The potential protective effect of alcoholic extracts of some herbs on hepatotoxicity induced by paracetamol in experimental rats

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Abstract

There are many plants which are used as herbs by many people such as *Laurus nobilis* and *Elettaria cardamomum*. The purpose of this study was to find out protective alcoholic Extract's effect of *Laurus nobilis* and *Elettaria cardamomum* on hepatotoxicity induced by paracetamol in experimental rats. 30 healthy adult rats of male albino weighing (150±10 g) were used and divided into 6 equal groups, one of them was kept as a negative control, Group (2): was fed on basal diet as a positive control group, All groups were fed on basal diet + orally doses of herbs as the following: (3 and 4) treated with *Laurus nobilis* extract 100 and 200 mg/Kg b.w. respectively; (5 and 6) treated with *Elettaria cardamomum* extract 100 and 200 mg/Kg b.w. respectively Once a day for twenty-eight days on twenty-one day, during the administration of the respective treatments, all animals of groups 2, 3, 4, 5 and 6 were administered with paracetamol 400 mg/kg orally for one week. At the end of experiment, biological evaluation was calculated. Liver enzymes, bilirubin, proteins and lipid profile were determined in serum. In liver tissue (GPX, SOD, CAT, MDA and α –TNF) were determined. Livers of rats were histopathologically examined. The results showed that alcoholic extract of *Laurus nobilis* and *cardamomum* improved biological evaluation, liver functions, and antioxidant enzymes compared with positive control groups. It could be concluded that take *Laurus nobilis* and *cardamomum* have markedly protected against the harmful effects of paracetamol on liver.

**Key words**: *Laurus nobilis*, *Elettaria cardamomum*, paracetamol, Liver functions and antioxidant enzymes.
INTRODUCTION

As one of the body's most vital organs, liver has vital roles to perform (Mayuren et al., 2010). To maintain normal physiological function, the liver must be kept in a healthy condition. It is believed that drugs are the primary cause of liver damage, which leads to major medical issues worldwide (Lakshmi et al., 2018). Paracetamol is recognized as acetaminophen as the greatest usually used painkillers (Sheen et al., 2002). Paracetamol is known to be relatively non-toxic in a therapeutic dose (Siemionow et al., 2016). Its toxicity is caused by a lone or frequent high dosage or chronic intake of the liver and kidneys is regarded as an example many drug therapy troubles (Cepan et al., 2018). Cytochrome P450 and glucuronidation or sulfations mainly lead to the metabolisation of the swallowed curative dosage of PCM. Fatigued GSH levels are depleted; NAPQI can bind freely to other cellular proteins that are responsible for oxidative stress and necrosis (Hamza and Al-Harbi, 2015). Many diseases can be prevented and improved with the use of herbs and spices. Such as liver disease, which require immediate care and priority treatment modern medicines do not always help to increase renewable activity and prevent fibrosis and cirrhosis development. The search for new drugs including herbal products with a broad range of pharmacological activities and economic affordability has increased in recent years (Singh et al., 2013). Various researchers have positively evaluated and explored a broad range of pharmacological activities among them Laurus nobilis ethanolic extract has antioxidant and wound healing effect (Vardapetyan et al., 2013). In the treatment of gastrointestinal, rheumatism, migraines, and diuretic, antimicroscopic, improving appetite and digestion are also used (Santos & Rao, 2000). The volatile laurel oils suppress tuberculosis development and stimulate immunity. Phytoncides are abundant in the leaves of L. nobilis, a large number of necessary tannins and trace elements which assistance to control body poisons (Rukhkyan et al., 2013). L. nobilis is help to prevent and treat type II diabetes as lowers blood serum glucose levels. L. nobilis lowers total cholesterol (TC) and LDL levels in
body (Khan et al., 2009). And cardamomum is the member of the Zingiberaceae family. Its dried fruit is one of the world's most expensive spices. The dehydrated fruit is used as a flavouring agent in whole or in ground form and also for flatulence intake medicines. Cardamomum was used in curative herbs for urinary and kidney diseases and in addition to culinary purposes (Ballabh et al., 2008). Cardamom extracts have antimicrobial properties, according to studies. (Hammad et al., 2014 & Winarsi et al., 2014) hepatoprotective activity, anti-hyperlipidemic and antidiabetic effect (Kandasamy et al., 2010, & Chacko et al., 2012).

