



Acrylamide Levels in Some Food Products in Egyptian Market and Protective Effect of Extra Virgin Olive Oil

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ACRYLAMIDE (ACR) is one of the most important contaminants occurring in foods heated at high temperatures. The aim of this study is to study the different levels of acrylamide in some food products in the Egyptian market and investigate the protective efficacy of extra virgin olive oil (EVOO) against hepatotoxicity induced by ACR. Forty-eight male rats were divided into eight groups. Group A1 was fed on a basal diet as a control group. Groups A2, A3, and A4 were fed on diets containing ACR at 500, 750, and 1000 µg/kg BW rat/day, respectively. The other groups (A5, A6, and A7) were fed on the same diet used in groups A2, A3, and A4, respectively, and supplemented with EVOO (0.5 mL/kg BW rat/day). Group A8 was fed a basal diet and supplemented with extra virgin olive oil (0.5 mL/kg BW rat/day). After 28 days, the rats fed with EVOO and ACR rats significantly decreased their levels of glucose. The treatment also resulted in a significant improvement in the lipid profile, liver functions, kidney functions, and total antioxidants. However, EVOO significantly increased the activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPS) in the blood of ACR rats. The treated groups showed a significant decrement in thiobarbituric acid-reactive substances like malondialdehyde (MDA) in their serum. Treatment with EVOO reduces the histopathological and liver abnormalities associated with ACR since the study of the induction of redox enzymes is considered to be a reliable marker for evaluating the antiperoxidative efficacy of EVOO.

Key words :Extra virgin olive oil (EVOO), Phenolic component, Acrylamide (ACR), Rats, Liver Function, Kidney Function, Histopathological.

Introduction

Acrylamide (ACR) is a chemical compound that is frequently utilized in the production of polyacrylamides and other industrial products. ACR is formed naturally during the Maillard browning reaction between reducing sugars (glucose and fructose) and free amino acids, mainly asparagine, in addition to industrial and laboratory applications (Krishnakumar & Visvanathan, 2014). (Fodor et al., 2015) found high levels of ACR in foods heated at high temperatures, especially potato products such as

French fries, potato chips, cereals, and roasted coffee. ACR accumulates at higher levels in the blood than in any other tissue after exposure via oral consumption or inhalation (Ghorbel et al., 2017). CYP2E1 can quickly distribute it to all tissues and convert it to glycidamide (GA), a more poisonous form than ACR (Ghanayem et al., 2005). The latter is one of several CYPs that have been identified as causing bio-activation while metabolising a variety of exogenous substances, including ACR. GA can be converted to glyceramide by hydrolysis or glutathione conjugation, resulting in two mercapturic acid

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metabolites discharged in the urine (Fennell et al., 2005). Furthermore, ACR affects biological molecules by interacting with their vinyl group, SH, and NH₂ of proteins, mainly haemoglobin (Ghorbel et al., 2017). It has been shown to be neurotoxic, genotoxic, and carcinogenic. In rats, ACR has been shown to cause disturbances in redox status and enzyme activity changes (Abd Eldaim & Hassan, 2015). Increased oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and antioxidant capacity, which plays a key part in the toxicity caused by ACR (Zhao et al., 2015). In order to combat oxidative stress, antioxidants and plant phenolics are regarded as chemoprotective agents against oxidative stress-related disorders. Extra virgin olive oil (EVOO) has long been recognized for its antioxidant qualities and anti-oxidant benefits (Rossi et al., 2017). Previous research has shown that EVOO can change the shape of cellular membranes and prevent oxidative damage (Ghorbel et al., 2017). The high oleic acid content of olive oil, as well as its great antioxidant potential due to the presence of phenolic compounds, has been credited with its favourable health effects (Gorzynik-Debicka et al., 2018). EVOO appears to be a functional food with nutritional benefits due to numerous components, such as monounsaturated fatty acids (MUFA). It contains 80% omega-9 MUFA, oleic acid, and other minor ingredients including aliphatic alcohols, sterols, and polyphenols (α -tocopherols, tyrosol, oleuropein, and hydroxytyrosol) (Schwingshackl & Hoffmann, 2014).

Ethephon (EP) may cause nephrotoxicity and kidney impairment by increasing oxidative stress and causing renal tissue necrosis. However, pretreatment with virgin olive oil (VOO) produced renoprotective effects against EP due to its antioxidant and free radical scavenging characteristics. As a result, it's possible that VOO could be a beneficial dietary supplement for EP-exposed individuals (Mokhtari, et al., 2020).

According to the World Health Organization (WHO), total daily acrylamide consumption in various European Union countries ranged from 0.3 to 2.0 $\mu\text{g}/\text{kg}$ body weight. The maximum limits for acrylamide content in food products have not yet been determined. In accordance with European Union legislation, the Environmental Protection Agency (EPA) regulations in the USA say the maximum acrylamide level is 0.5 $\mu\text{g}/\text{dm}^3$

(official Journal of the European Union No 330 of 5 December 1998; European Council Directive (98/83/EC) (Zyzelewicz et al., 2010).

Consumer advocacy group Safe Food Advocacy Europe (SAFE) is calling for the EU to enact mandatory acrylamide limits after several Italian potato crisp brands were tested as containing more of the potential carcinogen than recommended. In 2017, the EU enacted a new regulation setting an acrylamide benchmark level of 750 $\mu\text{g}/\text{kg}$ of product and making mitigation (Serena, 2018). The aim of this work is to identify acrylamide levels in some consumable food products in Egyptian markets and investigate the protective effect of extra virgin olive oil (EVOO) and examine the nutritional and protective effects of EVOO against liver damage and histological changes in ACR treated rats at different levels, including the recommended level of acrylamide (750 $\mu\text{g}/\text{kg}$) by Serena, (2018).

Materials and Methods

Materials

Acrylamide was purchased from Merk-Schuchardt Chemical Company (Hohenbrunn, Germany), with purity of 99%. Albumin used as white egg albumin and cysteine were obtained from Sigma Chemical Company. All other chemicals were of analytical grades.

Methods

Preparation of sample for HPLC analysis

The samples were prepared according to the procedure given by Gökmen & Enyuva (2006).

Analysis of the Acrylamide (ACR) using HPLC-UV:

A high-performance liquid chromatography (HPLC) system equipped with a variable wavelength detector was used (Agilent Technologies, 1200 series, Berlin, Germany). The HPLC was also provided with an autosampler, quaternary pump degasser and column compartment set at 35 C. Analyses were performed on a C18 reverse phase (BDS 5 lm, Labio, Czech Republic) packed in stainless-steel column (4 x 250 mm, I.D.).

Refractive index, Acidity, peroxide value, UV absorption characteristics, K₂₃₂nm (conjugated dienes) and K₂₇₀nm (conjugatedtrienes) and ΔK [$\Delta K = k_{270} - (k_{266-4}) + (k_{274+4})/2$] were carried out following the analytical methods described by A.O.A.C. (2016).

Determination of fatty acids profile

Fatty acids were carried out by preparation of methyl ester followed by the identification of methyl esters using an Agilent 6890 series gas chromatograph apparatus equipped with a DB23 (60 m × 0.32) (ISO, 2011).

