HEPATOPROTECTIVE EFFECTS OF OLEUROPEIN, THYMOQUINONE AND FRUIT OF PHOENIX DACTYLIFERA ON CCL₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Chronic liver diseases commonly result in liver fibrosis and eventually liver cirrhosis. It has been demonstrated that oxygen-derived free radicals and lipid peroxidation play a critical role in the pathogenesis of various liver diseases including hepatic fibrosis. Our research aim was the evaluation of the Active ingredients of Olive, Nigella Sativa, and fruit of Phoenix dactylifera as hepatoprotective against hepatocellular damage induced by hepatotoxins. In this study the effect of Thymoquinone, Oleuropein and fruit of Phoenix dactylifera were investigated in rats affected by hepatotoxicity and cirrhosis as a result of administration of a chemical substance (CCL₄). Rats were divided into five groups 10 rats in each. The level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was determined, regarding its relation to liver damage. Liver tissues were investigated to compare between healthy and infected ones. It was found that Thymoquinone, Oleuropein and Phoenix dactylifera fruit significantly revised the changes produced by CCL₄ on hepatic cells and enzymes.

Key words: Oleuropein, Nigella sativa, Phoenix dactylifera, Hepatotoxicity.

INTRODUCTION

It is well known that the hepatotoxic effect of Carbon Tetra Chloride CCL₄ is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of
the mechanisms of hepatoprotective drugs[1]. CCl4 also known as Macrophage inflammatory protein-1β (MIP-1β) is a CC chemokine with specificity for CCR5 receptors. It is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells[2]. CCL4 Carbon tetrachloride (CCl4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals[3]. The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in adequate, so far not yet any therapy has successfully prevented the progression of hepatic disease[4]. Liver disease is a serious health problem because the liver is an important organ for the biotransformation and detoxification of endogenous and exogenous harmful substances. Steroids, vaccines, and antiviral drugs, which have been used to treat liver diseases, have potential adverse effects, especially when administered long-term [5].

*Nigella sativa* (of the family ranunculaceae) is a plant that is synonymous with nigella cretica and is commonly called black cumin, fennel flower, or nutmeg flower despite being wholly unrelated to the common cumin (Cuminumcyminum), fennel (Foeniculumvulgare), and nutmeg (the Myristica genus). Other names include Kalonji seeds[6], and Ajaji, black caraway seed, and HabbatuSawda[7]. It appears to be a fairly well regarded medicinal herb, with some religious usage calling it the 'remedy for all diseases except death' (*Prophetic hadith*) [8], and Habatul Baraka "the Blessed Seed". [7]. The seeds are the main medicinal component, although a seed oil pressed from the seeds (Black Cumin Oil or Black Seed Oil) also shares the same bioactives.

Medicinal usage of these seeds mostly centers around diarrhea and abdominal pain, dyslipidaemia,[9] asthma and coughs,[7] headache, dysentery, renal calculi,[7] infections, obesity, back pain, hypertension, and dermatological problems [10,11,12].

The seed of *Nigella sativa* L (NS), an annual *Ranunculaceae* herbaceous plant, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma. NS contains more bthan 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% Thymoquinone (TQ) and 46% many monoterpenes such as p-cymene, and α-pinene [13].

TQ has an antioxidant potential [14] and possesses superoxide anion radical-scavenging ability in vitro and vivo [15]. It was reported that TQ protects isolated rat hepatocytes against CCl4-induced hepatotoxicity by preventing the depletion of intracellular GSH and thus
maintain the integrity of cell membrane [16,17]. The aqueous extract of *N. sativa* has been suggested to possess antioxidant property and reduces the hepatotoxicity against CCl4-induced liver damage [18].