Current study is undertaken to examined the protective effects of alcohol extracts of Laurus nobilis & Elettaria cardamomum on liver toxicity and hematological parameters of rats suffering from hepatotoxicity induced by paracetamol.

**Materials and Methods**

**Materials:**

Laurus nobilis and Elettaria cardamomum were purchased from the Cairo-based Agricultural Seeds, Herbs, and Medicinal Plants Company. Thirty adult male albino rats (Sprague Dawley strain) were procured from the Helwan farm animal colony, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt. El-Gomhoria Company for Trading Drugs, Chemicals, and Medical Appliances, Cairo, Egypt, supplied all essential chemicals. We purchased casein, vitamins, minerals, cellulose, and choline chloride from El-Gomhoria Company in Cairo, Egypt.
Methods:

Chemical analysis of herbs

The polyphenolic compounds of plants extract were fractionated and identified for phenolic compounds by HPLC (Tarola et al., 2013).

Preparation of herbs:

Dried herbs were purchased then heated in oven for ten minutes then triturates into a well powder and saved till for preparation alcohol extract.

Preparation of alcohol herbs extract:

Approximately 200 g of ground herbs were steeped for 3 days in 70% ethanol at room temperature (23–25 °C) with intermittent shaking. It went through a muslin cloth and then a Whatman qualitative grade 1 filter paper. The combined filtrates were evaporated on a rotary evaporator at reduced pressure (760 mmHg) after this procedure was done twice (Chacko et al., 2012).

Experimental animal

Thirty mature white Albino rats of an average body weight 150±10 g of Sprague Dawley Strain was used in this study. Rats were fed on basal ration supplying the essential vitamins and trace elements and water supply was given ad-libitum.

Preparation of Experimental diet

Basal diet was prepared from fine ingredient per 100 g as follows: Casein (≥ 80% protein) 14%, Sunflower oil 4 %, salts mixture 4%, vitamins mixture 1%, DL-methionine 0.3%, choline chloride 0.2% which was added at the expense of corn starch up to 100g (Reeves et al., 1993).
Experimental design

Rats were divided into six groups 5 animals each as a following:

**Group (1):** was fed on basal diet as a negative control group (G-).

**Group (2):** was fed on basal diet as a positive control group (G+).

**Groups (3 and 4):** were fed on basal diet and treated daily orally with *Laurus nobilis* extract (100 and 200 mg / Kg body weight).

**Groups (5 and 6):** were fed on basal diet and treated daily orally with *Elettaria cardamomum* extract (100 and 200 mg / Kg body weight). Once a day for twenty-eight days. On twenty-one day, during the administration of the respective treatments, all animals of groups 2, 3, 4, 5 and 6 were administered with paracetamol 400 mg/kg orally for one week according to *Ravindran et al., 2013*. Body weight and feed intake were checked once a week. At the end of experimental period animals were weighed, fasted overnight, and then sacrificed under very light ether anaesthesia.

**Biological evaluation**

Body weight gain, feed intake, feed efficiency ratio, and relative organs weight were calculated at the end of the experiment according to *Chapman et al., 1959*.

**Biochemical analysis of serum:**

After sacrifice of rats, blood samplings were collected from hepatic portal vein of each rat in dried clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 round per minute (rpm) for 15 minutes at room temperature, transferred into dry clean ebendorf tubes, then kept frozen at -20°C for later determinations.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to
Bergmeyer et al., (1986), serum (ALP) was determination according to the colorimetric method of Roy (1970), albumin according to Drupt, (1974) total protein according to Sonnenwirth and Jaret, (1980). Globulin was calculated according to Busher, (1990) using the following equation:

\[
\text{Globulin} = \text{Total protein} - \text{Albumin}.
\]

Total bilirubin was determined in the serum according to Doumas et al., (1973), direct and indirect bilirubin were determined in the serum according to Chary and Sharm (2004).

Total cholesterol was determined in the serum according to the method described by Allain et al., (1974). Triglycerides were determined in the serum according to the method described by Trinder and Ann (1969). HDL-C was determined in the serum according to the method described by Lopes- Virella et al., (1977). Serum LDL-c and VLDL-C were calculated according to Friedwald et al., (1972).

**Organ sampling:**

Livers was removed from rats by careful dissection, washed in saline solution (0.9%), dry using filter paper and independently weighed. A specimen from liver was kept at (-20 °C) for preparation of tissue homogenate for determination of antioxidant parameters. The homogenation was centrifuged at 1000 r.p.m for 10 minutes.