Total phenolic content

Total phenol content was calorimetrically quantified (Ranalli et al., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (20 mL) with 30 mL of a methanol-water mixture (60:40, v/v). The Folin-Ciocalteu reagent was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725 nm was measured. Values are given as milligrams of gallic acid per kilogram of oil (Gutfinger, 1981).

Animals

Forty eight male Albino rats, average weight of 160 ± 0.5 g were obtained from the animal house of the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. The rats were kept in normal healthy laboratory condition (temperature was adjusted at 25 ± 2 °C for 12 h, light – dark and Animals were adapted on free access of water) and were fed for one week basal diet before the initiation standard of the experimental. The Composition of the basal diet (g/kg) : Casein, 21.7%; sunflower oil, 15%; corn starch, 58.1%; cholic acid 0.2%; salt mixture, 4% and vitamin mixture, 1% according to Campbell (1963); Salemi & Kamall (2012), respectively.

Experimental design

Eight equal groups six rats each were housed in wire cages in an equal room temperature of 25 ± 2 °C with normal healthy conditions maintained. Food consumption was monitored, and the weekly weight gain was determined. The first group of rats (A1) was fed on basal diet and was considered as control negative group (normal control). The other six groups were given ACR. Groups A2, A3 and A4 fed on diets containing ACR at (500, 750 and 1000 µg/kg BW rat/day, respectively) the other groups A5, A6 and A7 fed on the same diet used in group A2, A3 and A4, respectively and supplemented with extra virgin olive oil (0.5 mL/kg BW rat/day). Group A8 fed on basal diet and supplemented with extra virgin olive oil (0.5 mL/kg BW rat/day).

Growth of rats

The gained weight was calculated by: the final body weight – the initial body weight.

FER = Body weight gain / Food intake.

Biochemical assay

When the 28 days was finished (the period of experimental), the blood samples were collected from the eye plexuses of the animals. Then they were put into a dry clean centrifuge glass tube without any coagulation to prepare serum. The samples were left for 15 min at normal of the temperature of room, after that the tubes were centrifuged for another 15 minutes at 300 rpm. From then until the time of analysis the clean supernatant serum was kept frozen at -20 °C. Determination of serum glucose level was done by Trinder (1969). Methods of Watson (1960); Assmann (1979); Fossati & Prencipe (1982) and Wallach (2007) described the determination of the levels of Total cholesterol, high density lipoprotein, low density lipoprotein, very low-density lipoprotein (VLDL- cholesterol) and triglycerides, respectively. Liver function: Alanine aminotransferase and aspartate aminotransferase activities were described methods of Bergmeyer & Harder (1986). Alkaline phosphatase activity was measured at 405 nm of paranitrophenol from para-nitrophenylphosphate of the formation as a substance using the method of Varley et al., (1980). Kidney function: creatinine was measured using the method of Henry (1964) urea was measured using the method of Fawcett & Scott, (1960) while uric acid was measured using the method of Caraway (1955). The activity of lipid peroxidation level (Malondialdehyde, MDA) was determined in serum by the colorimetric method described by Meltzer et al. (1997). Glutathione reduced (GSH), glutathione peroxides (GSH-PX) superoxide dismutase (SOD) and Catalase were measured calorimetrically in erythrocyte according to the method of Ellman, (1959); Nishikimi et al., (1972) and Aebi, (1984), respectively.

Histopathological examination

At the end of the experiment samples from livers of the rats of all groups were collected and fixed in 10% neutral buffered formalin, the samples were dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4 µ thick Hematoxyline and eosin stained sections were prepared (Yoon et al., 2001).

Molecular docking

Auto Dock tools 4.2 by docking computations applying Gasteiger partial charges added to ligand (designed drug) atoms were used. On the ligand–protein pattern, the calculations were executed. Non-polar hydrogen atoms were conjoined, and

rotatable bonds were clarified. After the addition of fundamental hydrogen atoms, Kollman united atom type charges and salvation parameters the Auto Dock tools were applied (Abdelsalaam et al., 2019). Determining van der Waals and electrostatic terms was performed by Auto Dock parameter set and distance-dependent dielectric functions, respectively. Simulative docking was executed by Solis & Wets local search method and Lamarckian genetic algorithm (Dobbs & Hehre, 1987). Incidentally, initial position, orientation and torsions of the ligand molecule were set.

Statistical analysis

The obtained results were subjected to statistical analysis using the standard analysis of variance as outlined by Snedecor & Cochran (1980).

Result and Discussion

Quality indices of extra virgin olive oil

The physicochemical properties of olive oil are presented in Table 1. The refractive index (RI) has an important role in the identification of the nature of oils due to differences in saturation, unsaturation and chain length of fatty acids. (Ibrahim, 2012) The RI of olive oil sample was 1.4670.

Free acidity is an important quality factor and has been extensively used as a traditional criterion for classifying olive oil in various commercial grades. Free acidity value of olive oil was 0.25. The FFA value of the oil was lower than 0.80, which is met the limits set for extra virgin olive oil quality (IOC, 2015).

The oxidative state of oils is determined using the peroxide value and specific extinction at 232 and 270 nm, respectively. The peroxide value (PV) of oil is a valuable index to determine oil quality. If the peroxide value becomes higher than 20 meqO₂/kg oil, it indicates oxidative corruption in oil (IOC, 2015). As seen in Table 1, the amount of PV in studied variety is 4.76 which represent good extraction and maintenance conditions. This result indicates that olive oil can be stored for a long time without deterioration since oils become rancid when the peroxide value ranges from 20 to 40 meqO₂/Kg oil (Nehdi, 2011).

The specific extinction coefficients at 232 nm, 270 nm and ΔK are related, respectively, to the degree of primary and secondary oxidation of the oils and thus directly correlate to the amount of peroxide (Maskan & Bağcı, 2003 and Ku & Mun,

2008). The values of K232 (1.21), K270 (0.028) and ΔK – 0.0041, respectively. These values were within the limits specified by (IOC, 2015).

Fatty acid composition of extra virgin olive oil

Extra virgin olive oil (EVOO) is widely used in human diets, particularly in the Mediterranean (MED), and has long been known for its numerous health-promoting characteristics. (López-Miranda et al., 2010 and Caramia et al., 2012). Its consumption has been linked to a lower risk of various chronic conditions (Montani et al., 2004). These health benefits are due to the presence of bioactive substances such as phenolic compounds as well as the high MUFA concentration. Olive oil contains a high amount of oleic acid, which makes it a healthy fat when compared to other vegetable oils (Molina-Garcia et al., 2017). Table 1 shows the fatty acids composition of olive oil. This oil was found to contain a high level of oleic acid (70.30%), palmitic acid (15.23%) linoleic acid (6.10%), palmitoleic acid (2.80%), and stearic acid (4.11%), respectively. High oleic acid in olive oil makes it desirable in terms of nutrition and high stability during cooking and frying. Therefore, olive oil with a high proportion of oleic acid is more stable than other oils. In addition, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the linoleic acid. In addition, linolenic acid not exceeds 1% as a polyunsaturated fatty acid whereas arachidic acid represents 0.34 %. In the same context, the values of the total saturated fatty acids, total monounsaturated fatty acids and total unsaturated fatty acids of the olive oil were 19.73%, 73.17% and 7.10%, respectively. These results are in agreement with those reported by Boukachabine, (2011).