Oleuropein is a phenylethanoid, a type of phenolic compound found in olive leaf from the olive tree together with other closely related compounds such as 10-hydroxyoleuropein, ligstroside, and 10-hydroxyligstroside. All these compounds are tyrosolesters of elenolic acid that are further hydroxylated and glycosylated. It is one of the main natural phenols found in argan oil [19]. Oleuropein and its metabolite hydroxytyrosol have powerful antioxidant activity both in vivo and in vitro and give extra-virgin olive oil its bitter, pungent taste. Oleuropein preparations have been claimed for several pharmacological effects [20], among them strengthening of the immune system [21]

*Phoenix dactylifera* (Palmae) have been a staple food of the Middle East and the Indus Valley for thousands of years, No fewer than 800 uses are recorded for the date palm [22]. In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative [23].

Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats [22]. The pollen grains of date palm have been used by Egyptians to improve fertility in women [24]. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of estrogens [25] or testosterone [26]. Recent studies indicate that the aqueous extracts of dates have potent antioxidant activity [27]. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic, sinapic acids, flavonoids and procyandins [28]. Of late, more attention has been paid to the role of natural antioxidants mainly phenolic compounds, which may have more antioxidant activity than vitamins C, E, β-carotene [29,30].

**MATERIALS AND METHODS**

**Plant Materials**

Active ingredients of the *Nigella sativa* and *Olea europaea* used in this study were purchased from the international market (Thymoquinone, and Oleuropein) and the fruit of *Phoenix dactylifera* was purchased from local market.
Animals
A total No of 50 adult Wistar male rats weighting 180-220g were purchased from animal unit, college of pharmacy, King Saud University- Riyadh, they were kept under standard controlled conditions in the laboratory for two weeks acclimatization period before starting the experiment. The study protocol was approved by the deanship of scientific research,Northern Border university. an informed consent was gotten from the ethical committee in the college of pharmacy Northern Border University.

Experimental Design
The 50 animals were randomly divided into five groups ten in each. The first group served as control. Rest of the groups received Single dose of CCl₄ (0.625ml/kg i.p) for two weeks. the dose was based on previous study[31]. Group (I) was kept without treatment. During administration of the doses the groups were treated as follows: group (II) treated with Oleoripein, group (III) treated with Thymoquinone , group (IV) treated with fruits of Phoenix dactylifera.

Sacrifice of Animals
After twenty four hours of CCl₄ administration, the control group and group (I) animals were sacrificed, blood was collected by heart puncture, and kept in heparinized tube to obtain serum for biochemical analysis. After two weeks of treatment for different groups, the rest of animals were sacrificed manually after deep anaesthesia with chloform by cervical dislocation, blood samples were collected and allowed to clot at room temperature, the samples were centrifuged to separate serum for biochemical analysis. The liver for each animal was harvested and kept in a jar containing formaldehyde solution 10% for histological investigations.

Biochemical Analysis
Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in serum in serum of rats was measured using commercial enzymatic diagnostic kits.

Histopathological analysis
Liver tissues were fixed in 10% formaldehyde solution and were sent for Histopathological analysis to King Fahad Medical Research Center Jeddah.
Statistical analysis
All results are presented as mean ± SEM. Data were compared by one-way analysis of variance (ANOVA). A calculated P-value less than 0.05 considered statistically significant. The statistical analysis were performed using statistical package SPSS IBM version 19.

RESULTS AND DISCUSSION
The mean values ± SEM of biochemical indicators of liver function of rats ALT, AST, and ALP, are presented in (Table 1). Significant increase in the enzyme levels was observed after the administration of CCl4 (GI) and a highly significant decrease was noticed when the animals were treated with Oleoropein (GII) compared to other groups P < 0.05.

