Hepatoprotective Effect of Olive and Coconut Oils against Oxidative Stress-Induced by 2, 4-Dichlorophenoxyacetic Acid

1. INTRODUCTION
Oxidative stress produced by free radicals has been linked to the development of several diseases such as cardiovascular, cancer, and neurodegenerative diseases [1]. However, reactive oxygen species (ROS) are constantly formed by by-products of normal metabolic reactions and their formation is accelerated by accidental exposure to occupational chemicals like pesticides.

Since 2, 4-dichlorophenoxyacetic acid (2, 4-D) is a common herbicide used around the home and garden, on golf courses, ball fields, parks, in agriculture and forestry [1]. It is a moderately persistent chemical with a half-life between 20-200 days. Unfortunately, the herbicide does not target only weeds. It can cause low growth rates, reproductive problems, changes in behavior, or death in non-tagged species [2]. 2, 4-D is easily absorbed into the human organism from the alimentary tract and skin and is subsequently excreted in the urine in nearly unchanged form [3]. Several reports have shown that 2, 4-D produces oxidative stress and/or depletes antioxidants both in vitro and in vivo. Recently, there is growing evidence that ROS contribute to organ injury in many systems including heart, liver and central nervous system. Erythrocytes are equipped by many defence systems representing their antioxidant capacity. This protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione, glutathione peroxidase (GPx), glutathione-S-transferase, and glutathione reductase (GR). However, the cellular antioxidant action is reinforced by the presence of dietary antioxidants [4].

Olive oil is an integral ingredient in the Mediterranean diet. There is growing evidence that it may have great health benefits including the reduction in coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses [5, 6]. Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of Phytochemicals, including polyphenolic compounds [7, 8]. It is known that an increased consumption of monounsaturated fatty acids (MUFA) instead of polyunsaturated fatty acids (PUFA) reduces the risk of atherosclerosis because it decreases the circulating lipoprotein's sensitivity to peroxidation [9]. Furthermore, the dietary MUFA healthy effects were attributed to decreased endothelial activation [10], and LDL susceptibility to oxidation [11]. In recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. In experimental studies, olive oil phenolic compounds showed strong antioxidant properties against lipids, DNA and LDL oxidation [12]. Hydroxytyrosol (2-(3-4 dihydroxyphenyl) ethan-1-ol, DPE), one of the phenolic compounds present in extra virgin olive oil, has been suggested to be a potent antioxidant, thus contributing to the beneficial properties of olive oil [13].

Coconut oil has been used for many centuries as a cooking medium in many Asian countries. Considering its high content of saturated fatty acids, it is classified as mainly saturated oil. The oil contains approximately 92.1% saturated fatty acids of medium chain fatty acids in the form of triglycerides, 6.2% monounsaturated fatty acids, and 1.6% polyunsaturated fatty acids consisting of oleic and linoleic acids as triglycerides. Coconut oil administration was shown to increase the activity of the antioxidant enzyme SOD, which is known to be protective against reactive oxygen species [14].

Studies done on native diets high in coconut oil consumption show that this population is generally in good health. The oil has small amounts of tocopherols and tocotheriols and phyto-sterols and it gets easily absorbed in the body [15].

Virgin coconut oil (VCO) is one type of coconut oil that has recently gain a lot of attention due to various claimed medicinal values, such as antioxidant, antimicrobial, antiviral, antihypercholesterol and antithrombotic activities. Moreover, administration of VCO is capable of increasing antioxidant enzyme activities and reduces lipid peroxidation content [16].

This study was conducted to investigate the effect of dietary supplementation of olive and coconut oils on oxidative stress and liver enzyme activities of 2,4-D-treated rats by assessing some markers of oxidative stress and also some liver function tests.

MATERIALS AND METHODS
Supplements and chemicals:
Olive and coconut oils were purchased from local markets, Cairo, Egypt.