Total phenol contents of extra virgin olive oil

The amount of phenolic compounds in extra virgin olive oil is an important factor when evaluating olive oil quality, given that the natural phenols improve its resistance to oxidation and the nutritional properties and flavor of olive oil (Anastasopoulos et al., 2011; Dag et al., 2011).

Phenolic compounds have been proved to be responsible for antioxidant activity; it is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Total phenols content of olive oil was 300 mg /kg. This result was found in agreement with results reported by Boukachabine, (2011).

TABLE1. Physicochemical properties and fatty acid composition of extra virgin olive oil

Physicochemical properties	Olive oil
Refractive index at 25 ^o C	1.4679
Acidity(%as oleic acid)	0.25
Peroxide value (meq.O2/Kg oil)	4.76
UV absorbance at K232nm	1.21
UV absorbance at K270nm	0.028
DK	-0.0041
Total phenol mg /kg	300
Fatty acidscomposition %	
C16:0	15.23
C16:1	2.80
C17:0	0.05
C17:1	0.07
C18:0	4.11
C18:1	70.30
C18:2	6.10
C18:3	1.00
C20:0	0.34
SFA	19.73
MUFA	73.17
PUFA	7.1

Acrylamide content in analyzed investigated food products

It is obvious that acrylamide is formed in many types of food cooked at high temperatures. Acrylamide was detected in high amounts in fried and baked starch-based foods. Such foods constitute the bulk of dietary intake and they are now widely spread globally. Consequently, concern about acrylamide has become a priority issue for most countries (Naewbanij, 2002). At those times, nothing was known about the sources of acrylamide in foods. According to the data published thus far, its formation requires a temperature greater than 100 °C (Becalski et al., 2003).

Acrylamide is not found in natural (raw) foods like raw potatoes. It is formed during the heat processing (heat preparation) of carbohydrate-rich foods at temperatures of 120°C (248°F) or higher, as in frying, broiling, baking, roasting, grilling, and toasting (Tareke et al., 2002).

Acrylamide content in all sample types studied are shown in Table 2. It was noticed that the highest acrylamide level values was in potato chips (1400

µg/kg) which was a highly value compared with all of the other samples, followed by Rosted chips snack product (1034 µg/kg, followed by breakfast cereal (1000 µg/kg) followed by tortilla chips (890 µg/kg) followed by Bread bites (603 µg/kg) on the other hand the lowest value found in bread (111 µg/kg)

These results are in agreement with Youssef et al. (2004) and Arribas-Lorenzo & Morales, (2009).

Effect of EVOO on Initial weight, Final weight, net gain, Food intake and relative liver weight induced by ACR in rats

Effect of feeding on ACR for 28 successive days with or without mixed with EVOO (0.5 mL/kg BW rat/day) on rat body weight gain, and the results were reported in Table 3. Table 3 shows that the initial body weights did not differ significantly among the groups, and that at the end of the experiment, regardless of the diet variation, there were increased significantly differences among all the tested rat groups except in case of the p. control (A2,A3 and A4, respectively) groups which were high significantly decreased

TABLE 2 . Acrylamide content in analyzed investigated food products ($\mu\text{g}/\text{kg}$).

Products	Acrylamide content ($\mu\text{g}/\text{kg}$)
Potato chips	1400
Cake	301
Biscuit	500
Breakfast cereals	1000
Bread	111
Noddles	466
snack product	Rosted chips)
wheat snacks	Rosted bread
tortilla chips	890
Bread bites	603

(14.10, 17.72 and 19.62%, respectively) in food intake and also decreased (59.50, 73.08 and 76.54 %, respectively) in body weight gain comparing to non-ACR control group (A 1). Meanwhile, EVOO at (0.5 mL/ kg BW rat/day) with ACR increased food intake significantly (11.26, 12.30 and 12.59 %, respectively) and also increased (171.95, 317.43 and 390.52%, respectively) in body weight gain comparing top. Control (A2, A3 and A4, respectively) groups. On the other hand, there were no- significant differences founded in liver on their relative weight (%) of rats among rats feed on (N. control) normal group (A 1) and EVOO at (0.5 mL / kg BW rat/day) group (A 8) throughout the feeding periods 28 days (Table 3). Feeding of ACR for 28 successive day's significant decrease in body weight gain and food intake. The decline of body weight could be explained by a metabolic disorder causing energy metabolism pathways which interfere with ACR. These results agree with (Youssef et al., 2004 and Arribas-Lorenzo & Morales, 2009;). The former suggested that such decrease may be related to the accompanied significant decrease in reduced GSH levels (Mårtensson et al., 1990). Reported that GSH is essential for the function and structural integrity of the gut, thus the deficiency of GSH showed severe degradation of jejunum and colonic mucosa accompanied with weight loss and diarrhea. Feeding on EVOO with ACR appeared to reduce the inhibitory impact of ACR on body weight gain and foods intake (Ghorbela et al., 2017). The beneficial effect of antioxidant administration against ACR poisoning with respect to body weight observed in the present study confirms previous results obtained by Ghorbela et al., (2017) who concluded that feeding rats with antioxidants could play an important

role as a prophylactic against the toxic effects of ACR (Servili et al., 2014) found that EVOO contain several compounds such as phenolic components and flavonoids responsible for antioxidant effect. There was no difference in relative organs weight in rats feed on ACR with EVOO at (0.5 mL/kg BW rat/day). Rats fed ACR (A2, A3, and A4) had a higher relative organ weight (liver). The results correspond with those of El-Sheikh et al. (2008), who found that after rats ate ACR, relative organ weight increased considerably. They added that following ingestion, ACR is readily absorbed from the gastrointestinal tract and widely distributed into the body fluids through which ACR can be accumulated in liver and kidney tissues and consequently increase the relative weights of these organs. The dietary impacts of control and ACR on liver weight are in Table 3. When organ weight was compared to final body weight, ACR increased the hepatosomatic index, which causes liver damage (Eman & Amany, 2008). They found that the rat fed ACR consumed more potentially harmful components, which resulted in a higher hepatosomatic index.

Effect of EVOO on serum glucose levels and lipid profile levels induced by ACR in rats

The levels of serum glucose in normal and experimental animals are shown in Table 4. The data revealed a significant increase in blood glucose levels (98.22, 118.96, and 135.03 percent, respectively) in ACR (P. control) A2, A3 and A4 groups rats as compared to (N. control) normal rats. Supplemented provision of EVOO at (0.5 mL/kg BW rat/day) with ACR significantly decreased (32.05, 34.73 and 37.60%, respectively) the level of blood glucose compared to ACR (P. control) A2, A3 and A4, respectively groups of

TABLE 3. Effect of EVOO on Initial weight, Final weight, net gain, Food intake and relative liver weight induced by ACR in rats

