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The Potential Effect of Fruits and Vegetables on Liver Functions and Liver Alterations Induced by Acrylamide in Mice

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Abstract. The study aimed to assess the effect of some dried fruits and vegetables on liver functions and alterations against acrylamide that administered for Swiss adult male albino mice. A total of 49 mice (25±2g) were divided to seven groups. First group was considered as negative normal. The remaining mice were subjected for oral administration of 40 µg acrylamide / kg body weight daily for 8 weeks. Group 2 was considered as positive control. First and Second groups were fed on basal diet. Groups 3, 4, 5, 6 and 7 were given basal diets with 20% of raisins, apricot, figs, tomato and carrot, respectively. Inverse associations were observed between the consumption of vegetables and fruits and liver changes. These diets significantly reduced the activity of transaminases (ALT and AST) and Liver histopathological alterations compared to positive control.

Keywords: fruits, vegetables, liver function, histopathological alterations, acrylamide

1. Introduction

Up to 3-fold variation in colorectal cancer incidence across Europe, with an estimate of 372,000 new diagnoses and 203,000 deaths in 2002 was reported [1]. The estimates from the American Cancer Society and the International Union Against Cancer indicated that 12 million cases of cancer were diagnosed, with 7 million deaths worldwide that would be expected to double by 2030 (27 million cases with 17 million deaths) as noted in ref. [2]. In Egypt, liver cancer accounted to12.8% according to Age-Standardized Incidence Rates during the period from 1996 to 2001 [3].

Diets rich in fruits and vegetables reduced cancer risk, but definitive controlled trials on the prevention of specific cancers by antioxidants and phytochemicals were not completed [4]. Compelling evidence have been continue to strengthen the link between diet and cancer [5]-[8]. A variety of components in plant foods included micronutrients, polyunsaturated fatty acids, and secondary cancers. Consumption of vegetables, mainly onion and garlic, reduced intestinal stomach cancer risk but not associated with lung, prostate, and breast cancers [9]. Nevertheless, the review by an international panel of experts provided a limited evidence of this association [10].

Dietary factors accounted for about 35% of cancer deaths in the United States. Recent meta-analyses suggested an association of colorectal, prostate, and brain cancers with certain dietary patterns. Not only may food components be associated with cancer risk, but cooking methods, the direct impact of food on the human gastrointestinal mucosa, and individual susceptibility to dietary carcinogens could significantly increase cancer risk [11].

2. Materials and Methods

2.1. Materials

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Dried Fruits, Figs (*Ficus carica*) were purchased from Almarim Co. for Trading, Apricots (*Prunus mume*) from Badr Eldin Co. for Trading , and Raisins (*Vitis spp*) from Kiker Co. for Food Industry. Fresh vegetables, Carrot (*Caucus carota*), and Tomato (*Lycopersicum esculentum*) were purchased from local market Giza, Egypt.

Acrylamide was obtained from Sigma-Aldrich Laborchemikalien GmbH (Milwaukee, WI). Minerals, casein, cellulose, starch, vitamins, and ascorbic acid were purchased from El-Nasr Pharm. & Chem. Ind. Comp. Cairo, Egypt.

A total of 49 adult male Swiss albino mice strain with average body weight of 25 ± 2 g were obtained from the animal house of Research Institute of Ophthalmology, Ministry of Scientific Research, Giza, Egypt. Rats were caged individually in wire bottomed stainless steel cages and kept under normal healthy laboratory conditions at constant temperature (22 ℃ - 24 ℃). Water was consumed adlibitum.

2.2. Methods

2.2.1. Preparation of the raw materials

Carrot and tomato were separately washed with tap water, chopped into small pieces and blanched with water vapour then (freeze drying) according to ref. [12]. The dried materials were separately milled into powder and sieved through 100-mesh sieve, then packed in polyethylene bags at 4 ℃ till used.

2.2.2. Chemical analysis

Moisture, protein, crude fiber, fat and ash content of the prepared formulas were determined according to the method described in ref. [12]. Total carbohydrate was calculated by difference.

