Olive Oil Ameliorates Fries Potato Chips Associated Hazards Effects of Acrylamide

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Abstract: Acrylamide (ACR) is an important industrial chemical substance. ACR is also found in carbohydrate rich foods that prepared at high temperature (>120°C), such as fries potato chips. Studies approved that high levels of ACR cause several metabolic disorders such as oxidation in liver, small and large intestine tissues. ACR is metabolized by either conjugation with glutathione or oxidation to glycidamide. Hepatotoxicity of ACR is the motivation that leads us to carry out the study to demonstrate the impact of this research on liver. In a trial to overlap acrylamide toxicity, potato chips were fried in olive oil. Totally 24 adult male and female mice were divided into four groups: Group A includes control non-treated mice which were received normal saline. Group B includes mice which were received basal diet and ACR dissolved in the drinking water and they will held in cages in controlled environment. Group C (FPSO) includes mice which feed on basal diet of potato chips fried in sunflower oil. Group D (FPOO) includes mice which fed on basal diet of potato chips fried in olive oil in the same consumption quantity of sunflower oil group. After 45 days, the mice in all groups were sacrificed and a blood samples were collected. The effect of acrylamide on the liver enzymes (ALT and AST) was recorded. Also, lipid profile, total antioxidants were determined. Otherwise, pieces of liver were isolated and kept in formalin to be sent for histopathological examination. The present study exhibited that potato chips fried in olive oil protects against liver toxicity that may occur through ACR development more than sunflower oil usage.

Keywords: Acrylamide, Food processing, Hepatotoxicity, potato chips, olive oil, sunflower oil.

I. Introduction

The Consumption of high quantities of unhealthy food related with the rapid progress in lifestyle is associated with a major risk for the onset of different diseases. Fried potato (Solanum tuberosum) chips as a carbohydrates rich food are one of these most consumed snacks particularly by children. Nutrition field represented that fried potato chips involve considerable amounts of acrylamide (ACR) with its carcinogenic and neurotoxic characteristics (Ouhtit et al., 2014).

Acrylamide (ACR), a small organic molecule is highly soluble in water and a known neurotoxin, which is formed in starchy foodstuffs that are fried, baked or roasted at temperatures above 100°C such as chips, crisps and bread as well as biscuits, crackers and breakfast cereals, due to interactions between asparagines and reducing sugar in a series of non-enzymatic reactions given the name of the Maillard reaction. Asparagine typically accounts for approximately one-third of the total free amino acid pool in potato tubers and because asparagine is present at such a high concentration, sugar concentrations might be expected to be the limiting factor for acrylamide formation (Mutucumaru et al., 2013).

ACR has a higher tendency to bind with hemoglobin forming adducts, which reduces the surface for oxygen transportation and leads to cellular damage in tissues (Hogervorst et al., 2008). Two major metabolic pathways for ACR have been reported. One pathway is conjugation with glutathione to form urinary metabolites, N-acetyl-S-(3-amino-3-oxypropyl) cysteine and N-acetyl-S-(2-carbamoylthethyl) cysteine. The second pathway is epoxidation to glycidamide, which is more reactive than ACR toward DNA and proteins to form adducts, through the action of cytochrome P450 2E1 (Watzek et al., 2013).

Considering the prospective health hazard of ACR and its wide consumption in the diet, reliable alleviation strategies to minimize its levels in processed products have been undertaken. The selection of potato varieties having a low concentration of free carbohydrates, possibility to define time-temperature processing conditions which guarantee low acrylamide concentration, the addition of amino acids as glycine or glutamine to potatoes before processing all have a reducing effect on the content of acrylamide. In the past few years the acrylamide concentration has been significantly reduced in industrial products, but for potato products, domestic preparation remains a relevant source of acrylamide production (Kahkeshani et al., 2015).

Dietary antioxidants have been targeted as possible therapeutic and protective agents against free radicals to resist oxidative stress. Olive oil is recognized for its antioxidant properties and its positive effects against reactive oxygen species. Its beneficial role, concentrated on a decrease in the coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses. The mechanism proposed to explain the benefits of olive oil may be due to its contents of monounsaturated fatty acids (MUFA),

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Olive oil (omega-9) and other components, such as aliphatic alcohols, sterols and polyphenols (e.g., α-tocopherols and hydroxytyrosol). Olive oil, a widely applied omega-9 enriched dietary lipid, has attracted considerable interest in its effects against liver injuries (Ghorbel et al., 2015).

