Antioxidant Activity and Potential Hepato-Protective Effect of Saudi Olive Leaf Extract

Ismail Hamad

Abstract — The objectives of this study were to examine the antioxidant activity; the total phenol content of olive leaf methanol extract (OLME). The potential protective effect on hepatic damage induced by carbon tetrachloride in rabbits was also addressed. The antioxidant activity of OLME was evaluated using DPPH radical scavenging capacity assay and cupric reducing antioxidant capacity assay. Using rabbits as a model organism, the effects of OLME on scavenging capacity assay and cupric reducing antioxidant capacity antioxidative activity of OLME was evaluated using DPPH radical induced by carbon tetrachloride in rabbits was also addressed. The antioxidant activity; the total phenol content of olive leaf methanol extract showed high phenol content. The methanol extract showed a significant concentration dependent reducing activity. Pretreatment with OLE, as well as with Silymarin, significantly attenuated the CCl4 induced hepatotoxicity. Results presented here indicate that Olive leaf cultivated in Aljouf region, Saudi Arabia possess strong antioxidant activity, and has protective activity against CCl4-induced hepatotoxicity in rabbits, possibly related to its antioxidant activity.

Keywords — Antioxidant activity, Hepato-protective, Olive leaf.

I. INTRODUCTION

It is well established that, reactive oxygen species (ROS) can cause damages to biomolecules and thereby compromise cell viability [1].

Increased production of ROS plays an important role in the pathogenesis of many diseases including; cancer, cardiovascular diseases, Down's syndrome, Friedreich's ataxia, rheumatoid arthritis, autoimmune diseases and acquired immunodeficiency syndrome. Oxidative damage is also emerging as an important factor in mutagenesis, tumorigenesis, ageing and age-related disorders such as Parkinson's and Alzheimer's diseases[2].

The ability of exogenous antioxidants to prevent or suppress development of oxidative stress-related diseases are of confirmed by several epidemiological studies, therefore, there is a growing interest in natural phenolic antioxidants present in medicinal and dietary plants that might help preventing or decreasing oxidative damages without exerting harmful side effects [3].

Several studies have examined the antioxidant activity of OLE growing in various region of the world [4]-[5]. But there are no detailed reports on antioxidant activity of OLE growing in Saudi Arabia.

Animal studies using OLE or their constituents have showed hypoglycemic [6], cholesterol lowering [7], anti-atherosclerotic [8], antimicrobial [9], anti-viral [10], and anti-tumor [11] activities. OLE attenuates early diabetic neuropathic pain through prevention of high glucose-induced apoptosis [12]. Olive leaf extract also attenuates Cardiac, Hepatic, and Metabolic Changes in High Carbohydrate, High fat–fed rats [13].

The main objective of this study was to evaluate the antioxidant activity and the potential hepato-protective effect on CCl4-induced hepatic damage in rabbits of olive leaf extract from Aljouf region, Kingdom of Saudi Arabia.

II. MATERIALS AND METHODS

A. Materials

All chemicals used are of analytical grade and were obtained from Riedel and Scharlau, Germany and LobaChem, India. Olive leaves were collected from olive trees in Sakaka city, Aljouf region, Saudi Arabia.

B. Preparation of Olive Leaf Methanol Extract

Leaves were separated from branch, washed and air dried. Dried leaves were ground to fine powder by blender. 25 g of dried samples were mixed with 250 ml of 80% methanol and shaken at room temperature for 24 h using. The mixture was filtered and centrifuged (5000 rpm for 5 minutes). The supernatant was concentrated in oven at 60 °C. The dry extract was kept frozen at 4 °C in dark tubes.

C. Estimation of Total Phenol Content

Total phenol content of the methanol extract of OLME was estimated by using Folin-Ciocalteu reagent [14]. The original procedure was modified to use with total volume of 1.5 ml instead of 5 ml used in the original method. Briefly 0.15ml of the sample dissolved in MeOH was incubated with 0.75 ml of 10% FC reagent and 0.6 ml of 7.5% Na2CO3 for 30 min at room temperature. The absorbance of the color developed was measured at 765 nm against blank. The total phenolic content was expressed as Gallic acid equivalents (GAE) using a standard curve generated with Gallic acid as standard.

D. Radical Scavenging activity assay

The ability of OLME to scavenge the “stable” free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Cheung et al. [15] with slight modifications. Briefly, various concentrations of OLME (0.2 ml) were mixed with 0.8 ml of methanol solution containing DPPH radicals (0.1 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was determined at 520 nm. IC50 values, which represent the
concentration of the extract or Gallic acid that causes 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

E. The Cupric Ion Reducing Capacity (CURAC Assay)

The cupric ion reducing antioxidant capacity of OLME was determined according to the method presented by Apak et al. [16]. The original procedure was modified to use with total volume of 1.5 ml instead of 4.1 ml used in the original method. Briefly, 0.036 ml of sample extract was mixed with 0.366 mL each of Cu II acetate solution (10mM), neocuproine alcoholic solution (7.5 mM), and NH4Ac(1M, pH 7.0) buffer solution and 0.366 mL of water to make the final volume 1.5 ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of ascorbic acid

F. Animals

24 adult male white New Zealand rabbits were obtained from Aljouf University animal house and housed individually in stainless steel wire bottom cages with a control environment (25 °C, 50–60% humidity, 12 h light per day) for one week of acclimatization. The animals will be fed a standard laboratory diet. Tap water will be supplied in free access.