2.2.3. Biological evaluation

For biological evaluation 49 male mice were individually housed in stainless steel wire-bottom cages under hygienic condition and fed on a basal diet for three weeks. Basal diet was composed according to [12]. After adaptation period animals were randomly divided into seven groups, (each comprised of 7 mice). Group 1 served as normal rats where fed on basal diet (BD) during the study period (8 weeks). The first group (7 rats) was considered as negative control (group 1). The other six groups (42 mice) were subjected for daily oral administration of acrylamide (40 µg/1kg body weight) daily for 8 weeks. The second group was considered as positive control fed on basal diet (BD) throughout the experimental periods. The other five groups were given diets with the different formulas. These groups (3, 4, 5, 6 and 7) were fed on basal diet with 20% of raisins (RD), dried apricot (DAD), dried figs (DFD), dried tomatoes (DTD), and dried carrots (DCD), respectively. The compositions of experimental diets are shown in Table I.

Table I: Composition of experimental diets

Ingredients	Experimental diets (g/kg)					
	Basal diet (BD)	Raisins (RD)	Dried Apricots (DAD)	Dried Figs (DFD	Dried Tomato (DTD)	Dried Carrots (DCD)
Casein	140	132.38	132.38	132.58	111	129.14
Soy bean oil	40	36.19	36.19	36.29	39.02	37.74
Sucrose	100	100	100	100	100	100
Salt mix.	35	31.28	29.22	30.82	14.98	24.68
Vitamin mix.	10	10	10	10	10	10
Cellulose	50	-	-	-	-	-
Corn starch	620.7	485.85	487.91	486.01	520.7	494.14
Raisins	-	200	-	-	-	-
Dried Apricots	-	-	200	-	-	-
Dried Figs	-	-	-	200	-	-
Dried Tomato	-	-	-	-	200	-
Dried Carrots	-	-	-	-	-	200

After elapse of experimental period, rats were fasted 12 h, blood samples were with-drawn from eye vein samples were left to clot and centrifuged for 15 minutes at 5000 rpm to obtain serum, and then put into dry clean Wasserman tubes, using a Pasteur pipette and kept at −20°C in clean glass well-stoppered vials till analysis. Then the mice were anaesthetized and sacrificed. Serum aspartate aminotransferase (sAST) and serum alanine aminotransferase (sALT) activities were measured colorimetrically [13].

2.2.4. Histopathological changes of examined organs

Examinations of liver samples were histopathologically examined at the Histology Laboratory, Faculty of Veterinary Medicine, Cairo University according to the method [14].

2.2.5. Statistical analysis

The data were expressed as means ± S.D. The statistical analysis was performed using SPSS for Windows (SPSS, Inc.). P values less than 0.05 was considered to be significant [15].

3. Results and Discussion

3.1. Liver Functions

Serum AST and ALT values as illustrated in Table II showed some significant differences ($P<0.05$) among the experimental groups. The positive control had the highest significant values. The increases in the liver enzymes following liver damage in albino mice were noticed confirming those of ref. [16], [17].

Table II: AST and ALT in mice of the different experimental groups

Liver enzymes (µ/L)	Groups						
	NC	PC	RD	DAD	DFD	DTD	DCD
AST(GOT)	78 ± 2.64^{b}	86 ± 2.64^{a}	62 ± 1.73^{c}	54 ± 2.64^{d}	58 ± 3.60^{cd}	37 ± 2.64^{e}	61 ± 3.60^{c}
ALT(GPT)	126 ± 1.00^{a}	127 ± 2.00^{a}	101 ± 1.73^{b}	86 ± 3.60^{d}	96 ± 1.73^{c}	64 ± 2.64^{e}	100 ± 2.64^{bc}

NC: negative control, PC: positive control, RD: basal diet with 20% raisins, DAD: basal diet with 20% dried apricots, DFD: basal diet with 20% dried figs, DTD: basal diet with 20% dried tomatoes, and DCD: basal diet with 20% dried carrots. Values is expressed as means ± SD. Means with the different letter superscripts in the same row denote significance at $P < 0.05$.

