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Research Article

Ameliorative and Synergistic Effect of Red Raspberry and Lycopene Against Hepatotoxicity Induced by Acrylamide in Male Mice

Fawziah A.Al-Salmi

Department of Biology, Faculty of Science, Taif University, 888 Taif, Saudi Arabia

Abstract

Background and Objective: Acrylamide (ACR) is a chemical substance formed when starchy foods, such as potatoes and bread are cooked at high temperatures (above 160°C). It can be formed when foods fried and baked. The study, designed to illustrate the biochemical responses and histopathological alterations besides the genotoxicity of ACR on the liver of mice and possible protection of either Lycopene (LYC) and/or red raspberry (RR) when administrated in co-treatment. **Materials and Methods:** Animals were separated into eight groups into control, ACR (500 Fg kg⁻¹ day), LY (10 mg kg⁻¹/day), RR (300 mg kg⁻¹), LY combined with RR, ACR plus LY and ACR plus RR and the last group treated with ACR with a combination of LY and RR. . Treatment was I.P for 30 consecutive days. The ACR raised activities of some enzymes of liver markers and disturbed the lipid profile levels. It is obvious that hepatic glutathione peroxidase (GPx) level was diminished, marker enzymes of antioxidant activities, as well as possible and increased the lipid peroxidation levels. **Results:** The results revealed that ACR significantly increased hepatic enzymes and marker of lipid peroxidation incomparable to control in a dose-dependent. Both LY and RR prohibited the ACR-induced liver damage as specified by enhancing all the previous parameters. Results of histopathological and electron microscope proved the biochemical feedback and the improvement of either LY and RR effect on liver toxicities that was observed. **Conclusion:** Co-treatment of LY and RR induced different improvement mechanisms against ACR-induced liver impairment. So, the ACR intake should be regulated and taken with either LY and/or RR when it is consumed in different food or drink to reduce its oxidative stress, histopathology and TEM of the liver.

Key words: Acrylamide, food preservatives, lycopene, red raspberry, oxidative stress, hepatotoxicity

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Corresponding Author: Fawziah A. Al-Salmi, Department of Biology, Faculty of Science, Taif University, 888 Taif, Saudi Arabia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acrylamide is an α , β -unsaturated carbonyl compound with a significantly high chemical activity. It is extensively used in many fields from industrial manufacturing to laboratory personnel work, so it is often absorbed during occupational exposure¹.

It is the monomer, from which polyacrylamides are synthesized. The latter is used in the treatment of water, cosmetics and paper packaging. Acrylamide does not occur naturally. It was found in various fried, deep-fried and oven-baked foods. It concerned foods that are regularly consumed throughout the years, like chips (French fries), crisps and bread but also biscuits, crackers and breakfast cereals².

Acrylamide might be formed through the Maillard reaction from amino acids (e.g., asparagine) and reducing sugars (e.g., glucose)³. Glycidamide, a metabolite of acrylamide, binds to DNA and can cause genetic damage. Prolonged exposure has induced tumours in rats but cancer in man has not been convincingly shown. The International Agency for Research on Cancer (IARC) has classified acrylamide as a probably carcinogenic to humans⁴. Barber *et al.*⁵ reported that the rate of acrylamide conversion to its epoxide metabolite glycidamide is higher during subchronic dosing conditions. LoPachin *et al.*⁶ indicated that peripheral axon degeneration is a product of acrylamide intoxication and occurs independent of behavioural and functional neurotoxicity.

Bioactive constituents in red raspberries, include polyphenolic compounds, antioxidants, vitamins, minerals and fibre. A targeted metabolomics study has identified approximately 50 phenolic compounds in red raspberry cultivars⁷, though the most abundant seem to be anthocyanins and the ellagitannins, sanguine H-6 and lambertianin C⁷.

The absorption and metabolism of raspberry polyphenolics occurs in the upper gastrointestinal tract and non-absorbed free and fibre-bound polyphenolics are metabolized and absorbed in the colon⁸.

Lycopene (LYC), a bioactive compound is a fat soluble naturally carotenoid pigment that is widely found in red foods such as tomato, and watermelon. The structure of LYC (C₄₀H₅₆) is an acyclic isomer of β -carotene with 11 conjugated double bond⁹. It is one of the most effective antioxidants in the carotenoid family and its activity against biological reactive oxygen species may prevent or ameliorate oxidative damage to cells and tissues both *in vivo* and *in vitro*¹⁰.

Lycopene has been shown to have the ability to reduce the risk of cancers, such as breast, prostate and gastric cancer¹¹. Studies have demonstrated that LYC is a common substance of chemoprevention, preventing the toxicity of a variety of toxicants. The LYC can protect against the toxic effects of malathion¹². It also reduced deltamethrin effects induced thyroid toxicity in albino rats¹³. Nevertheless, whether LYC has the potential to prevent the toxicity of ACR is still unclear.

The current understanding of acrylamide toxic effects on the liver tissues of animals is somewhat limited; therefore, the present study was aimed out to examine the liver of mice, in order to clarify the possible alterations, due to the oxidative stress of acrylamide induced on hepatic tissues, combined with the histological and TEM alterations of the hepatic tissues and to understand the accurate effect of acrylamide. Moreover, the objective was to evaluate the ability of Lycopene (LYC) and/or Red raspberry (RR) to decrease the toxicity in the presence of doses of acrylamide.

