INVESTIGATION THE ANTIOXIDANT PROPERTY OF CLOVE LEAF EXTRACT ON ALCOHOLIC LIVER DISEASE

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ABSTRACT

The present study aimed to investigated the protective activity of clove leaf extract by the physiological changes in alcoholic liver disease. Forty male rats taken were weighing 250-300 gm. All rats were subdivided: (were divided into four subgroups according to the type of drug administered) for each group. 10 male rats: rats were treated daily for thirty days. The first group: is the (control negative) will be administrate DMSO for 4 weeks. And The second group: is the (control positive) will be administrated clove leaf extract by Intragastric oral. And The third group: will be administrated Ethanol by Intragastric oral to induce liver alcoholic disease and The fourth group: will be administrated ET + Clove leaf extract by Intragastric oral. Result revealed that clove leaf extract significant decrease in serum AST, GGT, Conjugated bilirubin and Unconjugated bilirubin, while the levels of SOD, GST, Vitamin E, Vitamin C and GSH were increase. Based on our data clove leaf extract ameliorates the physiological changes in alcoholic liver disease.

Keywords: Antioxidant property, Investigation, Alcoholic Liver Disease, Extract

I. INTRODUCTION

Alcoholic liver disease (ALD) is a severe health hazard that accounts for roughly 4% of all deaths and 5% of all disabilities worldwide each year. Despite significant scientific progress in hepatology and related fields, liver disease is continually on the rise. (1). However, there are no specific management or therapy techniques available. The majority of therapy options come with substantial side effects and other health risks. (2). Alcohol is primarily processed in the liver. Its by-products, such as acetaldehyde, have been shown to be more hazardous than alcohol. (3) Long-term, excessive alcohol use can harm tissue, including the central nervous system, muscles, cardiovascular system, and endocrine system, gastrointestinal tract, haemopoiesis, and bone metabolism (4). Also harms the liver, causing clinical manifestations such as alcoholic fatty liver, which can lead to alcoholic steatohepatitis, fibrosis, and finally cirrhosis. (5). The mechanism of the antioxidant defense systems can be harmed by an excess of free radicals produced during alcohol metabolism. Atherosclerosis is the health effect of ethanol that has been studied the most. This is one of the leading causes of disease and mortality around the world. (1) Because of their antioxidant activity, a variety of medicinal herbs, including clove, are being utilized to treat alcoholic liver damage. Cloves are a type of plant that grows in the (S. aromaticum), which is used as a food preservation, is a member of the Myrtaceae family, which is used for a variety of therapeutic purposes all over the world. (6) Cloves are a rich source of dietary polyphenols and have a long history of therapeutic usage. (7). Clove contains compounds such as eugenol, eugenyl acetate, gallic acid, -caryophyllene, 2-heptanone, humulenol, -humulene, and others. (8) Also Cloves contain vitamin A (retinol), beta-carotene (9), and vitamins K, B6, B1 and C (10) Clove essential oil and clove extracts, on the other hand, have been demonstrated to have hepatoprotective properties via lowering liver damage. (11)(12)

II. MATERIALS AND METHODS

Preparation of Clove leaf extract

Clove extraction. The extraction was carried out according to the procedure characterized by Cortes-Rojas et al..(13) At a point, the clove leaves were macerated in 70% ethanol. a 5:1 ratio (sample : solvent). The sample was soaked in order to a 24-hour period, stirring every 12 hours. Subsequent to the harvest The method was replicated twice with the macerated material. By using the same solvent volume, Then Material was By the use of the rotary
evaporator, collected and condensed (45°C) one hour from now. An extract derived from ethanol was Obtained in a dark brown paste.

**Chemicals**

Serum AST, GGT, Conjugated bilirubin and Unconjugated bilirubin were measured by use commercial ELISA kits (Biolabo). According to the instructions of manufacture. Also estimation the levels of SOD, GST, Vitamin C, Vitamin E and GSH were performed according to procedures described by (Elabscience Biotechnology).

**Experimental design**

Foutry adult male albino rats, aged three months and their weight range 250-300 gm. In this investigation, they were used. Rats were raised in metal cages with free access to food and water. Male rats were distributed into four groups (10 rats each group) were dispersed randomly into four groups: 1-The first group: is the (control negative) will be administrate DMSO for 4 weeks 2-The second group: is the (control positive) will be administrated clove leaf extract 100 m g/ Kg body weight by Intragastric oral gavage for 4 weeks. (14). 3-The third group: will be administrated Ethanol (6 g/Kg body weight of 20% (V/V)) ET by Intragastric oral gavage for 4 weeks to induce liver alcoholic disease (15). 4-The fourth group: will be administrated ET + Clove leaf extract by Intragastric oral gavage for 4 weeks.

The trial will be extended for another 30 days, and the following parameters will be measured: levels of enzymatic antioxidants in the liver (SOD, GST), levels of non-enzymatic antioxidants (Vitamin C, Vitamin E, GSH), and liver function markers (AST, GGT, unconjugated bilirubin, conjugated bilirubin).