3.2. Histopathological Examination

In liver, there was no histopathological alteration observed and the normal histological structure of the central vein and surrounding hepatocytes were recorded in (Figure 1). However, very severe alterations were observed in positive control (Figure 2 and Figure 3). Fatty change was observed in the hepatocytes surrounding the dilated central veins (Figure 4). There was no histopathological alteration in liver (Figure 5). Diffuse kupffer dells proliferation and inflammatory cells infiltration were detected inbetween the karyocytomegalic hepatocytes in liver (Figure 6). Few inflammatory cells infiltration was detected surrounding the central vein with degeneration in the hepatocytes (Figure 7). In liver, the hepatocytes showed swelling and ballooning degeneration (Figure 8).

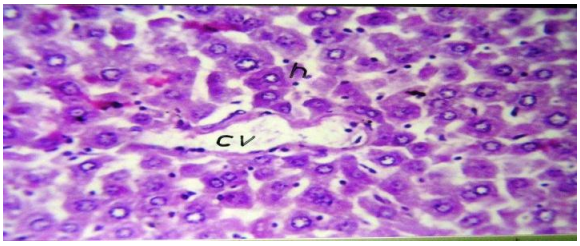

Fig. 1: Liver of mice in group 1 (NC) showing normal histopathological structure of the central vein (cv) and surrounding hepatocytes (h) (H and E X 80).

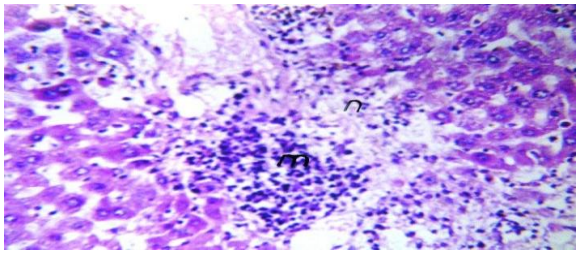

Fig. 2: Liver of mice in group 2 (PC) showing focal inflammatory cells aggregation (m) in the degenerated necrosed hepatocytes (n) (H and E X 40).

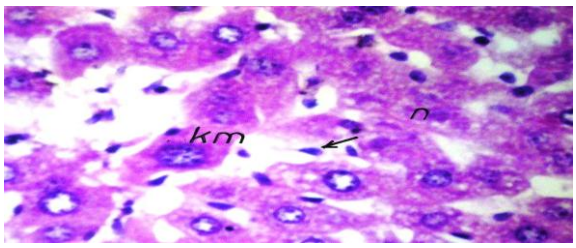

Fig. 3: Liver of mice in group 2 (PC) showing kupffer cells proliferation (arrow) in between necrosed (n) and karyocytomegaly (km) hepatocytes (H and E X 160).

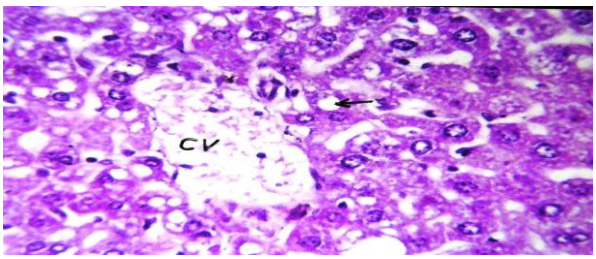

Fig. 4: Liver of mice in group 3(RD) showing Fatty change in the hepatocytes (arrow) surrounding the dilated central veins (cv) (H and E X 80).

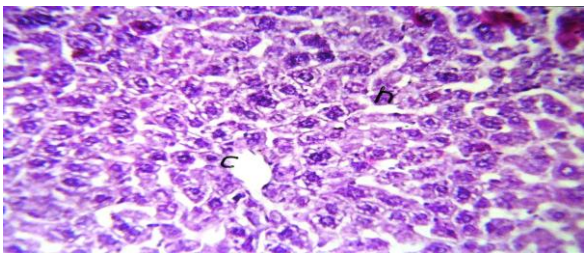

Fig. 5: Liver of mice in group 4 (DAD) showing normal histopathological structure of the central vein (cv) and surrounding hepatocytes (h) (H and E X 64).