KEYWORDS
2, 4-dichlorophenoxyacetic acid, olive, coconut, liver function

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ABSTRACT
The effect of dietary olive and coconut oils on liver damage and oxidative stress induced by 2, 4-Dichlorophenoxyacetic acid (2, 4-D) in rats was evaluated. 48 male adult albino rats were divided into 6 groups (8 for each group). Group 1: rats fed on basal control diet, group 2: rats fed on control diet and administrated orally 24 mg 2, 4-D/kg b.wt/twice a week, groups 3 and 5: rats fed on virgin olive diet or coconut diet respectively, groups 4 and 6: rats fed on olive diet or coconut diet respectively and administrated 25 mg 2, 4-D/kg b.wt/ twice a week for six weeks. Results: A significant liver damage was observed in rats treated with 2, 4-D via an increase in serum transaminases and alkaline phosphatase enzymes activities, hepatic lipid peroxidation and liver free fatty acids, while serum total protein and albumin, hepatic superoxide dismutase and glutathione peroxidase enzymes activities were significantly reduced with the presence of some inflammmations and necrosis in liver sections of 2, 4-D treated rats. However, virgin olive and coconut oils administration during 2, 4-D treatment induced an improvement in hepatic antioxidant enzymes, serum transaminases activities and liver free fatty acids levels. Biochemical results were confirmed by the liver histopathological examination. Conclusion: olive oil and coconut oil have protective effect against oxidative stress and liver damage induced by 2, 4-D.
2, 4-Dichlorophenoxyacetic acid was obtained from Sigma-
Aldrich. Co.

Diets: The diets were prepared according to the formula of (AIN-76) and modified by Benson and Devi [17]. Dietary oil was used at a dose of 10% (w/w). Three diets were used in this study as follows:

Diet 1: Control diet (CO diet) which contain corn oil, diet 2 (OL diet) and diet 3 (COC diet) contain the same previous components of control diets, while containing either virgin olive oil or virgin coconut oil respectively instead of the oil of the control diet. Diets were prepared freshly every week and were stored in a refrigerator at (2-8°C).

Experimental animals: Forty eight male Sprague Dawley rats weighing from 150-180 g were obtained from the Egyptian Organization of Biological Products and Vaccines (Helwan, Egypt). Upon arrival, the rats were allowed to acclimatize for one week. They were fed a control basal diet and water ad libitum. Animals were housed in stainless steel caged and light-controlled room.

Experimental design: Animals were divided into 6 groups (8 for each group) as follows:

Group 1: served as normal control and kept on basal diet (CO diet). Group 2: was fed on (CO diet) and administrated 24 mg 2, 4-D/kg body weight/ twice a week orally (representing 1/16.7 of oral DL 50) dissolved in 1 ml sunflower oil [18]. Group 3: was fed on (OL diet). Group 4: was fed on (OL diet) and administrated orally 24 mg 2, 4-D/kg body weight twice a week. Group 5: was fed on (COC diet). Group 6: was fed on (COC diet) and administrated 24 mg 2, 4-D/kg body weight twice a week orally. Rats had free access to food and water for 4 weeks. At the end of the experiment, all rats were sacrificed after fasting overnight; the blood samples were collected from portal vein into tubes to separates serum by centrifugation and kept at -20°C for biochemical analysis.

The rat’s Liver were removed immediately and washed in normal saline and homogenized in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 4000×g for 10 min at 4°C and supernatant were used for measurements. A portion of the liver was fixed in 10% buffered formalin for histopathological assessment.