Treatments	Initial (g)	Final (g)	Weight gain	Gain %	Food intake (g)	FER	Liver %
(A1(N.Control	160.40 ^a ±2.40	180.65 ^c ±1.65	20.25 ^c ±0.75	12.62 ^c ±0.66	442.40 ^a ±4.40	0.04577 ^{ab} ±0.002	2.25 ^c ±0.150
A2(ACR)	160.10 ^a ±3.10	168.30 ^d ±4.30	8.20 ^d ±1.20	5.12 ^d ±0.601	380.00 ^d ±15.00	0.02153 ^{bc} ±0.002	5.20 ^{abc} ±0.700
A3(ACR)	160.45 ^a ±4.45	165.90 ^e ±2.90	5.45 ^{de} ±1.55	3.39 ^d ±1.060	364.00 ^e ±12.00	0.01510 ^c ±0.005	5.45 ^{ab} ±0.650
A4(ACR)	159.50 ^a ±3.50	164.25 ^e ±2.25	4.75 ^e ±1.25	2.97 ^d ±0.85	355.60 ^e ±16.60	0.01350 ^c ±0.004	5.80 ^a ±1.450
A5(ACR +)	159.90 ^a ±2.90	182.20 ^{bc} ±2.20	22.30 ^{bc} ±0.70	13.94 ^{bc} ±0.69	422.80 ^b ±12.80	0.05280 ^a ±0.003	4.35 ^d ±0.350
A6(ACR +)	159.70 ^a ±1.70	182.45 ^{bc} ±3.45	22.75 ^{bc} ±1.75	14.24 ^{bc} ±0.94	408.80 ^b ±8.80	0.05563 ^a ±0.003	4.65 ^{cd} ±0.650
A7(ACR +)	160.30 ^a ±3.30	183.60 ^b ±5.60	23.30 ^b ±2.30	14.53 ^b ±1.135	400.40 ^b ±5.40	0.05817 ^a ±0.005	4.90 ^{bcd} ±0.900
A8(EVOO)	160.60 ^a ±1.60	188.75 ^a ±3.75	28.15 ^a ±2.15	17.52 ^a ±1.65	445.20 ^a ±2.20	0.06320 ^a ±0.004	2.22 ^e ±0.220
LSD	1.666	2.264	2.823	1.701	9.224	0.03033	0.7326

- Means, within the same column, followed by the same letter are not significantly different- Means are followed by the corresponding standard deviation

rats. Throughout the 28-day feeding period, there were no significant differences in glucose levels between rats fed on (N. control) normal group (A 1) and EVOO (0.5 mL/kg BW rat/day) group (A 8). The ACR significantly increased serum total cholesterol, low-density lipoprotein-cholesterol, triglyceride, and very low-density lipoprotein-cholesterol elevations (55.47, 69.80, and 74.08; 192.78, 235.86, and 264.89, 68.75, 99.49 and 111.51%, respectively) in ACR (P. control) A2, A3, and A4 groups rats compared to as shown in Table 4, HDL-cholesterol levels were significantly lower (39.49, 32.55, and 43.64%) in ACR (P. control) A2, A3, and A4 group rats compared to (N. control) normal rats.

The ACR groups received 0.5 mL/kg BW rat/day of the tested EVOO, which improved or returned these values to normal. On the other hand, serum total cholesterol, HDL cholesterol, low density lipoprotein cholesterol, triglyceride, and very low density lipoprotein cholesterol levels were not significantly different between rats fed the normal group (N. control) (A 1) and EVOO at (0.5 mL/kg BW rat/day) group (A 8) throughout the feeding periods 28 days (Table 4). The present findings demonstrated that there was no significant difference in serum glucose concentrations in rats fed EVOO at (0.5 mL/kg BW rat/day) group (A8) and normal control group (A1) , while glucose concentrations were higher in the serum of ACR (P. control) A2, A3 and A4 groups of rats. These results agree with that of Ghorbel et al. (2015). As reported by Raja et al. (1992) after a parquet treatment, an increase in blood glucose may indicate a disrupted carbohydrate metabolism due to increased breakdown of liver

glycogen, possibly mediated by an increase in the adrenocorticotrophic and glucagon hormones and/or reduced insulin activity. According to Lin et al. (2013), the increase in serum glucose level was due to a decrease in blood insulin and insulin resistance status following ACR treatment. Taken together, these findings show that EVOO's antioxidative characteristics aid in the prevention of ACR-induced damage in rats. The good effects of EVOO may be linked to the presence of apigenin rutinoside, elenolic acid, luteolin, flavonoid, and hydroxytyrosol in Chetoui extra virgin olive oil, all of which can help to maintain the integrity of biological membranes (Serreli & Deiana, 2020). The concentration of (TC) and (LDL-C) were significantly influenced by the ACR in all groups. Rats fed ACR (A2, A3 and A4) had higher concentrations of (TC) and (LDL-C) in their serum than those fed on (N- control) and all groups. Rats fed on diets with ACR and EVOO had lower concentrations of (TC) and (LDL-C) in serum than those fed on diets with ACR (A2, A3 and A4). Lipids are also believed to be among the most sensitive biological molecules in terms of ROS susceptibility. Changes in the lipid content can lead to various diseases, such as atherosclerosis, lung fibrosis, inflammatory bowel disease, and rheumatoid arthritis (Zabetakis et al., 2020). In addition to the fluctuating levels of serum marker enzymes and morphological liver alterations, treatment with acrylamide showed a marked perturbation of the plasma lipid metabolism. In fact, the treatment caused an important increase in the plasma TC and LDL-C levels and a decrease in HDL-C, which indicated a change in the permeability of hepatic cells, as reported by Allam et al. (2010)

TABLE 4. Effect of EVOO on serum glucose levels and lipid profile levels induced by ACR in rats.

Treatments	Glucose (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	T.G (mg/dl)
(A1(Control	84.35 ^f ± 4.35	87.60 ^e ± 1.6	46.85 ^a ±0.85	26.35 ^f ±0.35	13.89 ^e ± 0.29	69.45 ^e ± 1.45
A2(ACR)	167.20 ^c ±7.20	136.20 ^c ±5.20	34.25 ^d ± 2.25	77.15 ^c ± 1.65	23.44 ^e ± 1.04	117.20 ^c ±5.20
A3(ACR)	184.70 ^b ±4.70	148.75 ^b ±4.70	31.60 ^e ± 3.60	88.50 ^b ± 0.40	27.71 ^b ± 0.91	138.55 ^b ±4.55
A4(ACR)	198.25 ^a ± 8.25	152.50 ^a ± 2.50	26.40 ^f ± 4.40	96.15 ^a ± 2.421	29.38 ^a ±1.180	146.90 ^a ±5.90
A5(ACR +)	113.60 ^e ± 3.60	91.15 ^f ± 1.15	38.35 ^b ± 2.35	35.20 ^e ±1.453	17.08 ^f ± 0.48	85.40 ^f ± 2.405
A6(ACR +)	120.55 ^d ±5.55	94.70 ^e ± 1.70	37.60 ^b ± 2.60	36.65 ^e ±1.525	19.75 ^e ± 0.55	98.75 ^e ± 2.75
A7(ACR +)	123.70 ^d ±6.70	98.85 ^d ± 2.85	36.15 ^c ± 4.15	41.20 ^d ± 2.375	20.86 ^d ±0.86	104.30 ^d ±4.30
A8(EVOO)	84.60 ^f ±1.60	87.10 ^e ±1.10	47.30 ^a ±1.80	25.80 ^f ±1.028	13.80 ^e ±0.40	69.20 ^e ±2.20
LSD	3.753	2.778	2.130	2.553	0.570	2.806

- Means are followed by the corresponding standard deviation at <0.05.

