيرة محمد عزت،

مروة عاطف عليوة عبد المقصود

قسم علم الحيوان، كلية العلوم، جامعة بنها، بنها، مصر

أوضحت الدراسات السابقة ان الأطعمه التي تحتوي على الاكريلاميد تزيد من الأكسدة في الحيوانات و الأنسان. يستخدم الثوم في الطعام و يستخدم الكركاديه كشراب في حياتنا اليومية. صممتت الدراسة الحالية لإختبار التاثير الوقائي للثوم و الكركدية كمضادات للأكسدة. تم تقسيم 42 من الجرذان البيضاء التي تزن 130-150 جرام عشوائيا الي 6 مجموعات (7 حيوانات في كل مجموعة) على النحو التالي: المجموعه الأولى (الضابطة)، المجموعة الثانية (المعاملة بالاكريلاميد 50 ملى جرام/كجم/يومياً لمدة 5أيام متتالية)، المجموعة الثالثة (المعاملة بالثوم 54 ملى جرام/كجم/ يومياً لمدة 4 أسابيع)، المجموعة الرابعة (المعاملة بالكركدية 81 ملى جرام/كجم/ يومياً لمدة 4 أسابيع)، المجموعة الخامسة (المعاملة بالثوم كما في المجموعة الثالثة ثم الاكريلاميد كما في المجموعة الثانية) و المجموعة السادسة (المعاملة بالكركدية كما في المجموعة الرابعة ثم الاكريلاميد كما في المجموعة الثانية). وقد تمت جميع المعاملات عن طريق الفم. في نهاية التجربة تم تجميع الدم من جميع المجموعات وتم فصل المصل وتخزينه عند -20 درجة مئوية لحين الأستخدام. وقد أوضحت النتائج زيادة معنوية في كل من الكوليستيرول الكلى، ثلالثي الجليسريد، كوليستيرول اللبيدات البروتينية منخفضة الكثافة و كوليستيرول اللبيدات البروتينية شديد إنخفاض الكثافة وانخفاض معنوي فى كوليستيرول اللبيدات البروتينية عالية الكثافة فى مصل المجموعة المعاملة بالاكريلاميد. وأوضحت النتائج إنخفاض معنوي في الأنزيمات المضادة للأكسدة (سوبر اوكسيد ديسميوتيز، جلوتاثيون بيراوكسيديز و الكتاليز) فى هذه المجموعة مقارنة بالمجموعة الضابطة. إستخدام كلاً من الثوم والكركدية كمضادات أكسدة طبيعية قبل المعاملة بالأكريلاميد ساعد في أن تصل قيم معظم القياسات ما يقرب من القيم الضابطة. كلاً من الثوم والكركدية أوضح تأثير وقائي ضد الأكسدة المسببه بواسطة الاكريلاميد .

المحكمون:

أ.د. ناديه محمد البيه قسم علم الحيوان، علوم عين شمس

أ.د. اسماعيل صبري قسم علم الحيوان، علوم الاسكندرية