Biochemical analysis: Serum alanine transaminase (ALT), aspartate transaminase (AST) activities were assayed according to Henry et al. [19], serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities were assayed according to Young [20] and Saw et al. [21] respectively. Serum total protein and albumin were determined calorimetrically according to the methods described by Weichselbaum [22] and Doumas et al. [23] respectively. Total and direct bilirubin was measured in serum using colorimetric kits according to Young [24]; Serum total antioxidant capacity was assayed according to the method described by Koracevic et al. [25]. Liver lipid peroxidation levels was measured as thiobarbituric reaction substances (TBARS) following the methods described by Nichams and Samuelson [26], glutathione peroxidase (G PX) level was measured in liver according to the method described by Ursini et al. [27]. Superoxide dismutase was measured in liver according to the method described by Oyanagui [28]. Liver free fatty acids were assayed according to the methods described by Falholt et al. [29].

Histopathological examination Liver sections were fixed in 10% buffered formalin (PH: 7.4), dehydrated in graded ethanol cleared in xylene and embedded in paraffin. Sections 4-5µ thick was prepared stained with hematoxyline and Eosin (H&E) and examined under light microscopy at 400X magnification [30].

Statistical Analysis: The data were statistically analyzed using two way analysis of variance (ANOVA) according to Bailey [31]. The data are expressed as means ± SD. The difference among means were tested using the least significant differences (LSD). P-values ≤ 0.05 were considered statistically significant.

RESULTS

Serum AST, ALT, ALP and GGT enzymes activities of healthy and 2, 4-D- treated rats administered olive and coconut oils.

The extent of liver damage sustained following exposure to 2, 4-D is shown in Table 1. The serum alanine transaminases, aspartate transaminases, alkaline phosphatase and gamma-glutamyl transferase activities were used as biochemical markers for the early acute hepatic damage. The serum levels of AST, ALT, ALP and GGT activities were significantly higher in 2, 4-D-treated rats compared to healthy control group. The increases of their activities were markedly reduced in the presence of olive oil and coconut oil (p<0.05) while bringing back their rates towards the normal level found in the healthy control group. Although AST, ALT and ALP activities did not alter significantly following coconut oil administration alone to healthy treated rats, a significant decrease of GGT activity was noted in healthy animals treated with olive and coconut oils compared to the healthy control.

Serum total proteins, albumin, globulin, albumin/ globulin (A/G ratio), total and direct bilirubin of healthy and 2, 4-D- treated rats administered olive and coconut oils.

Exposure of rats to 2,4-D resulted in the significant decline of serum total protein, albumin and A/G ratio levels by -16.36%, -29.63% and -26.27%, respectively, compared to controls (Table 1). In contrast, treatment of either healthy or 2, 4-D treated rats with olive oil or coconut oil raised the levels of total protein, albumin and A/G ratio, but the increase was not statistically significant in case of total protein and A/G ratio, when compared to their corresponding control groups. Globulins levels showed no significant difference between all groups.

Our results also revealed that 2, 4-D caused a statistically significant increase in total and direct bilirubin compared to healthy control group. However, the administration of olive or coconut oils to healthy and 2, 4-D treated rats led to a statistically significant decrease in these values (p<0.05), compared to corresponding control group, while The decrease was not statistically significant in case of healthy rats treated with coconut oil as compared with healthy control.

Liver superoxide dismutase (SOD), glutathione peroxidase (GPX) activity and thio-barbituric acid reactive substances (TBARS) level of healthy and 2, 4-D- treated rats administered olive and coconut oils:

The oxidative stress in the liver tissue was assessed by measuring the levels of TBARS and antioxidant defense enzymes viz., SOD and GPX activities in treated groups. The levels of liver TBARS were significantly elevated in 2, 4-D treated animals by 44.26%, when compared to levels in normal healthy animals (Table 2). The elevated levels of TBARS were significantly reduced when olive and coconut oils were added to the diets of 2, 4-D treated groups by 10.61% and 7.2% respectively (Table 2). The activity of liver antioxidant defense enzymes viz., SOD and GPX were significantly decreased by 28 %, and 44.53% respectively in the liver tissue of 2, 4-D treated control animals, when compared to levels in normal healthy animals. When olive and coconut oils were administered to healthy and 2, 4-D treated groups, the levels of antioxidant enzymes were significantly increased as compared to corresponding control groups.
Serum total antioxidant capacity (TAC) of healthy and 2, 4-D- treated rats administered olive and coconut oils.