- Means, within the same column, followed by the same letter are not significantly different

after the ACR treatment. Acrylamide induces free radical production (Ghorbel et al., 2015), this may result in cellular cholesterol accumulation due to (1) increased cholesterol production and esterification, (2) decreased cholesteryl ester hydrolysis, and (3) decreased cholesterol efflux (Gesquière et al., 1999). In light of the present results, the occurrence of hypertriglyceridemia may result from the poor expression of lipolytic activity in the vascular bed during ACR treatment. Recently, the administration of EVOO to the treated rats improved the levels of all the parameters cited above. EVOO has been reported to improve the lipid profile through a decrease in TC, LDL-C, and an increase in HDL-C (Ghorbel et al., 2015). Phenolic chemicals may influence the development of atherosclerosis through a variety of mechanisms, including suppression of LDL oxidation, endothelial production of tissue factors, and adhesion molecules (Malekmohammad et al., 2019). The increase in HDL-C could be a result of a protective mechanism against oxidative stress generated by the ACR-containing diet, as well as a mechanism to prevent oxidative alterations in other lipoproteins like LDL (Brites et al., 2017). As shown in Table (4), the rats fed on ACR groups (P-control) (A2, A3, and A4) exhibited lower serum high-density lipoprotein cholesterol (HDL-C) concentrations than the other groups. (Ghorbel et al., 2015) found that antioxidant-rich foods increased the response to oxidative damage in the pathogenesis of many diseases and increased HDL-C. Triglyceride (TG) and very low-density lipoprotein cholesterol

(VLDL-C) levels in the blood As shown in table (4), the concentrations of (TG) and (VLDL-C) were studied. These data indicate that in rats fed on a diet containing ACR (P-control) groups (A2, A3 and A4), the concentration of (TG) and (VLDL-C) increased significantly. Meanwhile, the other groups (A 5, A 6 and A 7) fed on a diet containing ACR with EVOO (0.5 mL/kg BW rat/day) had a lower value compared with the group (P-control). These results are in agreement with (Ghorbel et al., 2017).

Effect of EVOO on serum liver functions levels induced by ACR in rats:

Liver function tests (LFTs) are used to detect liver disease, assess disease progression, and monitor the effects of hepatotoxic drugs and necrosis in animal livers. Prothrombin time, as well as serum aminotransferases and alkaline phosphatase, bilirubin, and albumin, are all included in the (LFTS) test (McFarlane et al., 2000). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used to determine the concentration of intracellular hepatic enzymes that have seeped into the blood. Moreover, the normal functioning of life is indicated by normal levels of ALT and AST, indicators of liver function. When the enzymes leak from the liver cytosol into the bloodstream, it leads to a higher level of ALP in the serum (El-Sharouny, 2016), which is indicative of the hepatotoxic activity induced by ACR in rats. Administration of ACR produced significant adverse effects on the liver functions of the rats, which is evidenced by a

significant increase in elevation (95.34, 109.71, 176.72, 88.60,192.29, 307.70 and 111.63, 147.95, 215.37%, respectively) in the actions of ALT; AST and ALP enzymes in ACR (P. control) A2, A3 and A4, respectively groups compared to (N. control) normal. The actions of ALT, AST, and ALP enzymes were improved in ACR rats treated with EVOO (0.5 mL / kg BW rat/day) compared to ACR (P. control) groups A2, A3, and A4, respectively. On the other hand, there were non-significant variances in the actions of ALT, AST and ALP enzymes among rats fed on (N. control) normal group (A1) and EVOO (0.5 mL/kg BW rat/day) group (A 8) throughout the feeding periods of 28 days (Table 5). The liver function could be detected in the state of the liver. Also, one of the advantages of the administration of EVOO to activity induced by ACR in rats was a reduction in ALP activity to its regular levels. High levels of ALP in the serum indicate liver damage. The decrease in ALP given EVOO in the current study indicates that documented liver damage, as a result of metabolic alterations such as toxin administration, liver cirrhosis, hepatitis, and liver cancer, resulted in increased serum ALT, AST, and ALP (Baghdadya, 2020). Thus, they can be used as markers to estimate the extent of liver damage. It was reported that oxidative stress mediates many of the effects caused by oxidized fats (Pizzino et al., 2017). The formation of reactive oxygen species (ROS) may increase the dietary oxidized fats and may cause increased damage of proteins in the liver by enhancing lipid peroxidation of the cell membrane and increasing the generation of (ROS) which can lead to calcium homeostasis

disturbances, increased membrane fluidity and cell death. On the other hand, (Abril et al., 2019) found that EVOO contains substances that delay the rate of oxidation by directing the breakdown of peroxides into stable substances that do not promote further oxidation or by sweeping free radicals away. A histopathological study of the liver showed kuffer cells activation, vacuolar degeneration of hepatocytes, nuclei pyknosis and congestion of blood vessels in the liver of ACR rats (AL-Mosaibih, 2013). Damaged membranes were recovered by the treatment with EVOO by enhancing antioxidants' status and decreasing lipid peroxidation (El-Sharouny, 2016).

Effect of EVOO on serum kidney functions levels induced by ACR in rats:

Creatinine is a primary waste product of creatine metabolism in muscles. The glomerulus in the kidneys filters it, and the tubules discharge it. Urea and uric acid are the main waste products of protein catabolism, in addition to free-creatinine, which is seen in blood serum (Stevens et al., 2006). They were made in the liver from ammonia that was created when amino acids were deaminated. A high-protein diet or higher endogenous catabolism as a result of famine or tissue damage might speed up the rate of synthesis (Ozturan al., 2014). A kidney function test can be used to measure the effectiveness of the kidneys. Table 5 shows that ACR had a significant negative impact on the rats' kidney functions, as evidenced by a significant increase in the actions of creatinine, urea, and uric in ACR (P. control) A2,A3andA4 groups rats compared to (N. control) normal rats (171.73, 302.17, 378.26,52.53, 69.57, 91.27 and 59.72, 64.58, 104.86 %, respectively).

TABLE5 . Effect of EVOO on serum liver functions and kidney functions levels induced by ACR in rats.

Treatments	Liver functions			Kidney functions		
	ALT (U/L)	AST (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Uric (mg/dl)
(A1(N.Control	24.70 ^e ±1.70	29.85 ^e ±1.85	68.30 ^e ± 2.30	0.46 ^e ±0.16	24.65 ^f ±1.65	1.44 ^e ±0.14
A2(ACR)	48.25 ^c ±3.25	56.30 ^c ±6.30	144.55 ^c ± 4.55	1.25 ^c ±0.35	37.60 ^e ±3.60	2.30 ^{bc} ±0.40
A3(ACR)	51.80 ^b ± 2.80	87.25 ^b ±7.25	169.35 ^b ±8.35	1.85 ^b ±0.65	41.80 ^b ±4.80	2.37 ^b ±0.37
A4(ACR)	68.35 ^a ± 3.35	121.70 ^a ±5.70	215.40 ^a ± 5.40	2.20 ^a ±0.40	47.15 ^a ±4.15	2.95 ^a ±0.75
A5(ACR +)	29.40 ^f ± 2.40	35.15 ^f ±5.15	84.75 ^f ± 3.75	0.80 ^d ±0.30	28.70 ^e ±3.70	1.70 ^d ±0.70
A6(ACR +)	34.85 ^e ± 4.85	41.60 ^e ±4.60	117.30 ^e ± 7.30	1.35 ^e ±0.55	32.45 ^d ±2.45	1.90 ^{cd} ±0.80
A7(ACR +)	37.15 ^d ±4.15	46.40 ^d ±2.40	137.10 ^d ± 6.10	1.90 ^{ab} ±0.60	33.20 ^d ±3.20	2.15 ^{cd} ±0.65
A8(EVOO)	24.45 ^e ±1.40	29.70 ^e ±2.70	68.85 ^e ±0.85	0.48 ^e ±0.18	24.90 ^f ±2.90	1.50 ^e ±0.20
LSD	2.048	3.461	4.386	0.3276	1.731	0.4533

- Means, within the same column, followed by the same letter are not significantly different at <0.05.