The present study examined the side effects of acrylamide produced from potato chips fried in sunflower oil on liver enzymes, lipid profile and oxidative stress and whether olive oil can prevent these toxic effects on exposed mice.

II. Materials and Methods

Animals
In this experimental study, 24 adult male and female mice with (18-20 g) were obtained from National Research Center (Cairo, Egypt) and maintained in wire cages 1 week for acclimatization with free access to food and water. Mice were divided into four groups with 6 mice in each. Group A served as controls and fed on basal diet, group B received basal diet and acrylamide (ACR) (0.5 mg/kg/day), which was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in the drinking water. Chemical analysis has shown that ACR remains stable in water for 1 week. Water consumption per cage was measured during the test to estimate the amount of ACR per kilogram of body weight in each mouse (Kermani-Alghoraishi, 2010). Group C received potato chips fried in sunflower oil (FPSO). Group D received potato chips fried in olive oil (FPOO). Potato consumption per cage was measured during the experiment to consider the amount of consumption (nearly 5.6gm/kg/day) of the mice weight during the experimental time of the study. The study was performed in accordance with the guidelines for the care and use of laboratory animals approved by Research Ethical Committee (Faculty of Pharmacy, Tanta University, Egypt).

After 45 days of acrylamide and potato chips consumption, the mice in all groups were sacrificed under light ether anesthesia and a blood samples were collected via cardiac puncture after overnight fast. Serum samples were collected for further biochemical analysis for liver enzymes (ALT and AST), lipid profile, and total antioxidants were determined. Otherwise, pieces of liver were isolated and kept in 10% formalin to be sent for histopathological examination.

1. Acute toxicity study
For choice of the working dose of acrylamide, its median lethal dose (LD50) was determined by an acute toxicity study. Swiss albino mice were assigned to 9 groups, with 7 animals in each group. The groups were treated with acrylamide in drinking water in dose range 0.2-1.8 mg/kg body weight. The mortality in each group was recorded within 24 hour after administration of acrylamide and the % mortalities were converted to probits by using Finney’s probit analysis table (Finney 1952). LD50 was determined graphically and was equivalent to 0.9 mg/kg body weight and therefore, a dose of 0.5 mg/kg body weight was chosen for the subsequent experiments.

2. Histopathology of the liver of mice:
Liver specimens were fixed in 10 % formalin for 24 hour. Five-micrometer slices (Microtome, Leica RM2155; Leica Inc., Nussloch (Germany)) were stained by hematoxylin and eosin (H&E). Histological analyses were done under light microscopy (Olympus Electron Microscope, Olympus (Japan)).

3. Measurement of liver and kidney markers:
Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were determined by an enzymatic colorimetric method. All the measurements followed the instructions of the manufacturer of kits (Diamond diagnostic, Inc.).

4. Measurement of serum total antioxidant:
Total antioxidants were determined by an enzymatic colorimetric method. All the measurements followed the instructions of the manufacturer of kits (Biodiagnostic, Egypt).

5. Measurement of lipids profile:
Enzymatic colorimetric determination of total cholesterol was according to Allain et al., 1974. Serum level of triglycerides was determined according to Bucolo and David, 1973. Both kits were obtained from Diamond diagnostic, England. High density lipoprotein cholesterol (HDL-C) was determined using kits obtained also from Diamond diagnostic, England. Low density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald formula (Friedewald et al., 1972).