**Statistical Analysis**

The Statistical Package for Social Sciences (SPSS, version 19) was used to analyze all of the data. ANOVA (one-way analysis of variance) was used. carried done in order to compare study groups and the least To determine significance, significant differences (LSD) are used. of the mean differences and the p-value of less than 0.05 was regarded as significant. The obtained data was reported as the mean minus the standard error.

### III. RESULT AND DISCUSSION

The results in Table (1), showed that the AST, GGT, Conjugated Bilirubin, Unconjugated Bilirubin levels of the ethanol are significantly increase in compare with the other groups. The present study agreed with results conducted by (16). This substantial rise is indicative of hepatotoxicity, which could be caused by leaking from cells due to peroxidative membrane damage. Increased serum marker enzyme levels in the liver indicate cellular leakage and a lack of cellular membrane functional integrity. (17).

Also The result in table (1) showed the levels of serum liver markers such as AST, GGT and the levels of bilirubin in (GIV) were significantly decrease (p ≤ 0.05) as compared to ethanol group (GIII). Clove leaf extract has strong antioxidant activity in vitro against a variety of antioxidant systems; additionally, clove buds can be employed as a convenient source of natural antioxidants, as a dietary supplement, or in applications for pharmaceuticals. (18)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST U/L</th>
<th>GGT U/L</th>
<th>Conjugated Bilirubin (mg/dl)</th>
<th>Unconjugated Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI. Negative control</td>
<td>75.125±4.32   B</td>
<td>0.64± 0.05 B</td>
<td>0.35±0.038 A</td>
<td>0.89±0.04 C</td>
</tr>
<tr>
<td>GII. Positive control (clove leaf extract)</td>
<td>77.25±6.18    B</td>
<td>0.62± 0.06 B</td>
<td>0.36±0.03 A</td>
<td>0.90±0.02 C</td>
</tr>
<tr>
<td>GIII. Ethanol</td>
<td>105.5±3.46    A</td>
<td>2.75± 0.09 A</td>
<td>0.82±0.29 B</td>
<td>2.35±0.042 B</td>
</tr>
<tr>
<td>GIV. Clove leaf extract +Ethanol</td>
<td>85.37±2.19    A</td>
<td>0.70± 0.03 B</td>
<td>0.37±0.036 B</td>
<td>1.19±0.32 A</td>
</tr>
</tbody>
</table>

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Values are expressed as mean ± E S

Number of rats in each group =6

The different litters refer to the significant change between groups (p ≤ 0.05)

In Table (2) illustrated there were a statistically significant (p ≤ 0.05) in cement in serum cholesterol and triglycerides in ethanol treated group (GIII) comparing to other groups. The higher serum enzyme levels reflect their release from injured hepatic cells after they have seeped into the bloodstream, confirming liver injury. The accumulation of triglycerides leads to fatty liver, which is caused by a decrease in apoprotein synthesis. (19).

Increased levels of a serum marker enzyme indicate cellular leakage and a lack of cellular membrane functional integrity in the liver. (20). This shows that oxidative stress causes lipid metabolism problems, which leads to lipid peroxidation, which causes a variety of ailments, including cardiovascular disease (21). Increased lipid peroxidation lowers the antioxidant defense system of the cell. (22). The predominant indicator of oxidative toxicity is lipid peroxidation, which is induced by the activation of oxidative destruction of membrane lipids rich in polyunsaturated fatty acids, resulting in the formation of malondialdehyde (MDA) (23).

Combined ethanol with clove leaf extract (GIV) in the same table caused significant (p > 0.05) decrement of serum cholesterol and triglycerides comparing to (GIII) group. A naturally occurring antioxidant would scavenge the free radical produced by ethanol, which could explain why mice treated with clove leaf extract had lower lipid peroxidation result. Clove, a natural antioxidant, would scavenge the free radicals generated by ethanol, explaining why rats administered clove showed reduced lipid peroxidation. (24)

Table (2) Effect of clove leaf extract on some lipid levels tests in male rats with induced alcoholic liver disease

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>cholesterol (Mg/dl)</th>
<th>triglycerides (Mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI. Negative control</td>
<td></td>
<td>239.99±3.396</td>
<td>330.92±7.83</td>
</tr>
<tr>
<td>GII. Positive control (Clove leaf extract)</td>
<td></td>
<td>242.80±2.44</td>
<td>335.67± 5.02</td>
</tr>
<tr>
<td>GIII. Ethanol</td>
<td></td>
<td>406.87±2.44</td>
<td>480.6±3.634</td>
</tr>
<tr>
<td>GIV. Clove leaf extract +ethanol</td>
<td></td>
<td>261.69±2.262</td>
<td>353.92±4.570</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± E S

Number of rats in each group =6

The different litters refer to the significant change between groups ((p ≤ 0.05)

There were statistically SOD and GST levels decreased significantly (p 0.05) in (GIII) as compare to other groups in table (3). Antioxidants are metal ions that have the ability to detoxify free radicals produced by oxygen molecules and are commonly seen in free radical form. Clove has a lot of antioxidants. (25)