MATERIALS AND METHODS

Chemicals: Acrylamide (ACR) (C₃H₅NO, >99% purity) was purchased from Sigma Chemical company. The dose of the treatment is 500 Fg kg⁻¹ b.wt., of acrylamide in drinking water for 10 weeks¹³.

The red raspberries (*Rubus idaeus* L.) were purchased from a local market in Taif. Fresh red raspberries were cleaned before extraction. The red raspberries were extracted using the method reported previously¹⁴. Briefly, 100 g of fresh weight of the red raspberries were weighed and homogenized with chilled 80% solvent (1:2, w/v) using a chilled waring blender for 5 min. The sample then further homogenized using a polytron homogenizer for an additional 3 min. The homogenates were filtered through filter paper. The filtrate was evaporated at 45°C until approximately 90% of the filtrate had been evaporated. The standardized raspberry extracts were frozen and stored at -80°C until use in the feeding study. Dose of red raspberry is 300 mg kg⁻¹ according to El-Baz *et al.*¹⁵.

Lycopene was obtained from sigma Aldrich company. Lycopene was given in a dose (20 mg kg⁻¹)¹⁶.

Experimental animals: The Wistar mice were obtained from King Fahad of animal research, Abd El-Aziz University. Male Wistar mice weighing 35-40 g and their ages about two months will be housed in metal cages and will be bedded with wood shavings and will be kept under standard laboratory

conditions of aeration and room temperature at about 25°C with 12/12 light and dark cycle. The animals will be allowed to free access to standard diet and water *ad libitum* throughout the experimental period. Following the European community Directive (86/609/EEC) and national rules on animal care in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. This study was approved by the research ethics committee of Deanship of scientific research by number: 39-31-0033. The animals were accommodated to the laboratory conditions for two weeks before being experimented.

Experimental groups: The male mice were separated into 8 experimental groups (8 animals in each group). Group I was considered as the control animals that were given saline as a vehicle. Group II was given Acrylamide (Acry) (500 Fg kg⁻¹ mg kg⁻¹) and group III was treated with Lycopene (Ly) (20 mg kg⁻¹). Group IV was administrated red raspberry (RR; 300 mg kg⁻¹). Groups V was administrated combination of LY and RR. While, groups VI and VII were administrated Acry+Ly and Acry+RR, respectively. The last VIII group was treated with Acry and a combination of both Ly and RR. All the rats were treated (I.P) for 30 consecutive days.

Sample collection of biomarkers hepatic functions assessment: Under ether anesthesia, the blood was collected from fasted animals over 12 h. They were centrifuged at 500xg for 15 min to collect the serum that kept at -80°C for liver biomarkers investigation.

Serum AST and ALT were estimated according to the methods of Reitman and Frankel, Serum of lactic dehydrogenase (LDH) activity was evaluated by King¹⁷. Activities of aminotransferases were assayed by Reitman and Frankel¹⁸. Alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ -GT) activities were estimated according to Choi *et al.*¹⁹ and Orlowski and Meister²⁰, respectively. Albumin and the total protein levels were assayed according to Bowers and Wong²¹ and Bradford²², respectively.

Hepatorenal biomarkers determination: The serum Total Cholesterol (TC) and triglycerides (TG) were determined by the method of Carr *et al.*²³. High density lipoprotein-cholesterol (HDL-c) was determined according to the methods of Warnick *et al.*²⁴. Serum low density lipoprotein-cholesterol (LDL-c) level will be calculated according to Friedewald *et al.*²⁵ formula: $LDLCc = 3 \text{ Total cholesterol levels} - (\text{Triglyceride concentration}/5) - HDLCc \text{ concentration}$. The protein content was determined by the method described by Bradford²² using bovine serum albumin as the standard.

Preparation of liver homogenate for redox state

assessment: Hepatic tissues were perfused through the hepatic vessels with a 50 mM sodium phosphate buffer (pH 7.4), 0.25 M sucrose and 0.1 mM ethylenediaminetetraacetic acid (EDTA). Then, homogenization of tissue was done (10%; W/V) in ice buffer/g tissue by using homogenizer. The homogenates were centrifuged and the supernatant was then put into Eppendorf in a deep freeze until used for determination of the following parameters.

The LPO was clarified as previously mentioned by Ohkawa *et al.*²⁶. Superoxide dismutase (SOD) and catalase (CAT) were obtained according to Marklund and Marklund²⁷ and Aebi²⁸. The glutathione peroxidase (GPx) activity was determined by Aebi²⁸. Myeloperoxidase (MPO) and xanthine oxidase (XO) activities were measured as Suzuki *et al.*²⁹ and Litwack *et al.*³⁰, respectively. Tissues reduced glutathione (GSH) levels were determined by Couri and Abdel-Rahman³¹. Total thiols level were determined according to Hu³².

Histological and Transmission Electron Microscopy (TEM)

evaluation: Liver specimens of the animals were fixed in 10% neutral buffered formalin for histological evaluation (H and E). Were assayed based on the histological criteria score.

Immediately after the animals sacrificing, small pieces of liver were fixed in 5% glutaraldehyde for 24 h and then complete other processing. Ultrathin sections were selected and were cut with a diamond knife using ultra-microtome.

The ultrathin sections were ascended on copper grids and the samples were examined by a transmission electron microscope (JEOL-Japan, JEM-1200) Egypt.