**Histopathological examinations**

The livers of sacrificed rats were submerged in a 10% formalin solution. After that, the fixed specimens were cut, cleaned, and dehydrated in increasing degrees of alcohol. They were then washed with xylol, fixed in paraffin, sectioned at 4-6 microns thickness, and stained with hematoxylin and eosin for examination of liver components according to Carleton (1979).
Statistical analysis:

Statistical analysis were carried out using one-way analysis of variance (ANOVA) test followed by Duncan test through the program of (SPSS). Results were expressed as mean± SD. The differences among means at p < 0.05 were considered significant (Snedecor and Cochran, 1989).

Results

Phenolic compounds of *Laurus nobilis* and *Elettaria cardamomum* by HPLC analysis

*Laurus nobilis* analyzed for their phenolic Compounds. The obtained results showed in table (1): *Laurus nobilis* recorded higher content of Gallic acid, Catechin, Chlorogenic acid, Naringenin and Ellagic acid while recorded lower content of Syringic acid, Vanillin, Coumaric acid and Pyro catechol.

*Elettaria cardamomum* analyzed for their phenolic Compounds. The obtained results showed in table (2): *Elettaria cardamomum* recorded higher content of Gallic acid, Chlorogenic acid and Rutin while recorded lower content of Methyl gallate, Vanillin, Cinnamic acid and Naringenin.

**Table (1): Phenolic compounds of *Laurus nobilis* (1g/15ml)**

<table>
<thead>
<tr>
<th></th>
<th>Area</th>
<th>Conc. (µg/ml)</th>
<th>Conc.(µg/15ml=µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>717.20</td>
<td>63.03</td>
<td>945.42</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>648.08</td>
<td>48.41</td>
<td>726.22</td>
</tr>
<tr>
<td>Catechin</td>
<td>512.49</td>
<td>61.41</td>
<td>921.09</td>
</tr>
<tr>
<td>Methyl gallate</td>
<td>1986.88</td>
<td>26.97</td>
<td>404.59</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>204.07</td>
<td>8.72</td>
<td>130.83</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>50.28</td>
<td>2.20</td>
<td>33.00</td>
</tr>
<tr>
<td>Pyro catechol</td>
<td>49.95</td>
<td>3.54</td>
<td>53.16</td>
</tr>
</tbody>
</table>
Rutin  
Ellagic acid  
Coumaric acid  
Vanillin  
Ferulic acid  
Naringenin  
Taxifolin

| Phenolic compounds of Elettaria cardamomum (1g/15ml) |
|-----------------------------------------------|-----------------|-----------------|
| Gallic acid                                   | 117.79          | 10.35           |
| Chlorogenic acid                              | 62.42           | 4.66            |
| Catechin                                      | 4.80            | 0.57            |
| Methyl gallate                                | 10.85           | 0.15            |
| Caffeic acid                                  | 24.70           | 1.06            |
| Syringic acid                                 | 35.01           | 1.53            |
| Rutin                                         | 22.78           | 3.10            |
| Ellagic acid                                  | 16.15           | 1.08            |
| Coumaric acid                                 | 104.24          | 1.77            |
| Vanilllin                                     | 12.03           | 0.27            |
| Naringenin                                    | 8.70            | 0.48            |
| Cinnamic acid                                 | 46.96           | 0.47            |

Biological evaluation:

Results of FI, BWG% and (FER) were shown there is significant decrease of these parameters in positive control.
compared to normal group. But, there was significant increase in the other treated groups when compared with positive control. Regarding to FI, the best results were found in groups *Laurus nobilis* (100 and 200 mg/kg). The best results for BWG% and FER was recorded for *Laurus nobilis* (200 mg/kg) which recorded non-significant with normal group.