The histopathological analysis of liver showed a variation in CCl4 toxicity group (GI)(fig.2 A) dilation and congestion, Necrosis haemorrhage and haemosiderin deposition, abnormal mitosis and degeneration (Fig 2 B). This group also showed Bile duct proliferation and portal tract cell infiltration, hepatic artery thickening, hepatocyte degeneration (fig. 2C, D). Some rats in Thymoquinone treated group G(III) showed moderate changes with decreased capsular and perivascular fibrosis, still degenerative changes near portal area with no signs of bile duct proliferation (fig.3 A,B, and C). In the Phoenix dactylifera treated group G (IV) examination showed absence of capsular and perivenular hepatocytes still showing cytoplasmic vacuolation and there was no improvement but enhanced hepatotoxicity (vacuolar degeneration) only absence of fibrosis (fig.4 A,B). Sections from the Oleuropein treated group G (II) showed normal surface with no fibrosis (thin black arrows), few cells undergoing Apoptosis (white arrows), and sinusoidal cells are prominent (dotted arrows) (fig.5 A). Normal non degenerated hepatocytes (black arrows) around central vein(CV). Prominent sinusoidal cells (dotted arrows) (fig.5 B) and normal hepatocytes (black arrows) near portal area which showing normal bile ducts (stars) (H&E stain) (fig.5 c).

In previous studies it was found that Oleuropein during ethanol treatment in rats resulted in a higher antiperoxidative enzyme activity, catalase, and inhibited toxicity to the liver, as monitored by the reduction in ALT and AST levels and TBARS concentration. It is suggested that Oleuropein possesses beneficial antioxidant effects against ethanol-induced liver toxicity[32]. Nigella sativa treatment positively protects the alterations in the biochemical variables in the CCl4 induced hepatotoxicity [33]. Hepatoprotective properties of Nigella sativa in liver damage of experimental rats by reducing oxidative stress are evident[34]. It is revealed that the treatment with aqueous extract of date flesh or pits significantly reduced
CCl4-induced elevation in plasma enzyme and bilirubin concentration and ameliorated morphological and histological liver damage in rats [35,36]. Results of our study are in conformity with previous studies conducted. The Oleoropein has promisingly reduced the hepatotoxic effects induced by CCl4 reversing the changes towards normal / control group. In the Phoenix dactylifera group (IV) other observations beside hepatoprotective effects were also made, like development of aggressive behavior amongst the group which resulted in killing /death of 40% animals and eating of the viscera of the dead animals by rest of the group members and increase in the size of male genitalia.

Table 1. Serum Levels Of Alt, Ast, And Alp After Two Weeks Treatment With Drugs

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.6 ±1.081</td>
<td>50.7 ±1.644</td>
<td>90.0 ±2.081</td>
</tr>
<tr>
<td>Group1 (CCl4)</td>
<td>85.8 ±1.664</td>
<td>125.4 ±1.913</td>
<td>185.3 ±2.338</td>
</tr>
<tr>
<td>Group2 (Oleoropein)</td>
<td>45.2 ±1.985</td>
<td>98.7 ±1.011</td>
<td>118.4 ±1.594</td>
</tr>
<tr>
<td>Group3 (Thymoquinone)</td>
<td>58.3 ±0.404</td>
<td>120.1 ±1.193</td>
<td>156.5 ±1.069</td>
</tr>
<tr>
<td>Group4 (Phoenix dactylifera)</td>
<td>51.6 ±1.967</td>
<td>108.3 ±1.249</td>
<td>130.1 ±1.014</td>
</tr>
</tbody>
</table>

Keys: Values are mean ± SEM for 9 animals in each observation, the experimental animals were compared to normal groups.

Fig 1. Histopathology sections of the control group (C)
Fig. 2. Histopathology sections of group (I)

Fig. 3. Histopathology sections of group (III)
Fig. 4 Histopathology sections of group (IV)

Fig. 5 Histopathology sections of group (II)
CONCLUSION
The results of the study show that the active ingredient of *Olea europaea* (Oleuropein), *Nigella Sativa* (Thymoquinone) and *Phoenix dactylifera* (date palm) possess significant hepatoprotective effects, specially the *Olea europaea* (Olive) can be the possible treatment of cirrhosis of liver as is evident in the histopathological examination results of this study in which it can be seen clearly that fibrotic changes have been reversed. It is suggested that all of these herbals are used by folklore since time unknown as staple food or for the treatment of different ailments, so clinical trials in humans must be conducted to observe their hepatoprotective effects. The probable discovery of these substances as cost effective hepatoprotective agents will be of great help to millions of people who are suffering from liver diseases in Asia and Africa specially.

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