Statistical analysis: Data are expressed as a Mean $\pm$ Standard Error (SE). All data were analyzed with the SPSS. Statistical significance was evaluated by One Way-Analysis of Variance (ANOVA). For each significant effect of treatment, the *post hoc* Tukey's test was used for comparisons. The criterion for statistical significance was set at $p < 0.05$.

RESULTS

Hepatic antioxidant levels: There were alterations in hepatic antioxidant enzymes and thiol level in male mice treated with ACR or/and LY and RR (Table 1).

The results revealed an increase of LPO in the liver of the ACR-treated group in a manner depending on a dose as demonstrated by the elevation of malondialdehyde levels in

Table 1: Effect of acrylamide, lycopene and red raspberry extract each alone and their combination on hepatic antioxidant capacities in male rats

Groups	Super oxide dismutase (SOD) (U g ⁻¹)	Malondialdehyde (MDA) (U g ⁻¹)	Glutathione peroxidase (GPx) (U g ⁻¹)	Catalase (CAT) (U g ⁻¹)
Control group	10.03±0.58 ^a	4.36±0.32 ^e	8.69±0.14 ^{ab}	4.25±1.36 ^a
Acrylamide treated group	2.58±0.02 ^d	18.69±1.36 ^a	3.58±0.47 ^e	1.02±0.36 ^f
Lycopene treated group	10.25±0.24 ^a	4.02±0.69 ^e	8.48±2.58 ^b	4.35±1.02 ^a
Red raspberry treated group	10.03±0.31 ^a	4.69±0.69 ^e	8.69±2.01 ^{ab}	4.01±0.69 ^b
Lycopene+Red raspberry treated group	10.13±0.21 ^a	4.56±0.82 ^e	8.15±0.64 ^b	4.45±1.08 ^a
Acrylamide+Lycopene treated group	5.69±1.03 ^d	12.98±1.69 ^{bc}	5.69±0.58 ^d	2.02±0.98 ^e
Acrylamide+Red raspberry	7.99±0.36 ^c	11.69±1.65 ^c	6.58±1.58 ^c	2.89±0.41 ^{de}
Acrylamide+Lycopene+Red raspberry	9.01±1.39 ^b	7.69±1.69 ^d	8.01±1.98 ^b	3.89±0.78 ^c

Values are expressed as Means ± SE, n = 8 for each treatment group, SOD: Superoxide dismutase, MDA: Malondialdehyde, Gpx: Glutathione peroxidase, CAT: Catalase, Significant difference was assigned alphabetically

the liver of adult mice. The Administration of LY with ACR mitigated LPO and significantly modulated the MDA levels in the liver.

In the liver of ACR-treated mice, SOD, CAT and GPx activities reduced clearly, when compared to control group (Table 1). Administration of LY with ACR ameliorated the antioxidant enzymes group as comparable with ACR-group.

A significant decline of GPx and catalase (CAT) of the liver was obvious in ACR-group compared to control group (Table 1). Giving LY with ACR to the mice ameliorated GPx and CAT levels when compared to ACR-group.

Activities of serum MPO and XO levels were elevated due to the administration of ACR while the thiol level decreased. They were improved when mice treated either with LY and/or RR and more improved in both and ACR.

Biomarkers of liver assessment: Serum ALT activities of ACR treated group was increased by 85 fold when compared with the control group (Table 2). Administration of 20 mg kg⁻¹ LY combined with ACR decreased both ALT and AST activities by 40 and 30% as compared with ACR treated group. The same notice has been recorded in the ALP, LDH and γ -GT activities that increased by increasing the dose of ACR and decreased after treatment of the rats with either LY and/or RR (Table 2). The total protein and albumin levels diminished in ACR-treated mice depend on the dose but elevated in the groups treated with the LY and/or RR (Table 2).

The lipid profile picture (TG, TC, LDL-c and VLDL-c) were elevated while the HDL-c was decreased when the mice exposed to treatment with ACR (Table 3). Administration of combination of LY and RR to the ACR-treated groups, restored about all the complete normal levels.

Histological and ultrastructure observation: The data in Fig. 1 showed the histological examination of (A) Control liver sections showing liver section showing polyhedral hepatocyte (Black head arrow) alternate with the blood sinusoids (S) lined

by endothelial cells and Von Kupffer cells arranged in cords around the Central Vein (CV), (B) Liver section of group treated with ACR showing Epithelioid Granuloma (EG) with mild lobular inflammation, (C) LY treated group showing normal tissues of the liver structure encountered by cords of polyhedral hepatocytes with eosinophilic cytoplasm; each hepatocyte has a distinct limiting membrane with centrally located nucleus and prominent nucleolus, (D) RR treated group showing normal hepatic tissues with normal sized Central Vein (CV) and normal portal vein with appearance of eosinophilic nucleus and cytoplasm, (E) Hepatic tissues of group treated with combination of LY and RR showing highly normal hepatic structure with normal Central Vein (CV) and appearance of polyhedral hepatocytes, (F) Hepatic tissues of group treated with a combination of ACR and LY showing distention of portal tracts by chronic inflammatory cells with moderate necrosis, (G) A liver tissue of a group treated with ACR+RR showing lobular aggregates of chronic inflammatory cell and (H) Hepatic tissues of group treated with ACR and combination of LY and RR showing great restoration of hepatic tissues architectures with moderate size nucleus.