All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals [17], and were approved by the Bioethical Committee of the Ajlouf University.

G. Experimental Design

The animals were randomly divided into five groups (A, B, C, D and E) with each consisting of 6 rabbits. OLME, CCl4 and Silymarin were administered to animals using gavage. Group A: basal diet for a period of 4 weeks, Group B: basal diet (with the addition of olive leaf extract) at doses of 50 mg/kg body weight for a period of 4 weeks. Groups C were given carbon tetrachloride at a dose of 1 mL in corn oil (50%), twice a week for a period of 4 weeks. Groups A and B were received corn oil as vehicle control. Groups D were administered olive leaf extract (50 mg/kg body weight) daily for 4 weeks and 2 hours before the administration of the oral dose of carbon tetrachloride. Groups E was administered Silymarin (standard hepato-protective drug, 100 mg/kg body weight) daily for 4 weeks and 2 hours before the administration of the oral dose of carbon tetrachloride.

After completion of treatment period, blood samples from all rabbits were collected at 4000g for 15min. Serum samples were stored at -4°C until analysis. Serum samples were analyzed using Chemistry Auto-analyzer (MINDRAY BS-300, CHINA) to determine aspartate transferase (AST), alanine transferase (ALT) according to the manufacturer’s instructions.

H. Statistical Analysis

Experimental analyses were performed in duplicate. Data were recorded as mean ± standard deviation and analyzed by Graphpad prism version 5

III. RESULTS AND DISCUSSION

Phenolic compounds have been shown to be responsible for the antioxidant activity of plant materials [18]. Therefore, the amount of total phenol in the methanol extract of olive leaves were determined using Folin-Ciocalteu using Gallic acid as a standard.

The methanol extract of olive leaves was shown to be very rich in phenol content (79.84 ± 13.45 mg GAEq/g dw). The amounts of phenols determined in the present study are in good agreement with Orak et al. [19] who studied the total phenol contents from 21 different olive tree cultivars and found that the highest total phenolic content was (102.69±1.63 mg GAEq/g dw) and the lowest content was (78.52±2.18 mg GAEq/g dw). Although it was lower than those reported by [20] in which total phenol content of olive leaf extract was 144 mg/g dw. Differences observed may be related to the use of different cultivars and different procedures of extraction.

In this study, different concentrations of the olive leaf extracts (50, 100 and 200 μg/mL) exhibited a strong DPPH scavenging activities and the EC50 values (half maximal effective concentration) of OLME was calculated (Table1) (EC50 value of Saudi OLME was 175.67μg/ml). The DPPH scavenging activity of Saudi Methanol OLE was more significant than that reported by Kaied et al. [12] who reported that EC50 value of olive leaf ethanol extract was 231.62μg/ml. This difference should be the result of differences between cultivars and the differences in the extracting solvents.

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<th>Sample</th>
<th>DPPH Scavenging Activity</th>
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<tr>
<td></td>
<td>Conc. (μg/ml)</td>
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<tr>
<td>OLME</td>
<td>200</td>
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<tr>
<td></td>
<td>100</td>
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<tr>
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<td>50</td>
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The reducing power of compound serve as a significant indicator of its potential antioxidant activity, because its associated with their ability to donate electron to free radical species, reducing them into more stable and un-reactive form.[21]. Therefore the reducing power of OLME was investigated using cupric reducing antioxidant capacity (CURAC) assay.

The reducing power of the OLME increased with concentration but it was below the reducing power capacity of ascorbic acid (Figure1). The obtained result is in a good agreement with Ferreira et al. [22] who investigated the reducing power of olive leaf extract using the ferricyanide assay.
Cupric reducing antioxidant capacity of OLME

CCl4 has been widely used for creating acute viral hepatitis in model animals [23]. Serum enzyme activities of aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) were measured to assess hepatic function. ALT and AST activities were measured using Chemistry Auto-analyzer (MINDRAY BS-300, CHINA), using Mindray kits, according to the manufacturer’s instructions. ALT and AST activities were expressed as U/L (Figure 2 and 3 respectively).

![Fig. 2 Effect of OLE on serum ALT in CCL4 intoxicated rabbits](image2)

![Fig. 3 Effect of OLE on serum AST in CCL4 intoxicated rabbits](image3)

IV. CONCLUSION

The obtained results showed that olive leaf methanol extract cultivated in Saudi Arabia have very high phenol content and possess strong antioxidant activity and significant effect on liver damages induced by CCl4 administration, which result in improved serum ALT and AST and increased serum total antioxidant capacity in comparison with CCl4 treated group.

REFERENCES


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