- Means are followed by the corresponding standard deviation.

Treatment of ACR rats with EVOO (0.5 mL/kg BW rat/day) improved kidney function when compared to ACR (P. control) A2, A3, and A4 rats. Meanwhile, there were non-significant differences in creatinine, urea and uric among rats fed on (N. control) normal group (A 1) and EVOO (0.5 mL / kg BW rat/day) group (A 8) throughout the feeding periods of 28 days (Table 5). (Moawadet al., 2019) confirm that ACR worsens early disease progression of renal disease. While treatment with EVOO showed a decrease in kidney disease and an improvement in the level of renal function tests (Ghorbela et al., 2017), In normal pathological states, EVOO has been described as an antioxidant that removes free radicals for cell protection (Serreli & Deiana, 2020).

Effect of EVOO on malonaldehyde, enzymatic antioxidants and non-enzymatic antioxidants activity induced by ACR in rats:

In normal and experimental rat groups, activity levels of malonaldehyde (MDA) in serum, enzymatic antioxidants (SOD), (CAT), (GSH-Px), and non-enzymatic antioxidants (GSH) in the blood were shown in Table (6). Serum malonaldehyde (MDA) activity was significantly increased (581.60, 1120.68, and 1905.74%, respectively) in ACR (P. control) A2, A3, and A4 groups rats compared to normal (N. control). While the activities of blood enzymatic antioxidants SOD, Catalase, (GSH-Px) and non-enzymatic antioxidants (GSH) were significantly decreased (58.69, 67.35, 75.83, 40.36, 45.49, 52.54, 34.48, 38.07, 48.06 and 50.38, 65.50,

69.89%, respectively) in ACR (P. control) A2, A3 and A4, respectively groups rats compared to (N. control) normal. Treatment of ACR rats with EVOO (0.5 mL/ kg BW rat/day) resulted in lower levels of malonaldehyde (MDA) and enhanced activities of enzymatic antioxidants such as SOD, Catalase CAT, GSH-Px, and non-enzymatic antioxidants such as glutathione (GSH-Px) (GSH). Throughout the feeding periods of 28 days, there were no significant variations in MDA, SOD, catalase, and non-enzymatic antioxidants (GSH) between rats fed on (N. control) normal group (A 1) and EVOO at (0.5 mL/kg BW rat/day) group (A 8). A stressful condition leads to the excessive production of free radicals, which results in oxidative stress an imbalance in the oxidant per antioxidant system. Free radical generation is an integral feature of normal cellular functions. In contrast, excessive generation and/or inadequate removal of free radicals results in destructive and irreversible damage to the cell (Lobo et al., 2010). Under normal circumstances, numerous enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) and non-enzymatic antioxidants such as Glutathione Reduced (GSH) create a natural defensive system that aids in the detoxification of free radicals. Since numerous diseases have been linked to oxidative stress, the use of antioxidant-rich foods or antioxidant-rich supplements has grown in popularity (Nakbi et al., 2010). Therefore, the objective of this research was to investigate the effect of EVOO on the antioxidant enzyme induced by ACR in rats after 28 days of treatment.

TABLE 6. Effect of EVOO on serum (MDA) and on erythrocyte (SOD, Catalase, GSPx and GSH) levels induced by ACR in rats.

Treatments	SOD (U/ml)	Catalase (U/ml)	GSPx (U/ml)	GSH (mg/dl)	MDA (nmol/ml)
(A1(Control	287.15 ^a ± 2.15	178.50 ^a ± 1.50	179.65 ^b ± 3.65	38.70 ^a ±2.70	4.35 ^f ± 1.35
A2(ACR)	118.60 ^c ± 8.60	106.45 ^c ± 6.45	117.70 ^f ± 7.70	19.20 ^c ±4.20	29.65 ^c ± 4.65
A3(ACR)	93.75 ^f ± 9.75	97.30 ^f ± 7.30	111.25 ^g ±6.25	13.35 ^d ±3.35	53.10 ^b ± 8.10
A4(ACR)	69.40 ^g ± 4.40	84.70 ^g ± 4.70	93.30 ^h ± 3.30	11.65 ^d ±2.65	87.25 ^a ± 7.25
A5(ACR +)	219.25 ^b ± 9.25	163.10 ^b ±3.10	151.20 ^c ± 6.20	31.30 ^b ±6.30	14.20 ^e ±4.20
A6(ACR +)	164.35 ^c ± 4.35	148.20 ^c ± 4.20	144.75 ^d ± 4.75	27.80 ^b ±7.80	22.15 ^d ± 2.15
A7(ACR +)	130.10 ^d ± 8.10	119.65 ^d ± 2.65	129.40 ^e ±7.40	21.55 ^c ±3.55	32.70 ^c ± 4.70
A8(EVOO)	291.40 ^a ± 4.40	179.75 ^a ± 3.75	186.70 ^a ± 2.70	41.65 ^a ± 1.65	4.15 ^f ± 1.15
LSD	5.012	3.377	3.352	3.591	4.518

- Means, within the same column, followed by the same letter are not significantly different at <0.05.

- Means are followed by the corresponding standard deviation.

Pathological effects of different organs:

The livers of the experimental rat groups were examined microscopically (Fig 1). (Control N.) The hepatic lobule slide of an untreated rat group had a normal histological appearance (1). Meanwhile, the liver of rats from group ACR (A2) showed fibroplasia in the portal triad slide (2) and fibroplasia in the portal triad and portal infiltration with mononuclear inflammatory cells slide (3). However, the liver of rats from group ACR (A3) showed perivascular focal hepatic necrosis associated with mononuclear cell infiltration slide (4) and hyperplasia of the epithelial lining of the bile duct, portal fibrosis associated with the appearance of newly formed bile ductules slide (5). While the liver of a rat from group ACR (A4) demonstrated cytoplasmic vacuolization of hepatocytes and fibroplasia in the portal triad slide (6) fibroplasia in the portal triad and portal infiltration with mononuclear inflammatory cells slide (7). Meanwhile, the livers of rats from group ACR treated with EVOO (0.5 mL/kg BW rat/day) (A5, A6, and A7) showed slight Kupffer cell activation and a few inflammatory cell infiltration in the portal triad slide (8), slight congestion of the hepatic sinusoids slide (9) and slight Kupffer cell activation and focal hepatic necrosis associated with mononuclear cell infiltration slide (10). In addition, the liver of a rat from group EVOO at (0.5 mL/kg BW rat/day) (A8) showed no changes with apparent normal hepatocytes slide (1).