Data are presented as mean ± standard error (SE) and were analyzed by Microsoft software (EXCEL 2000) and Statistical Package for Social Science (SPSS) version 17. The experimental data were analyzed for significant differences by paired t-test.
III. Results

1- Effect on Histopathology of acrylamide and vegetable oil treated liver mice:

Figure 1 represents pathological changes in mice liver. Liver mouse supplemented with fried potato chips in sunflower oil (FPSO) shows central vein congestion, sinusoidal dilation and kupffer cell hyperplasia. Also, liver section reveals portal inflammation, bile duct proliferation and piecemeal necrosis (Figures 1b, 1c). As the same, treatment with acrylamide (ACR), liver mouse shows hepatic steatosis and hydropic change. Also, liver reveals portal inflammation, central vein congestion, kupffer cell hyperplasia and piecemeal necrosis (Figures 1d, 1e). Olive oil supplementation in group (FPOO) leads to reduction of pathological changes with normal hepatocytes and portal tract shows bile duct, portal vein and hepatic artery. Also, hepatocytes with round eosinophilic cytoplasm separated by thin sinusoidal blood vessels are represented (Figure 1f, 1g). These results were compared with normal control liver mouse, showing no pathological changes (Figure 1a).

Figure 1. Histopathological examination of mice liver from different groups (H&E x 200) a: Section of normal control liver mouse, showing no pathological changes. b: Section of fries chips in sunflower oil (FPSO) liver mouse shows central vein congestion, sinusoidal dilation and kupffer cell hyperplasia. c: Section of FPSO liver mouse reveals portal inflammation, central vein congestion, bile duct proliferation and piecemeal necrosis d: Section of acrylamide (ACR) liver mouse shows hepatic steatosis and hydropic change. e: Section of ACR liver mouse reveals portal inflammation, central vein congestion, kupffer cell hyperplasia and piecemeal necrosis f: Section of fried potato chips in olive oil (FPOO) liver mouse shows normal hepatocytes with the portal tract shows bile duct, portal vein and hepatic artery. g: Section in FPOO group shows normal hepatocytes with round eosinophilic cytoplasm separated by thin sinusoidal blood vessels.

2- Effect on liver enzymes in mice serum:

AST and ALT levels were estimated in serum. Liver enzymes levels were both significantly (P<0.01) increased by treatment with acrylamide compared to control normal group. Figure 2 illustrates FPSO group showed that both liver enzymes AST and ALT were significantly increased (226.8±7.5, 70.7±6.6 P<0.01) compared to control normal group (175.6±7.1, 61.4±2.3 P<0.01) respectively. On the other hand, supplementation by potato chips fried in olive oil (FPOO) resulted in significant decrease in ALT (10.7±2.04, P<0.001) compared to control normal, ACR treated (76.5±1.8, P<0.001) and also FPSO group. AST significantly decreased (220.0±2.5, P<0.01) compared to the ACR treated group (308.3±30.8, P<0.01) in spite that it significantly increased compared to control normal group and non-significantly changed compared to FPSO supplemented group.
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Figure (2): Data are presented as mean±SE. AST: aspartate aminotransferase, ALT: alanine aminotransferase. 
a: Significant versus normal control at P<0.01. b: Significant versus acrylamide treated group at P<0.001. c: Significant versus potato chips fried in sunflower vegetable oil treated group at P<0.01. ACR: Acrylamide, FPSO: Fried potato chips fried in sunflower oil, FPOO: Fried potato chips fried in olive oil.

3- Effect on total antioxidant in mice serum:
The results showed a significant decrease in total antioxidant level in ACR treated control group (0.8±1.1 mM/L, P<0.05) when compared to normal control group (2.65±0.1 mM/L, P<0.05). Although there was a significant decrease in total antioxidant level (1.64±0.4 mM/L, P<0.05) in FPSO group compared to normal control, a significant elevation when compared to ACR group was obtained. On the other hand, a significant elevation in FPOO group (2.3±0.9 mM/L, P<0.05) when compared to both ACR control group and FPSO group. 

Figure (3) presents serum total antioxidant concentration in the studied groups.

4. Lipid profile in the studied groups
ACR treated mice showed significant increase (P<0.05) in serum TC, LDL-C and TGs levels compared to the control normal group but a significant decrease (P<0.05) in serum HDL-C. The level of TC, LDL-C and TGs were 25.0±5.8, 20.0±0.7 and 408.6±4.8 mg/dL respectively in control group, increased to 145.0±7.9.
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130.2±0.6 and 1029.1±6.8 mg/dL respectively in ACR treated group. On the other hand, HDL-C was significantly decreased from 5.7±0.5 mg/dL in control group to 3.9±0.6 mg/dL in ACR treated group (Table 1).