As data in Fig. 2 illustrated TEM of (A) showing control group; with normal appearance hepatocytes with spherical bi-nucleus (N), nucleolus, spherical mitochondria, M and Rough Endoplasmic Reticulum (RER) and normal distribution of chromatin (CH) and glycogen granules (5 Fm), (B) An electron micrograph of ACR treated liver showing severely haemorrhage (Black arrow heads) with appearance of red blood cells (RB) with fatty changes (White head arrow) and appearance of a lot of fat droplets (F) with distingeneration of some hepatic tissues (Blue head arrow) (5 Fm), (C) Hepatocytes of LY group showing normal nucleus (N) with regular boundaries (white arrow heads), Endoplasmic Reticulum (ER) and normal mitochondria (M) (5 Fm), (D) Hepatocytes of RR group showing normal nucleus (N) with regular boundaries (white arrow heads), Endoplasmic Reticulum (ER) and normal mitochondria (M)

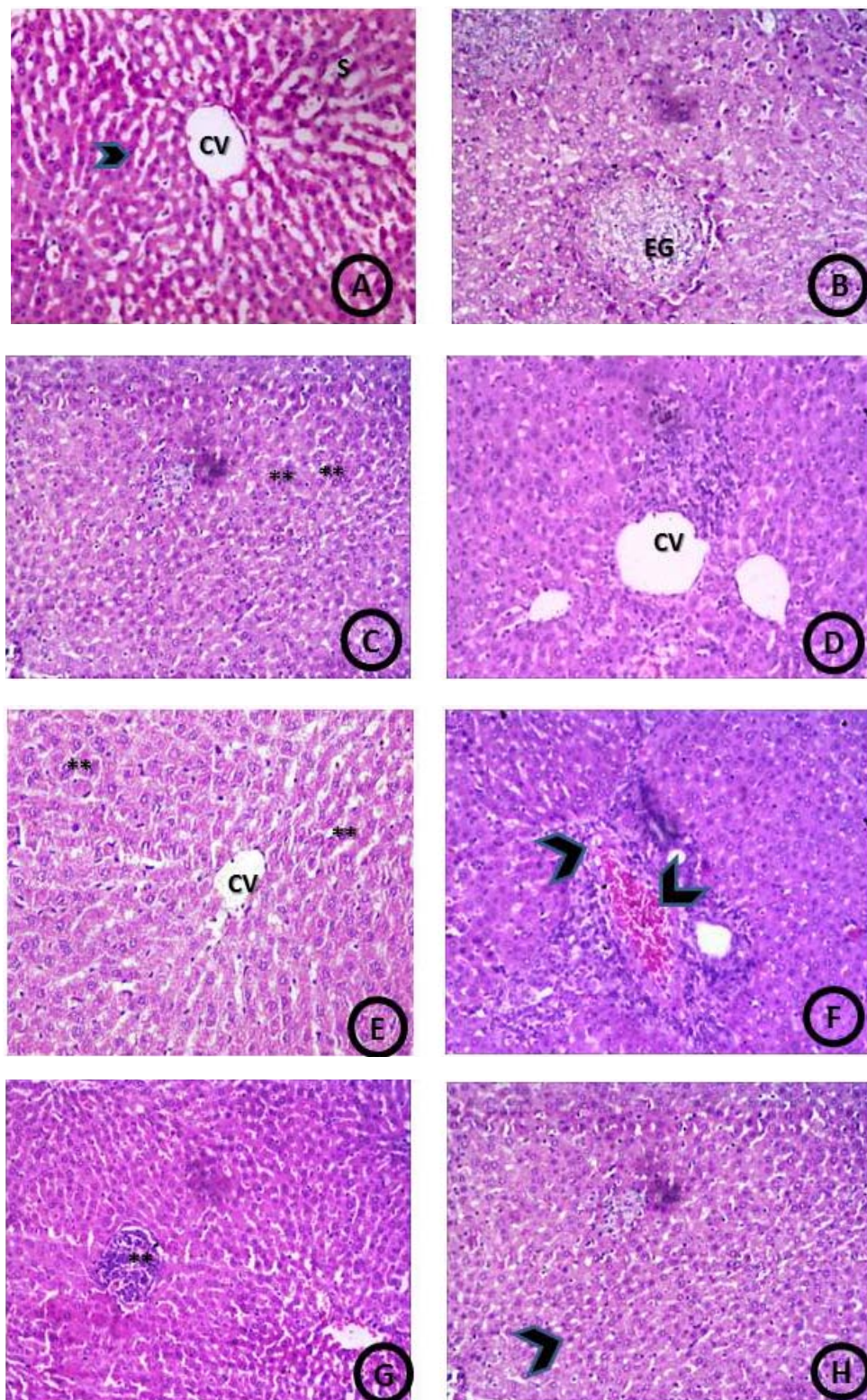


Fig.1(A-H):Photomicrograph of liver sections, (A) A portal area of control group, (B) Liver section of group treated with ACR, (C) A liver section treated with LY (20 mg kg⁻¹), (D) Group treated with RR, (E) Hepatic tissues of group treated with combination of LY and RR, (F) Hepatic tissues of group treated with a combination of ACR and LY, (G) A liver tissue of a group treated with ACR+RR and (H) Hepatic tissues of group treated with ACR and combination of LY and RR

Table 2: Effect of acrylamide, lycopene and red raspberry extract each alone and their combination on changes in hepatic and renal functions in male rats