As cited in table (2), TAC concentration was markedly decreased in 2, 4-D treated rats (0.93±0. 06 mmol/l) (p<0.05) as compared to normal control rats (1.44±0.09 mmol /l). Treatment with either olive or coconut oils prohibited 2, 4-D-induced small production of reactive species and leads to significant increase in TAC level by about 30% and 20.4% respectively as compared to 2, 4-D treated group alone. Furthermore the addition of olive and coconut oils to the diets of healthy rats caused statistically significant increase in TAC level as compared with healthy control.

Liver free fatty acids (FFAs) in healthy and 2, 4-D - treated rats administered olive and coconut oils.

As cited in table (2), FFAs concentration were increased in 2, 4-D treated rats (443.18±9.47 ng / g wet tissue) (p<0.05) as compared to healthy control rats (362.76±3.51 ng / g wet tissue). Treatment with either olive or coconut oils prohibited 2, 4-D, which induced lipids breakdown and leads to significant decline in FFAs level as compared to 2, 4-D treated control group. Healthy rat’s administrated olive or coconut oils at the same level also showed a significant decline in liver FFAs concentration.

Histopathological examination of liver tissues in healthy and 2, 4-D- treated rats administered olive and coconut oils:

The histopathological examinations of liver sections are presented in fig. (1-4). Fig. (1) showed that liver sections of healthy rats looked normal with regular cellular architecture. In the hepatotoxic group, the histology (fig. 2) confirmed liver damage as evidenced by the presence of inflammation and necrosis in the respective liver sections. The rats in olive and coconut oils-treated groups (fig. 3 and 4) exhibited significantly lesser pathology as compared to the extensive liver damage found in the hepatotoxic group. Olive and coconut oils administration prevented the swelling, lymphocytes infiltration, hepatic necrosis, and fibrous connective tissue proliferation induced by 2, 4-D. Consequently, the liver tissue preserved its nearly normal hepatic lobular architecture with central veins and radiating hepatic cords. These results reconfirmed the protective functions of olive and coconut oils against 2, 4-D induced liver damage. Healthy rats treated with olive and coconut oils showed no histopathological changes as compared with healthy control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Olive oil</th>
<th>Coconut oil</th>
<th>Control</th>
<th>Olive oil</th>
<th>Coconut oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein(g/dl)</td>
<td>Healthy rats</td>
<td>6.54±0.29a</td>
<td>6.92±0.6b</td>
<td>6.59±0.46c</td>
<td>5.47±0.47b</td>
<td>5.77±0.53c</td>
<td>5.94±0.50b</td>
</tr>
<tr>
<td></td>
<td>2,4-D treated rats</td>
<td>3.51±0.33b</td>
<td>3.92±0.25c</td>
<td>3.53±0.26b</td>
<td>2.47±0.21d</td>
<td>3.02±0.21c</td>
<td>2.84±0.23c</td>
</tr>
<tr>
<td></td>
<td>L.S.D (P≤0.05)</td>
<td>3.04±0.38</td>
<td>3.0±0.77</td>
<td>3.06±0.63</td>
<td>3±0.59</td>
<td>2.75±0.51</td>
<td>3.1±0.58</td>
</tr>
<tr>
<td></td>
<td>A/G ratio</td>
<td>1.18±0.24ab</td>
<td>1.39±0.37a</td>
<td>1.22±0.36bc</td>
<td>0.87±0.27c</td>
<td>1.13±0.23abc</td>
<td>0.96±0.24bc</td>
</tr>
<tr>
<td></td>
<td>Total bilirubin(mg/dl)</td>
<td>0.36±0.03d</td>
<td>0.3±0.04e</td>
<td>0.32±0.03e</td>
<td>0.99±0.07f</td>
<td>0.59±0.03g</td>
<td>0.66±0.02h</td>
</tr>
<tr>
<td></td>
<td>Direct bilirubin(mg/dl)</td>
<td>0.18±0.01i</td>
<td>0.163±0.01j</td>
<td>0.174±0.003ik</td>
<td>0.472±0.03l</td>
<td>0.263±0.02m</td>
<td>0.303±0.01n</td>
</tr>
</tbody>
</table>

There were no significant difference between means had the same letter in the same row (p≤0.05).