Docking studies

Molecular docking:

Auto Dock and vina dock tools were applied to explain the toxicity anticancer features of drugs and favor the experimental products. Molecular docking with the crystal structure of proteins have been undertaken to identify the level of toxicity and

anticancer activities of Acrylamide-Ligand over liver and kidney in which growth factor receptor. (Abdelsalaam et al., 2019)

The interaction of Acrylamide with liver proteins like (PDB code: 4RT7) it react with amino acids : 4rt7-h/A1/A/ASP`829/HN with hydrogen bond length 2.2 Å°, 4rt7-h/A1/A/VAL`675/O with hydrogen bond length 2.7 Å° and 4rt7-h/A1/A/ILE`827/O with hydrogen bond length 2.8 with binding energy -6.1(kcal/mol)shown in Figure 1.

The interaction of Acrylamide with kidney proteins like PDB code: 2gbi it react with amino acids: 2gbi-h/A1/A/ARG`123/1HH1 with hydrogen bond length 2.2 Å°, 2gbi-h/A1/A/GLU`203/OE2 with hydrogen bond length 2.2 Å°, 2gbi-h/A1/A/ARG`123/1HH2 with hydrogen bond length 2.5 Å° and 2gbi-h/A1/A/TYR`663/OH with hydrogen bond length 2.5 Å° with binding energy -3.6(kcal/mol) (Mannhold et al., 2008 and Zhao et al., 2001) shown in Figure 3.

The choice of all proteins depend on the (X, Y, Z) cavity, value of rmsd, crystallography, method, resolution and other parameters of protein. It clears that the indole capable to release the toxicity effect either in liver, kidney and heart. Carcinogenicity is a type of toxicity which can lead to the growth of cancer in the body. The carcinogenicity test typically uses rats or mice exposed to a molecule or drug candidate (Hou, et al., 2004 and Zhao et al., 2001). The molecular structure of the investigated molecules plays a key role in determining the properties and their pharmacokinetic behavior. The scrutiny of toxicity is a vital feature in the conception of drugs, as it can foretell the mutagenicity and carcinogenicity of new compounds.

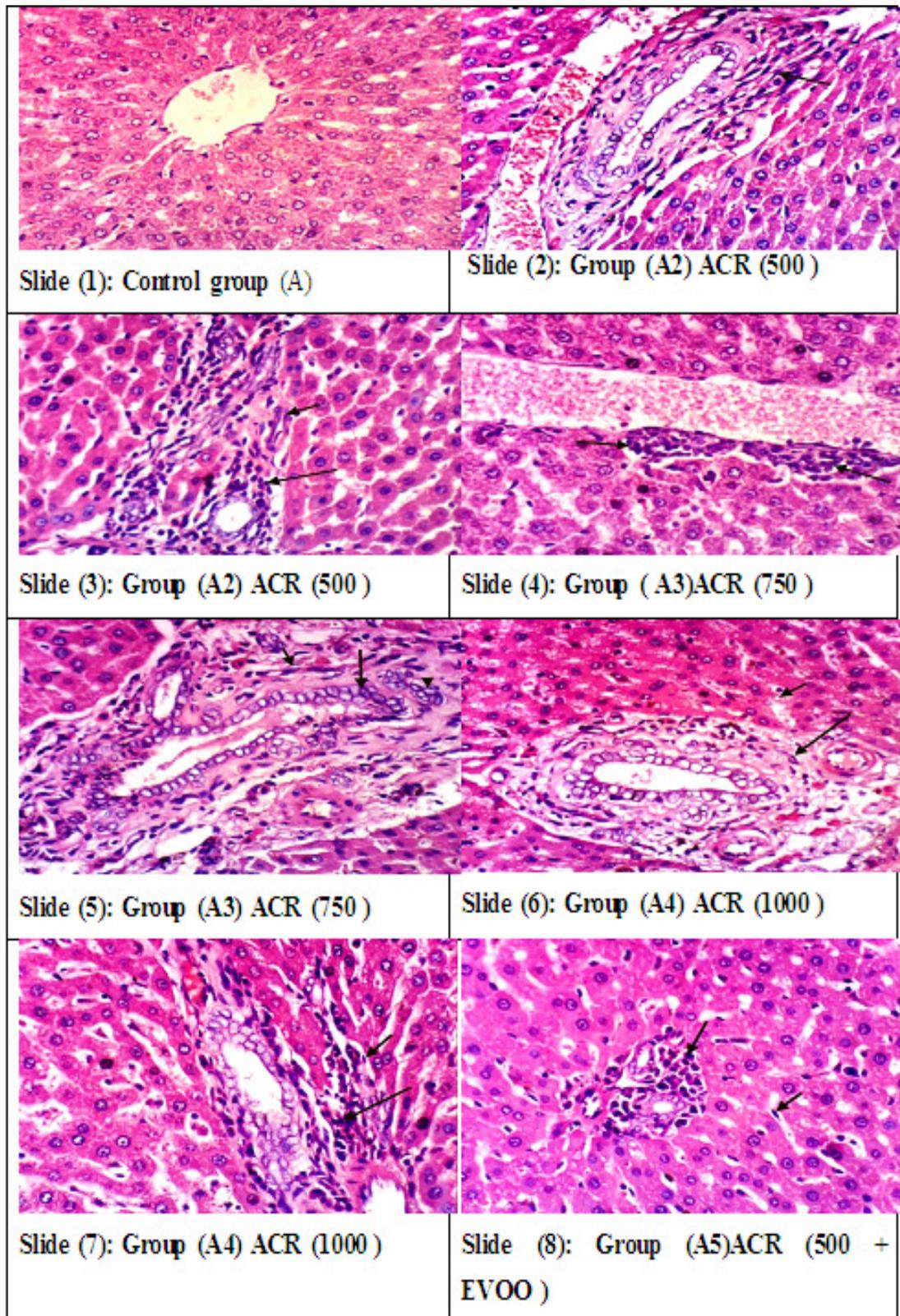


Fig. 1. Photomicrographs of liver organ (1):Control group (A), (2): ACR (500 μ g), (3): ACR (500 μ g), (4): ACR (750 μ g), (5):ACR (750 μ g), (6): ACR (1000 μ g),(7): ACR (1000 μ g),(8): ACR (500 μ g + EVOO), (9): ACR (750 μ g + EVOO), (10): ACR (1000 μ g + EVOO).