Parameters	Control group	Acrylamide	Lycopene	Red raspberry	Lycopene+Red raspberry	Acrylamide+Lycopene	Acrylamide+Red raspberry	Acrylamide+Lycopene+Red raspberry	Acrylamide+Lycopene+Red raspberry
ALT (U L ⁻¹)	13.65±1.25 ^f	89.27±2.65 ^a	14.06±1.25 ^{ef}	13.69±1.89 ^f	13.15±0.87 ^f	49.58±4.25 ^c	59.98±3.25 ^b	59.98±3.25 ^b	20.69±3.25 ^d
AST (U L ⁻¹)	18.69±2.65 ^f	78.69±5.69 ^a	19.65±2.36 ^{ef}	18.47±2.08 ^f	18.29±2.25 ^f	48.69±2.58 ^c	49.86±5.25 ^{bc}	49.86±5.25 ^{bc}	23.69±1.25 ^d
ALP (U L ⁻¹)	20.69±1.58 ^f	29.69±4.25 ^a	21.65±3.65 ^e	21.36±2.69 ^e	20.49±1.18 ^f	24.69±2.39 ^c	25.69±2.66 ^b	25.69±2.66 ^b	23.59±2.28 ^d
LDH (U L ⁻¹)	52.36±4.02 ^g	324.69±8.69 ^a	60.25±1.54 ^f	62.05±5.25 ^{ef}	52.16±0.57 ^a	187.69±5.69 ^c	210.69±8.25 ^b	210.69±8.25 ^b	70.25±5.25 ^d
γ-GT (U L ⁻¹)	33.68±1.36 ^d	97.98±5.96 ^a	33.69±3.52 ^d	32.05±3.86 ^e	33.38±0.98 ^d	42.02±3.69 ^c	45.02±3.25 ^{bc}	45.02±3.25 ^{bc}	32.69±1.69 ^e
Total proteins (g dL ⁻¹)	8.09±1.36 ^c	6.38±1.25 ^e	9.05±1.98 ^{ab}	8.89±2.35 ^b	8.49±1.02 ^b	7.01±1.02 ^d	6.98±1.25 ^e	6.98±1.25 ^e	7.69±1.02 ^d
Albumin (g dL ⁻¹)	4.56±0.69 ^c	1.59±0.69 ^g	4.69±0.87 ^{bc}	4.96±0.68 ^a	4.26±0.41 ^c	3.58±1.25 ^e	3.01±0.98 ^f	3.01±0.98 ^f	4.02±0.69 ^d

Values are expressed as Means±SE, n = 8 for each treatment group, ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase, γ-GT: Gamma glutamyl transferase, Significant difference was assigned alphabetically

Table 3: Changes in lipid profile in male rats treated with acrylamide, lycopene and red raspberry extract on male rats

Parameters (mg dL ⁻¹)	Control group	Acrylamide	Lycopene	Red raspberry	Lycopene+Red raspberry	Acrylamide+Lycopene	Acrylamide+Red raspberry	Acrylamide+Lycopene+Red raspberry	Acrylamide+Lycopene+Red raspberry
Triglycerides (mg dL ⁻¹)	60.25±4.65 ^{ef}	109.69±2.58 ^a	59.34±4.25 ^f	61.67±5.66 ^e	60.45±2.25 ^e	89.68±4.25 ^b	79.25±4.25 ^c	79.25±4.25 ^c	68.69±3.69 ^d
Total cholesterol (mg dL ⁻¹)	78.95±3.02 ^{ef}	145.69±4.25 ^a	79.25±6.58 ^e	74.95±5.25 ^f	78.15±2.02 ^e	120.39±5.25 ^b	115.69±4.25 ^c	115.69±4.25 ^c	89.58±4.25 ^d
HDL-c (mg dL ⁻¹)	40.95±2.69 ^b	29.69±5.69 ^d	41.36±4.58 ^a	41.25±3.65 ^a	40.55±2.19 ^b	33.69±3.25 ^c	32.98±2.56 ^{cd}	32.98±2.56 ^{cd}	40.69±5.25 ^b
LDL-c (mg dL ⁻¹)	27.69±2.58 ^{cd}	34.69±4.25 ^a	26.58±2.69 ^d	26.98±5.69 ^d	27.39±2.02 ^{cd}	30.25±4.25 ^b	29.78±3.25 ^c	29.78±3.25 ^c	28.01±3.65 ^c
vLDL-c (mg L ⁻¹)	12.05±1.58 ^e	21.93±2.54 ^a	11.86±1.89 ^f	12.33±1.58 ^e	12.15±1.18 ^e	17.93±3.65 ^b	15.85±2.05 ^c	15.85±2.05 ^c	13.73±2.5 ^{de}

Values are expressed as Means±SE, n = 8 for each treatment group, HDL-c: High density lipoprotein of cholesterol, LDL-c: Low density lipoprotein of cholesterol, vLDL-c: Very low density lipoprotein of cholesterol, Significant difference was assigned alphabetically

Table 4: Changes in serum hepatic antioxidant enzymes and thiol level in male rats treated with acrylamide, lycopene and red raspberry extract on male rats