Table (2): Effects of olive and coconut oils on liver (SOD), (GPX), (TBARs) , free fatty acids and serum ( TAC) . (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Olive oil</th>
<th>Coconut oil</th>
<th>Control</th>
<th>Olive oil</th>
<th>Coconut oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD(U/mg tissue)</td>
<td>Healthy rats</td>
<td>222.74±7.07b</td>
<td>238.44±3.71a</td>
<td>223.15±4.56a</td>
<td>160.35±3.30c</td>
<td>193.66±2.95c</td>
<td>188.93±3.22d</td>
</tr>
<tr>
<td></td>
<td>2,4-D treated rats</td>
<td>5.3±0.09c</td>
<td>5.86±0.09a</td>
<td>5.54±0.14b</td>
<td>2.94±0.27d</td>
<td>4.13±0.13d</td>
<td>3.59±0.11e</td>
</tr>
<tr>
<td>GPX (µmol/mg tissue)</td>
<td>Olive oil</td>
<td>0.183±0.003d</td>
<td>0.173±0.007f</td>
<td>0.177±0.002g</td>
<td>0.264±0.003h</td>
<td>0.236±0.003i</td>
<td>0.245±0.003j</td>
</tr>
<tr>
<td></td>
<td>Coconut oil</td>
<td>362.76±3.51d</td>
<td>34.34±3.22e</td>
<td>351.15±1.93c</td>
<td>443.18±9.47i</td>
<td>408.09±4.26i</td>
<td>418.75±4.3h</td>
</tr>
<tr>
<td>TBA(µmol/mg tissue)</td>
<td>Olive oil</td>
<td>1.44±0.86h</td>
<td>1.59±0.102i</td>
<td>1.54±0.08j</td>
<td>0.93±0.06k</td>
<td>1.21±0.07l</td>
<td>1.12±0.06m</td>
</tr>
<tr>
<td></td>
<td>Coconut oil</td>
<td>0.87±0.06c</td>
<td>1.59±0.102i</td>
<td>1.54±0.08j</td>
<td>0.93±0.06k</td>
<td>1.21±0.07l</td>
<td>1.12±0.06m</td>
</tr>
</tbody>
</table>
Fig (1): Liver of rat from healthy control group showing no histopathological changes (H&E x400)

Figure (2): Liver of rat from 2, 4-D treated rats showing Kupffer cells activation and necrosis of sporadic hepatocytes (H&E x 400)

Fig (3): Liver of rat from 2, 4-D treated rats with olive oil showing slight activation of Kupffer cells (H&E x 400)

Fig (4): Liver of rat from 2, 4-D treated rats with coconut oil showing slight activation of Kupffer cells (H&E x 400)

DISCUSSION

2, 4-Dichlorophenoxyacetic acid is a selective, systemic auxin–type herbicide most widely and successfully used throughout the world. Sources of exposure to 2, 4-D is during manufacturing conditioning and in agriculture during spraying the fields [18]. Several reports have shown that 2, 4-D produces oxidative stress and/or depletes antioxidants in vitro and in vivo [32].

The increasing popularity of olive oil and coconut oil is mainly attributed to its antioxidant and anti-inflammatory effects which may help prevent disease in humans [33]. In the current study, an attempt has been made to assess the hepatoprotective potential and the antioxidant effects of olive oil and coconut oil supplementation for animals' subjected to 2, 4-D intoxication.