Supplementation of mice with potato chips fried in sunflower oil in FPSO group produced a significant increase in TC, LDL-C and TGs levels (P<0.05) when compared to control normal group but significantly decreased when compared to ACR group. The levels of lipid parameters in FPSO group were 90.8±3.2, 80.3±0.5 and 852.6±5.3 mg/dL for T-C, TGs and LDL-C, respectively. On the other hand, TC, LDL-C and TGs were significantly decreased to become 25.8±6.0, 20.6±0.5 and 412.3±4.0 mg/dL respectively in FPOO group when compared to both ACR treated group and FPSO group (Table 1).

Table (1): Lipid profile in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Control normal (n=6)</th>
<th>ACR control (n=6)</th>
<th>FPSO group (n=6)</th>
<th>FPOO group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>25.0±5.8</td>
<td>145.0±7.9</td>
<td>90.8±3.2</td>
<td>25.8±6.0</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>20.0±0.7</td>
<td>130.2±6.0</td>
<td>80.3±0.5</td>
<td>20.6±0.5</td>
</tr>
<tr>
<td>TGs (mg/dL)</td>
<td>408.6±4.8</td>
<td>1029.1±6.8</td>
<td>852.6±5.3</td>
<td>412.3±4.0</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>5.7±0.5</td>
<td>3.9±0.6</td>
<td>4.4±0.5</td>
<td>4.7±0.6</td>
</tr>
</tbody>
</table>

Data are presented as mean±SE.

ACR: Acrylamide, FPVO: Fried potato chips in vegetable oil, FPOO: Fried potato chips in olive oil TGs: Triglycerides, TC: Total cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol.

a. Significant versus normal control group at P<0.05.
b. Significant versus ACR treated group at P<0.05.
c. Significant versus FPVO supplemented group at P<0.05.

IV. Discussion

Despite the marketing requirements of fries potato chips as snacks meal; its acrylamide (ACR) metabolic activation leads to the generation of reactive oxygen species (ROS), in which the consumption of the chips is associated with some side effects. Reports from different studies have provided to the presence of ACR and its active metabolites which may be generated during the heating of specific foodstuffs as a result of Millard reaction between amino acids and sugars (Muttucumaru et al., 2013; Watzek et al., 2013).

Histological examination of liver sections of mice showed pathological changes of hepatocytes in ACR-treated groups as same as FPSO when compared to the control suggesting a cell damage caused by ACR intoxication. Smith et al since (1984), mentioned that the cellular hurt caused by toxic materials is mostly joined by enhancement in cell membrane permeability. Other biochemical point of view, Allam et al., (2010) explained that structural changes in the liver may be due to oxidative stress and disturbance of lipid and protein metabolism of rats treated by ACR. Veenapani et al. (2010) suggested that, following acute exposure to ACR, the hepatocytes are able to get damaged, while Kovac et al. (2015) reported that after sub-chronic consumption of ACR to immature rats prominently alter the microstructural features and functional status in the hepatocytes, with potentially very harmful hepatotoxic effects. This, together with ours, indicates possible cytotoxic effects of ACR in the mice liver.

Supplementation with FPOO resulted in a marked improvement in the hepatic histological overview, highlighting its protective potential. The results also indicate that inhibition of Kupffer cell hyperplasia plays an important role in the ameliorative effects of olive oil on liver fibrosis which may be occur by ACR consumption. Kupffer cells are the resident macrophages of the liver, which upon activation, release toxic cytokines and ROS that participate in ACR-induced liver injury. These findings are coincided with other different studies decided that agents selectively block Kupffer cell activation may provide effective prevention against the progression of fibrosis (Fang et al., 2008; Eidli et al., 2012).