Parameters	Control group	Acrylamide	Lycopene	Red raspberry	Lycopene+Red raspberry	Acrylamide+Lycopene	Acrylamide+Red raspberry	Acrylamide+Lycopene+Red raspberry	Acrylamide+Lycopene+Red raspberry
Myeloperoxidase (MPO) (nmol min ⁻¹ mL ⁻¹)	12.36±1.07 ^e	37.67±2.62 ^a	12.00±1.36 ^e	12.46±1.69 ^e	12.30±1.03 ^e	25.06±2.36 ^{bc}	24.03±1.25 ^c	24.03±1.25 ^c	13.85±1.01 ^d
Xanthine oxidase (XO) (U g ⁻¹)	14.36±1.09 ^f	41.07±4.36 ^a	14.53±2.36 ^f	15.20±0.36 ^{ef}	14.30±0.38 ^f	25.69±3.03 ^c	26.35±2.02 ^{bc}	26.35±2.02 ^{bc}	17.36±2.03 ^d
Hepatic thiol (μmol g ⁻¹)	10.25±1.36 ^a	3.07±1.03 ^e	10.39±2.03 ^a	10.78±2.34 ^a	10.20±0.87 ^{ab}	5.14±1.56 ^d	7.69±1.96 ^c	7.69±1.96 ^c	9.58±1.69 ^b

Values are expressed as Means±SE, n = 8 for each treatment group, LY: Lycopene, ASP-LD: Low dose of aspartame, ASP-HD: High dose of aspartame, HDL-c: High density lipoprotein of cholesterol, LDL-c: Low density lipoprotein of cholesterol, VLDL-c: Volatile low density lipoprotein of cholesterol, Significant difference was assigned alphabetically

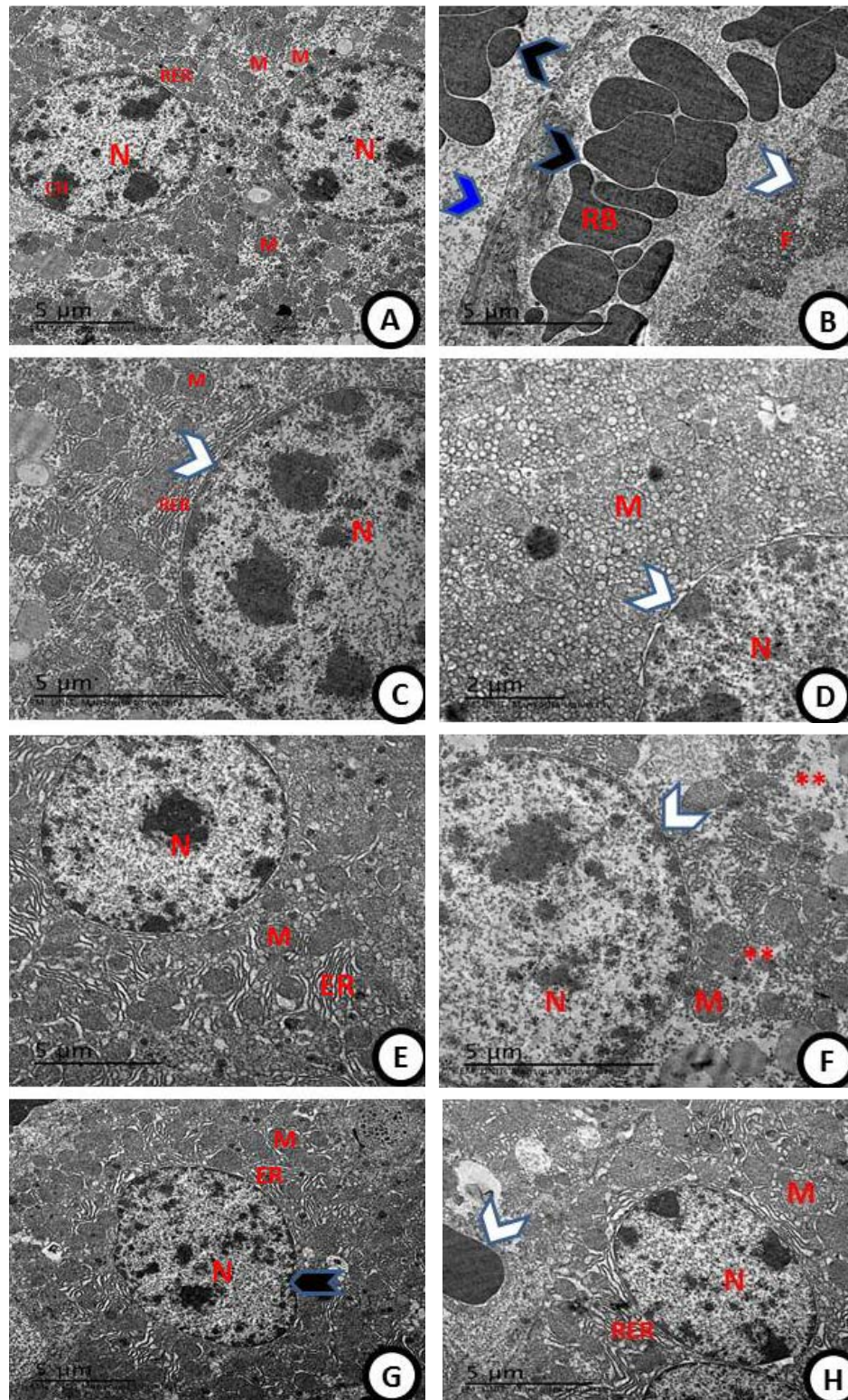


Fig. 2(A-H): Electron micrographs of liver sections, (A) Control group; with normal appearance, (B) An electron micrograph of ACR treated liver, (C) Hepatocytes of LY group, (D) Hepatocytes of RR group, (E) Hepatocytes of group treated with a combination of LY and RR, (F) Hepatocytes of group treated with ACR and LY, (G) Hepatocytes of group treated with ACR and RR and (H) Hepatocytes of RR group