Our study confirmed that treatment with 2, 4-D resulted in structural liver damage by the increase of serum AST, ALT, ALP and GGT activities and changes in antioxidant enzyme leakage by increasing TBARS level with a decrease in SOD and GPX activities in liver and decreasing serum TAC. Also the increased level of total and direct bilirubin in association with decreased serum total protein and albumin revealed hepatic damage in the 2, 4-D treated group.

In agreement with our results Celik et al. [34] found that the administration of 1.5 and 3mg/d of 2, 4-D for 25 days to rats might affect antioxidant potential enzymes.

It has been reported that liver reduced and total glutathione statistically decreased after 6 months of herbicide 2,4-D exposure. Also whole blood SOD activity raised in toxic exposed group compared to controls, and this is due to the effect of 2, 4-D which enhances lipid peroxidative processes [18].

The study of Palmeira et al. [35] have looked at the in vitro effects of 2, 4-D on the generation of oxidative stress, either at the mitochondrial level in hepatocytes or in red blood cells.

In fact, the enhanced activities of transaminases (AST and ALT), ALP and GGT, and the increased level of total bilirubin revealed hepatic dysfunction in the 2, 4-D-treated groups [36].

The administration of olive and coconut oils to 2, 4-D treated rats caused a modulation in the activity of the above enzymes and lipid peroxidation, which may have resulted from the stabilizing of plasma membrane as well as the repair of the hepatic tissue damage.

Some findings demonstrate that olive phenolics are powerful antioxidants, both in vivo and in vitro, and posses other potent biological activities that could partially account for the observed healthful effect of Mediterranean diet [37].

Several studies have demonstrated the ability of olive oil to inhibit oxidative stress in the liver through various mechanisms. The mechanism proposed to explain the positive effects of olive oil may be attributed to its richness in MUFA, mainly oleic acid which has different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria [38].

Coconut oil administration was shown to increase the activity of the antioxidant enzyme SOD, which is known to be protective against reactive oxygen species. In according with our findings Nandakumarani et al. [14] confirmed that coconut oil, by reducing oxidation of the LDL moiety mediated through reactive oxygen, could play a beneficial role in preventing the formation of plaques and could in fact be antiatherogenic.

The significant decrease in the levels of biochemical marker enzymes like ALT, AST, ALP and bilirubin in olive and coconut oils administered animals might be due to decreased leakage of the enzymes in liver cells. This suggests that the oils used in this study could repair the hepatic injury and/or restore the cellular permeability, thus reducing the toxic effect of 2, 4-D induced liver toxicity and preventing enzymes leakage into the blood circulation.

The hepatoprotective activity, which is seen at the highest concentration (10ml/kg) of virgin coconut oil (VCO) used, was also accompanied by the ability of VCO to reduce the serum level of ALT, AST, and ALP increased after pre-treatment with paracetamol [39].
The depletion of the elevated bilirubin level together with the suppression of ALP activity in the serum of rats treated with olive oil suggested that biliary dysfunction of the rat's liver during sub acute injury with 2; 4-D has been stabilized [36].

The significant decrease in the free fatty acid in liver of olive oil and coconut oil administrated groups might be due to decreased lipid breakdown, which corroborates with the results obtained where in a decreased lipid peroxidation and increased activity levels of antioxidant defense enzymes.

A marked improvement was detected in the hepatic fatty acid composition of rats supplemented with olive oil and the two fractions compared to controls and 2,4-D-treated animals [1].

Histopathological examination of rats liver in our study showed that olive and coconut oils caused an amelioration of liver damage induced by 2, 4-D administration.

Conclusion: The results of the present study showed that olive oil and coconut oil have protective effect against oxidative stress and liver damage induced by 2, 4-D by preventing excessive lipid peroxidation and by maintaining hepatic antioxidant enzyme activities at near normal concentrations.

REFERENCE