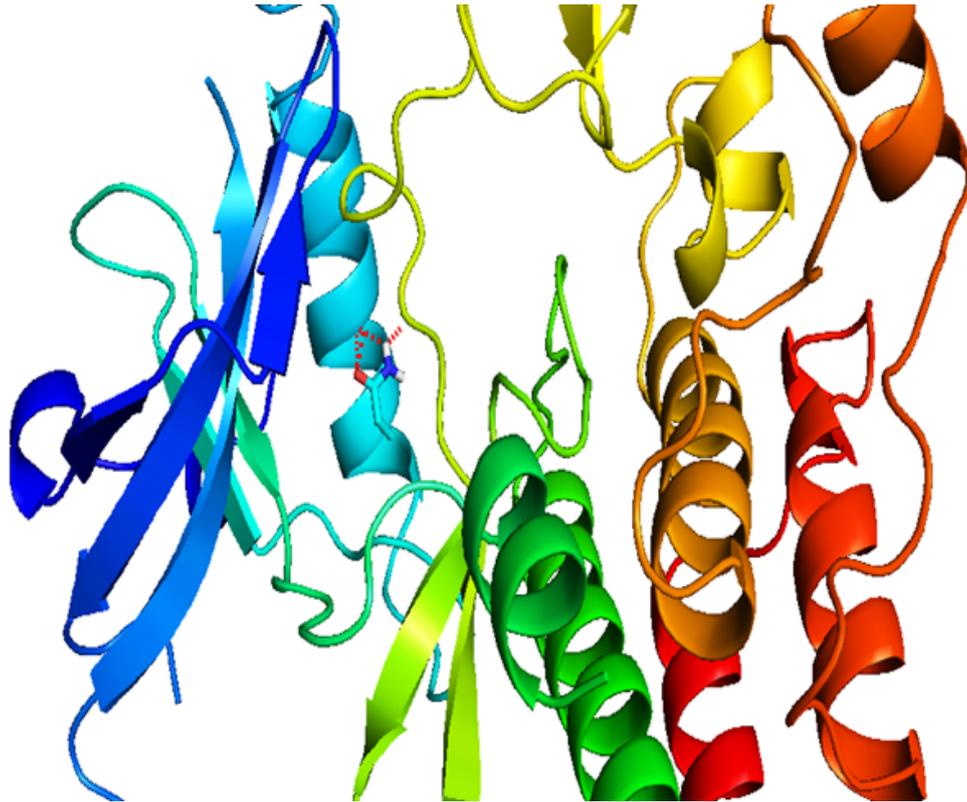


Fig. 2. Three dimensional plot interaction of Acrylamide ligand with liver protein (PDB code: 4RT7) receptor

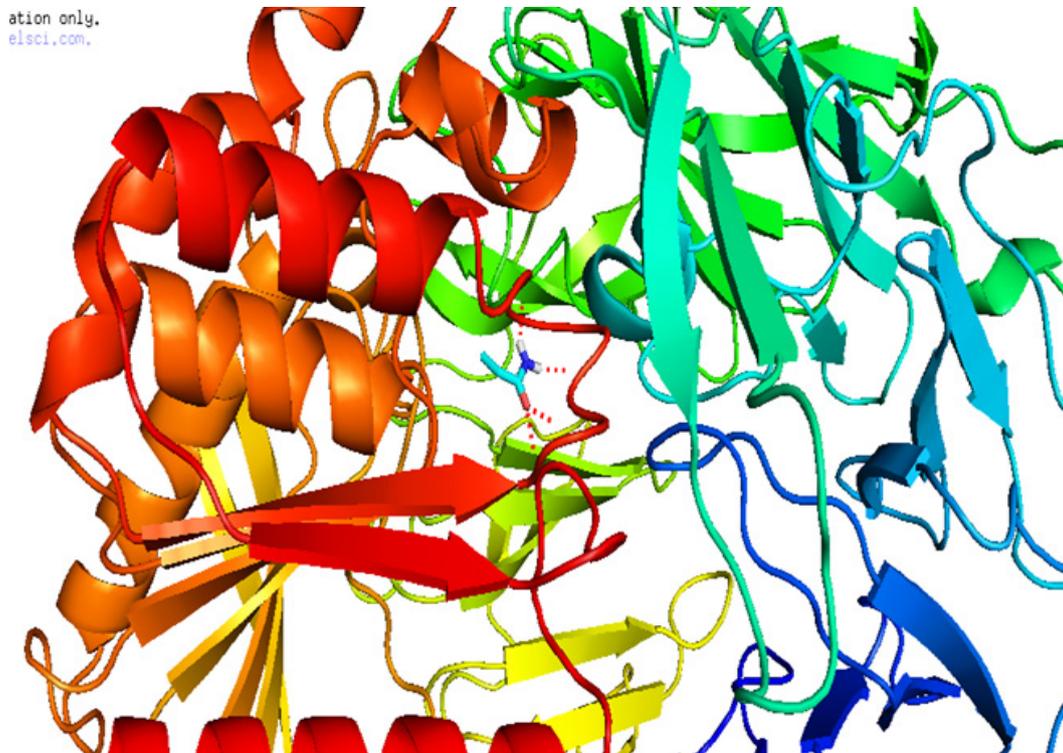


Fig. 3. Three dimensional plot interaction of Acrylamide ligand with kidney protein (PDB code: 2GBI) receptor.

Conclusion

The levels of acrylamide in certain Egyptian food products were measured. The effect of extra virgin olive oil (EVOO) on liver damage and histological changes in ACR-treated rats was studied at various levels as well as biological assessment. Feeding EVOO improved the lipid profile, liver functions, renal functions, and overall antioxidant levels, according to our findings. The excess formation of reactive oxygen species, which causes oxidative stress, could be the underlying mechanism of acrylamide-induced hematotoxicity. Hematological indices, lipid and protein oxidation, and the loss of antioxidant status in erythrocytes were all significantly reduced when EVOO was co-administered. The anti-oxidant properties of EVOO, as well as the free radical scavenging activities of monounsaturated fatty acids and polyphenols, are likely to be responsible for these positive effects. More research and analysis are needed on this subject to lower the amounts of acrylamide in various food products during food processing technologies.

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مادة الأكريلاميد (ACR) هي واحدة من أهم الملوثات التي حدثت في الأطعمة التي يتم تسخينها في درجات حرارة عالية. الهدف من هذه الدراسة هو دراسة المستويات المختلفة من مادة الأكريلاميد في بعض المنتجات الغذائية في السوق المصري والتحقق من الفعالية الوقائية لزيت الزيتون البكر الممتاز (EVOO) ضد السمية الكبدية التي يسببها مادة الأكريلاميد. تم تقسيم ٤٨ من الفئران الذكور إلى ثمانية مجموعات. تم تغذية المجموعة A1 على نظام غذائي أساسي كمجموعة تحكم. تم تغذية المجموعات A2 و A3 و A4 على حمية تحتوي على مادة الأكريلاميد عند ٥٠٠ و ٧٥٠ و ١٠٠٠ ميكروغرام على التوالي. تم تغذية المجموعات الأخرى (A5, A6, A7) على نفس النظام الغذائي المستخدم في المجموعات A2 و A3 و A4 على التوالي. مع إضافة ٠.٥ مل من زيت الزيتون البكر الممتاز. تم تغذية المجموعة A8 بنظام غذائي أساسي ومكمل بزيت الزيتون البكر الممتاز (٠.٥ مل). بعد ٢٨ يومًا، وجد الفئران التي تم تغذيتها على مادة الأكريلاميد و زيت الزيتون البكر الممتاز إلى خفض مستويات الجلوكوز لديهم بشكل ملحوظ. أدى العلاج أيضا إلى تحسين كبير في الدهون. ووظائف الكبد. ووظائف الكلى. ومضادات الأكسدة الكلية. ومع ذلك، زاد EVOO بشكل كبير من نشاط ديسموتاز (SOD). الكاتالاز (CAT). الجلوتاثيون (GSH). والجلوتاثيون بيروكسيداز (GPS) في دم فئران أظهرت المجموعات المعالجة انخفاضًا كبيرًا في المواد التفاعلية لحمض الثيوباربيتوريك مثل malondialdehyde (MDA) في مصلهم. وجد استخدام زيت الزيتون البكر الممتاز EVOO من التشوهات النسيجية المرضية والكبدية المرتبطة باستخدام الأكريلاميد وجد أيضًا دراسة إنزيمات الأكسدة والاختزال تعتبر علامة موثوقة لتقييم فعالية مضادات الأكسدة لزيت الزيتون البكر