(5 Fm), (E) Hepatocytes of group treated with a combination of LY and RR showing normal appearance of hepatic structures with normal nucleus (N) and normal sized mitochondria (M) with normal endoplasmic reticulum (Normal space) (5 Fm), (F) Hepatocytes of group treated with ACR and LY showing restoration of normal euchromatic nucleus (N) with normal boundaries (white head arrow) and little condensed mitochondria (M) but with some small distingrated parts (5 Fm), (G) Hepatocytes of group treated with ACR and RR showing partial restoration of nucleus structure (N) with little pyknosis area (Black head arrow) with normal endoplasmic reticulum (ER) (5 Fm) and (H) Hepatocytes of RR group showing normal nucleus (N) with regular boundaries (white arrow heads), Endoplasmic Reticulum (ER) with mild large space and normal mitochondria (M) with condensed the haemorrhage area excessively (white head arrow) (5 Fm).

DISCUSSION

The current study concerned with the biochemical, histopathological and ultrastructure changes to evidence that either LY and/or RR has an improvement effect on ACR afforded hepatic toxicity. Increment the levels of AST, ALT, ALP, LDH and γ -GT in serum specified that an injury has taken in the membrane structure of hepatocytes that could be as a result of the presence of free radicals resulted during the oxidative stress. These results are congruent with Ahmed and El-Menoufy³³ found that ACR alter the biochemical parameters of rats and induced DNA damage, inflammation and oxidative stress in rat testes. besides Yousef and El-Demerdash¹³ who noticed that acrylamide exerted damage effects on enzymatic activities and lipid peroxidation depending on it's dose.

Hypercholesterolemia has been considered a risk factor for hepatic injury as all the total cholesterol was changed. The liver is the main organ responsible for cholesterol metabolism³⁴. The changes in lipid metabolism have been observed in ACR groups and this could be due to the evolution of hypercholesterolemia as noticed by Prokic *et al.*³⁵. Jang *et al.*³⁶ found that atherosclerosis was associated with increases in ROS, which responsible for causing cardiovascular diseases as the effect of ACR.

Many pathological alterations extending to cell injury occur if the balance between antioxidants and oxidants cannot be preserved in tissues. Oxidative stress damage with the elaboration of free radicals and overwhelming LPO is the reference to ASP toxicity. In the present study, MDA level in ACR group was elevated significantly and taken by an attendant decline in the activities of antioxidant enzymes

(SOD, CAT, GPx) besides total protein levels in the liver tissue as compared to control rats. These results were similar previously reported by Gedik *et al.*³⁷ who observed that acrylamide administration significantly decreased liver GSH and TAS levels when compared to the control group and it was also observed that AST, ALT, ALP, SOD and CAT activities and TOS and MDA levels increased as a result of acrylamide administration and concluded that acrylamide induced severe liver damage. Who demonstrated that ACR may induce an oxidative stress in the liver of rats. Elevation of LPO levels in ACR-treated rats indicated the generation of ROS and had been used as indirect biomarkers of oxidative stress. The SOD and CAT are significantly decreased in ACR treated groups and they considered being primary defense enzymatic antioxidants that prevent the oxidative damage by free radicals to macromolecules³⁸.

The CAT and GPx protect SOD against inactivation by H_2O_2 . Reciprocally, SOD protects CAT and GPx against superoxide anion through dismutation of endogenous cytotoxic superoxide anion to O_2 and H_2O_2 ³⁹. However, overload of ROS could disturb these reconciliations. This is in parallel with who indicated that formaldehyde exposure leads to a falling of hepatic SOD and CAT activities.

The GSH is considered to be one of the most important non-enzymatic antioxidant in living cells⁴⁰. After ACR treatment, GPx levels decreased hepatocytes which increase cell susceptibility to oxidative stress. In the present study, hepatic GPx is involved in the protection of the organism against ROS.

In the present study, the severe hepatic damage in hepatocytes structure and appeared clearly in TEM sections and histological sections are compatible with Gedik *et al.*³⁷ who proved that histopathological examinations demonstrated inflammatory cell infiltration, hepatocellular necrosis and hemorrhage areas in ACR group liver sections. In agreement with our explanation, acrylamide induced free radicals break down the structure of polyunsaturated fatty acids in cell membranes, especially those associated with phospholipids⁴¹. This leads to a deterioration of cell integrity and later a significant elevation in plasma ALT and AST levels. In the present study, elevated plasma ALT, AST and ALP enzyme levels were observed in acrylamide administered mice liver cells due to liver damage. This could be due to the introduction of enzymes such as ALT, AST and ALP into the circulation, which was induced by the destruction in the liver cell membrane due to oxidative stress and elevated plasma levels. In other studies that reported similar results, it was also observed that these enzymes were elevated in serum after CCl_4 induced liver damage⁴².