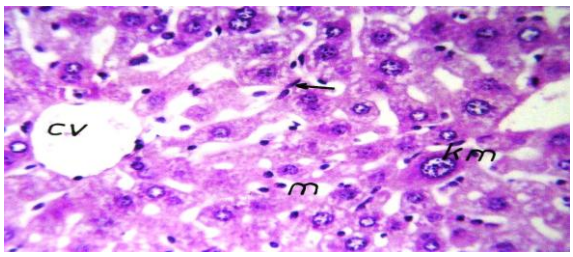

Fig. 6: Liver of mice in group 5 (DFD) showing Diffuse kupffer cells proliferation (arrow) and diffuse inflammatory cells infiltration (m) in-between the karyocytomegalic (km hepatocytes (H and E X 80).

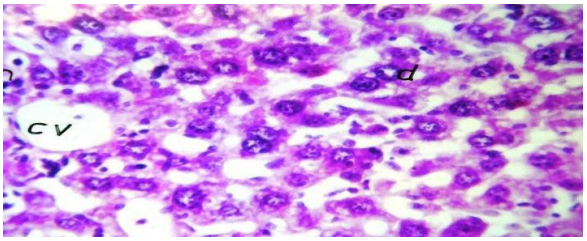

Fig. 7: Liver of mice in group 6 (DTD) showing inflammatory cells infiltration (m) surrounding the central vein (cv) with degeneration in the hepatocytes (H & E X 80).

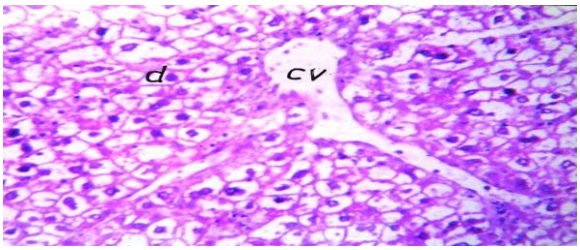

Fig. 8: Liver of mice in group 7 (DCD) showing ballooning degeneration in the hepatocytes (d) (H and E X 160).

Very severe effects of acrylamide adminestration on liver tissues in the positive . However, other groups fed on diets with dried fruits and vegetables demonstrated normal histopathological structures (group 4 fed on diet with apricots) or mild alterations in liver. adding dried fruits and vegetables to the diets ameliorated the effect of acrylamide and delayed its determenal damege on different tissues in mice. These findings might be related to lycopene and phenols in such diets. The purified fraction with the major glycolipids from some dried vegetables (included carrots) was an inhibitor of DNA polymerase α (pol α) in vitro and the proliferation of human cancer cells [18]. The anti-neoplastic effect might be exerted by induction of apoptosis and autophagy [19] showing the anti-neoplastic effect of MK615 , an extract from the Japanese apricot (*Prunus mume*), against colon cancer cells. The effects of apricot and β-carotene treatment might protect from the of oxidative stress impairment on methotrexate (MTX) induced intestinal damage in rats. It has been significantly more interest in plant and naturally available compounds for chemoprevention [20].These assured that apricot-rich diet ameliorated the oxidative status and detrimental effects of low-dose x-rays on in tubular histology of histopathological structures of different tissues [21]. adding dried fruits and vegetables to the diets ameliorated the effect of acrylamide and delayed its determenal damege on different tissues in mice. testis tissue due to the natural antioxidant activity and prevented the damage. Lycopene was also associated with a reduced risk of prostate cancer and decreased cell apoptosis rates [22].

4. Conclusion

Severe or very severe effects of acrylamide adminestration in the treated tissues of the positive control were noticed. However, in the other groups fed on diets with dried fruits and vegetables demonstrated that the severity of alterations was different (normal, mild and moderate).

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