The changes in the biochemical parameters are more sensitive clue and can share an important diagnostic tool in toxicological studies. The analysis of blood samples showed that both ACR and FPSO treatments altered the serum activities of AST and ALT enzymes when compared to the control normal. Serum ALT and AST are among most precise indicators of the liver damage. In a case of hepatocytes injury, the enzymes tend to leak out through the damaged cell membrane firstly into the extracellular space and then into the peripheral blood (Khalil et al., 2013). The AST enzyme is more indicative for mitochondrial damage, while ALT enzymes point to cell membrane impairment (Yousef and El-Demerdash, 2006). In addition, according to our results for FPOO group, the AST activity increased when compared to control normal group, decreased when compared with ACR group and non-significantly changed when compared to FPSO group that may be explained that mitochondria is subcellular organelle where ACR is still has a prominent toxic effect compared to olive oil and sunflower oil supplemented groups. It is important, furthermore, to point out that the levels of AST

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and ALT which ultimately reach the blood flow, and whose activities are lastly analyzed, may be conditioned either by their routes of clearance in the liver itself or by their different half-lives. It is suggested that the ALT is predominantly cleared by endothelial cells in liver while the clearance of AST uses more of Kupffer cells (Radi et al., 2011). If so, the results for AST presented in this study indicate to a decreased number of Kupffer cells in the mice liver which might be interpreted as hepatoprotective effect of olive oil.

A reduction of total antioxidant activity was observed in serum in ACR treated group, and this reduction was blocked in group ate potato chips fried in either sunflower oil or olive oil in this study. Veenapani et al., 2010 reported that ACR induced a reduction of glutathione levels in liver parenchymal cells which potentially may result in oxidative stress, leading to cell necrosis that was appeared also in the histopathological profile of liver in our study. By comparing FPOO group with FPSO group, there was a significant elevation in total antioxidant. It could be suggested that olive oil scavenges free-radical generation and inhibits ACR–induced injury in hepatic tissues more than sunflower oil.

However, Rangel-Zuñiga et al., 2016 recently has been shown that the content of polar compounds in frying oils has been associated with endothelial dysfunction, and that frying oils rich in phenolic compounds of olive origin, reduce inflammatory response as compared to sunflower oil intake. Also, It has also been shown that the use of sunflower oil as a frying oil increases oxidative stress as compared with virgin olive oil and with oil models with added antioxidants (Napolitano et al., 2008).

Juáñiz et al., (2016) represented that, the use of olive oil for frying resulted in a higher increase of flavonoids than the use of sunflower oil in which all heat treatments tended to increase the polyphenols content in vegetables suggesting a thermal destruction of cell walls and sub cellular compartments during the cooking process that favor the release of these compounds.

ACR treated mice in our study showed significant change in serum lipid profile levels compared to the control normal group which supported the change in permeability of cells as reported previously. Supplementation of mice with potato chips fried in sunflower oil in FPSO group produced a significant increase in TC, LDL-C and TGs levels when compared to control normal group but significantly decreased when compared to ACR group. On the other hand, TC, LDL-C and TGs were significantly decreased in FPOO group when compared to both ACR treated group and FPSO group. It is well established that fatty acids composition such as Omega-9 (oleic acid) or omega-6 ((linoleic acid) is crucial in explaining the differential effects of dietary oils on plasma parameters such as the lipid profile (Sebbagh et al., 2009). Omega-9 is a monounsaturated fatty acid found in olive oil and, Omega-6 is a polyunsaturated fatty acid found in sunflower oil both lowers the risk of heart attack as well as atherosclerosis. It also helps to prevent cancer (Oluwakemi et al., 2016). On the other hand, it has been reported that olive oil polyphenols can inhibit HMG-CoA reductase and may play an important role in the prevention of cardiovascular diseases more than sunflower oil (Farrás et al., 2013). It is well known that the increased consumption of MUFA instead of polyunsaturated fatty acids (PUFA) reduces the risk of atherosclerosis because it decreases the sensitivity of the circulating lipoprotein to peroxidation (Ghorbel et al., 2015).

V. Conclusion

The present study revealed that potato chips fried in olive oil protects against liver toxicity which may occur through ACR development. It could be recommended in accordance with ours and other studies that used olive oil or other antioxidant substances, such as colocynth oil (Amamou et al., 2015), n-acetylcysteine (Liu et al., 2016), and vitamins E and C, which all of decreased the severity of hepatic fibrosis.

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