Hepatoprotective drugs are used to reduce liver damage caused by hepatotoxic agents such as acrylamide or recover normal values⁴³. It was observed in the present study that either LY and/or RR alleviated the increment in plasma AST, ALT and ALP levels that are considered as application as a hepatoprotective agent. The reduction in ALT, AST and ALP levels was a result of the regulation of liver cell membrane integrity by either LY or RR and appear synergistically in both combined compounds due to their antioxidant and free radical scavenging properties. This clearly demonstrated the hepatoprotective effect of LY and RR.

The results of the present study was harmony with previous studies where the AST, ALT and ALP levels, which were elevated with hepatotoxicity induced by sodium fluoride⁴⁴ in animal models were reduced significantly with black berry administration which is similar to the current study.

The ultrastructure of hepatocytes that treated with ACR showed reduced size mitochondria and vacuoles in rough endoplasmic reticulum in a dose-dependent manner. Moreover, the appearance of severe haemorrhage, which is a real indication of damaging effect induced by ACR. Furthermore, the changes in the mitochondria could be taken as an early sign of apoptosis; this observation could be secondary to the elevation of LPO. The reduction of total protein could be attributed to the damage of the endoplasmic reticulum, which results in P450 cytochrome enzymes loss leading to depletion in protein and aggregation of triglycerides leading to the fatty liver⁴⁵. The LY and/or RR with ACR reinforced the synthesis of TP which protect the hepatocytes and indicates the hepatoprotective activity of either LY and/or RR.

These biochemical changes were supported by the current histological observations that go in line with previous studies on the hepatotoxic effect of ACR 36. The inflammatory response may be due to the increasing the production of ROS in the liver.

Lycopene (LY), a bioactive compound, is a fat soluble naturally carotenoid pigment that is abundant in red foods such as tomato, pink grape fruit and watermelon. The structure of LY (C40H56) is an acyclic isomer of β -carotene with a 40 carbon polyisoprenoid chain and 11 conjugated double bond structure⁴⁶.

The LY is one of the most effective antioxidants in the carotenoid family and its activity against biological reactive oxygen species may prevent or ameliorate oxidative damage to cells and tissues both *in vivo* and *in vitro* and LY has been shown to have the ability to reduce the risk of cancers, such as breast, prostate and gastric cancer¹⁰.

The significant elevations of ALT, AST, LDH and ALP in ACP groups were decreased in treated group with LY and ACR. The reversal of increased serum enzymes in ACR induced liver damage by LY may be due to the obstruction of the infiltration of enzymes by membrane stabilizing³⁴.

Red Raspberries are receiving highly interest as a healthy functional food, because their regular consumption has been reported to decrease the risk of chronic diseases⁴⁷. The nutritional and health benefits of red raspberries are usually attributed to their chemical composition. Fruits contain a wide range of phytochemicals including anthocyanins and other flavonoids, flavonols, tannins and phenolic acids.

The ACR induced hepatotoxicity and severe oxidative stress and this effect was alleviated by using Red Raspberry (RR) and previously. The effect of red raspberry was assessed in obesity-prone, Zucker Fatty rats as a model of cardiometabolic risk. The RR reduced fasting triglycerides and fasting glucose, low-density lipoprotein or body weight gain. The RR did significantly reduce heart rate relative to time-matched CON rats³⁸ and this explain the effect of RR on improving liver biomarkers as ALT, AST, ALP, LDH and lipid profile picture as reducing triglycerides level, LDL-c, vLDL-c and improving HDL-c and all the mentioned parameters reflect the effect of RR on hepatic structure and enhancing antioxidant capacities by elevating SOD, CAT, GPx and reducing marker of lipid peroxidation (MDA).

The histological observations supported the results of biochemical parameters. There was a significant encoring of these biomarkers on the administration of the LC.

The histological results showed that treatment of liver with LY or RR and ACR showed an improvement in hepatocytes. Therefore, it is possible that these antioxidant compounds could scavenge free radicals and produce advantageous effects against ACR damage in the liver.

There is still very mild damage in the liver at the cellular level in rats treated with either LY or RR and ACR that could be a result of the increase of ROS production as well as decreasing the intrinsic antioxidant by ACR in the cells. In addition to this, ACR caused DNA damage. The amelioration in hepatic ultrastructure in groups treated with the combination of ACR and LY and RR was noticed very well and this support the using of the two compounds together to alleviate the hepatotoxicity of ACR.

CONCLUSION

The study clarified that ACR causes Hepato-damage and then affects their functions as specified by remarkable biochemical besides histological and ultrastructural changes.

The results proved that the antioxidant status decrement is one of the main agents participating in ACR intoxication in liver tissue. The LY and RR combined synergistically induced improvement in these alterations and reduced the ACR injury. Therefore, intake of ACR could be limited and recommended with either LY and RR when it is used with especially fast food or highly cooked food to decrease its toxicity.

SIGNIFICANCE STATEMENT

This study discovers the hepatoprotective effects of both lycopene and red raspberry against oxidative stress induced by acrylamide. The current study reported a novel mechanism of ameliorative effect of either lycopene and/or red raspberry against especially hepatocellular alterations induced by acrylamide as many peoples exposed to this compound which are exceedingly produced during cooking food in very high temperature as fried or roasted food and almost fast food which is consumed excessively and thus giving these peoples either lycopene and/or red raspberry supplementation will protect them against these alterations with enhancing hepatic function biomarkers. Therefore, these results clearly demonstrate a protective role for both lycopene and red raspberry against hepatotoxicity induced by Acrylamide.

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