SOD, a naturally occurring oxidoreductase. The dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide is catalyzed by this enzyme. (26). Superoxides (O2 •*), which can produce hydroxy radicals (OH•), are harmful to cells and play a role in alcohol-induced liver damage (27). On ethanol treatment, we discovered a decrease in SOD and glutathione metabolizing GPx activities, as well as a contemporaneous depletion of GSH, indicating enhanced oxidative stress, which has been implicated in the pathophysiology of alcohol-related illnesses. Clove essential oil has been demonstrated in studies to be one of the most effective antioxidants, outperforming synthetic antioxidants such as BHT or butylated hydroxyanisole. (28; 29).
On the other hand the table (3) There was a statistically significant increase (p 0.05). in the levels of SOD and GST in (GIV) as compare to ethanol treated group. The antioxidant effects of clove leaf extract may be useful. As a result of direct ROS scavenging activity, this happened. These enzymes operate together to form an anti-ROS defensive squad. In experimental animals, lipid peroxidation, a ROS-mediated process, has been linked to the pathophysiology of diverse liver lesions and subsequent liver fibrogenesis. (30). Clove has a greatest ability to emit hydrogen and hence minimize lipid peroxidation. In terms of lipid peroxidation, clove oil's inhibitory action measured using a linolenic acid emulsion technique revealed that it had stronger antioxidant activity than normal BHT (Butylated hydroxyl tolvene). It also acts as an iron chelator and has a substantial inhibitory impact against hydroxyl radicals. (31)

Generally, the phenolic compounds (flavoniods) have antioxidant activity for neutralizing free radicals and preventing their production (32; 33).

Table (3) Effect of clove leaf extract on some enzymatic antioxidants in males rats with induced alcoholic liver disease

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD(U/mg)</th>
<th>GST(U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI. Negative control</td>
<td>6.96±0.157 A</td>
<td>7.10±0.200 A</td>
</tr>
<tr>
<td>GII. Positive control(clove leaf extract)</td>
<td>7.11±0.168 A</td>
<td>7.73±0.152 A</td>
</tr>
<tr>
<td>GIII. Ethanol</td>
<td>3.02±0.087 B</td>
<td>2.84±0.405 B</td>
</tr>
<tr>
<td>GIV. Clove leaf extract + Ethanol</td>
<td>7.12±0.125 A</td>
<td>5.65±0.44 C</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± E S
- Number of rats in each group =6
- The different litters refer to the significant change between groups (p ≤ 0.05)

Also table (4) illustrated there were a significant decrease in vitamin C, vitamin E and GSH in ethanol treated group (GIII) as compare with other groups, the result of the present study agree with the confirmation of several investigations in the liver, ethanol is metabolized via conjugation with GSH, which is performed by glutathione S-transferase (GST). GST-A1-1 catalyzes the process in the human liver. (34;35) The metabolism of ethanol, which involves both microsomal and mitochondrial systems, is linked to alcohol-induced oxidative stress. Similarly, with the help of the endogenous antioxidant GSH, GPx is an antioxidant enzyme involved in the detoxification of H2O2 . (36).

The table (4) also showed there were significant statistically (p 0.05) increment in serum Vitamin C, Vitamin E and GSFH in (GIV) as comper to ethanol group (GIII) due to the antioxidant property of clove leaf extract (37). Vitamin E contains both antioxidant and non-antioxidant characteristics, including the regulation of signal transduction pathway(38). Vitamin C protects lipids from oxidative damage caused by aqueous peroxyl radicals by acting as an antioxidant (39;40). Reduced glutathione (GSH) is a significant endogenous antioxidant that protects against free radical damage. GSH is well-known for its role in redox balance, quenching free radicals, and detoxifying activities, all of which contribute to the preservation of normal cell structure and function. (41). The CYP2E1-expressing cells lose viability when GSH is removed. This has been linked to mitochondrial dysfunction and a drop in mitochondrial membrane potential. Surprisingly, glutamate cysteine ligase transcriptional activation increases GSH levels in CYP2E1-expressing cells. CYP2E1-induced oxidative stress, mitochondrial damage, stellate cell activation, and GSH homeostasis are all involved in ethanol's harmful effects on the liver. (42).
<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Vitamin .C (mg/dl)</th>
<th>Vitamin .E (mg/dl)</th>
<th>GSH(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI. Negative control</td>
<td></td>
<td>1.90±0.14 A</td>
<td>1.88±0.032 A</td>
<td>25.55±0.25 a</td>
</tr>
<tr>
<td>GII. Positive control (clove leaf extract)</td>
<td></td>
<td>2.12±0.08 A</td>
<td>1.84±0.037 A</td>
<td>27.36±0.35 A</td>
</tr>
<tr>
<td>GIII. Ethanol</td>
<td></td>
<td>0.80±0.06 B</td>
<td>0.81±0.04 B</td>
<td>12.18±0.2 B</td>
</tr>
<tr>
<td>GIV. Clove leaf extract + Ethanol</td>
<td></td>
<td>1.84±0.07 A</td>
<td>1.70±0.03 A</td>
<td>26.51±0.32 A</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± E S
- Number of rats in each group =6
- The different litters refer to the significant change between groups (p ≤ 0.05)

REFERENCES