Table (3): Protective effect of *Laurus nobilis* and *Elettaria* on feed intake (FI), body weight gain (BWG %) and feed efficiency ratio (FER) in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FI (g/28 day)</th>
<th>BWG (%)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>(- ve) Control</td>
<td>507.40 ± 2.23</td>
<td>74.00 ± 3.33</td>
<td>0.145 ± 0.01</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>386.40 ± 1.25</td>
<td>40.58 ± 1.39</td>
<td>0.105 ± 0.00</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (100 mg /kg)</td>
<td>497.40 ± 1.83</td>
<td>62.36 ± 1.56</td>
<td>0.125 ± 0.00</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (200 mg /kg)</td>
<td>497.20 ± 2.18</td>
<td>71.45 ± 2.72</td>
<td>0.143 ± 0.01</td>
</tr>
<tr>
<td><em>Elettaria</em> (100 mg /kg)</td>
<td>471.20 ± 2.53</td>
<td>53.00 ± 2.23</td>
<td>0.112 ± 0.00</td>
</tr>
<tr>
<td><em>Elettaria</em> (200 mg /kg)</td>
<td>470.80 ± 2.45</td>
<td>55.00 ± 3.80</td>
<td>0.116 ± 0.01</td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

**Relative liver weight:**

Positive control group have increase in relative liver weight as compared with negative group. Relative liver weight showed significant decrease in all treated groups as compared to positive control. The best results were found in groups *Laurus nobilis* and *Elettaria* (200 mg/kg) because these treatments showed significant decrease, when compare with other treated groups as shown in (Table 4).
Table (4): Protective effect of *Laurus nobilis* and *Elettaria* on relative liver weight rats (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative Liver weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(- ve) Control</td>
<td>2.80 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>4.20 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (100 mg /kg)</td>
<td>3.72 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (200 mg /kg)</td>
<td>3.10 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Elettaria</em> (100 mg /kg)</td>
<td>3.75 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Elettaria</em> (200 mg /kg)</td>
<td>3.10 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

**Liver functions:**

Mean values of AST, ALT and ALP in the (+ve) control group showed significant increase as comparison with the (-ve) control group. All treated groups recorded significant decrease in all liver enzymes as compared to control (G+). Best results in ALT and AST were found in rats received *Laurus nobilis* (200 mg/kg) and *Elettaria* (200mg/kg), while *Laurus nobilis* (200 mg /kg) only recorded the best result in ALP.

Table (5): Protective effect of *Laurus nobilis* and *Elettaria* on serum liver enzyme (ALT, AST and ALP) in rats (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
<th>ALP (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(- ve) Control</td>
<td>43.29 ± 1.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.33 ± 3.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>137.00 ± 1.89&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>100.31 ±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.59 ± 1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160.46 ± 2.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (100 mg /kg)</td>
<td>60.35 ± 3.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.39 ± 1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>152.16 ± 2.38&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (200 mg /kg)</td>
<td>53.41 ± 2.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>77.26 ± 3.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>143.24 ± 2.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
The results revealed that mean value of total protein, albumin and globulin in paracetamol rats group (G+) was significantly decreased as compared with normal group (G-). While all treated groups recorded significant increase as comparison with control (G+). Highest level of total protein and globulin recorded by treated group with *Elettaria (200 mg/kg)*, best result in albumin found in groups *Elettaria (200 mg/kg)* and *Laurus nobilis (200 mg/kg)* as shown in (Table 6).

Table (6): Protective effect of *Laurus nobilis* and *Elettaria* on total protein, albumin and globulin in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TP (mg/dl)</th>
<th>Alb (mg/dl)</th>
<th>Glob (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-ve) Control</td>
<td>7.75 ± 0.01$^a$</td>
<td>4.07 ± 0.04$^a$</td>
<td>3.68 ± 0.03$^a$</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>4.86 ± 0.01$^f$</td>
<td>2.57 ± 0.02$^d$</td>
<td>2.29 ± 0.04$^d$</td>
</tr>
<tr>
<td><em>Laurus nobilis (100 mg/kg)</em></td>
<td>5.52 ± 0.04$^c$</td>
<td>2.89 ± 0.02$^c$</td>
<td>2.63 ± 0.01$^c$</td>
</tr>
<tr>
<td><em>Laurus nobilis (200 mg/kg)</em></td>
<td>5.90 ± 0.03$^c$</td>
<td>3.20 ± 0.15$^b$</td>
<td>2.70 ± 0.02$^c$</td>
</tr>
<tr>
<td><em>Elettaria (100 mg/kg)</em></td>
<td>5.74 ± 0.01$^d$</td>
<td>2.95 ± 0.01$^c$</td>
<td>2.79 ± 0.01$^c$</td>
</tr>
<tr>
<td><em>Elettaria (200 mg/kg)</em></td>
<td>6.50 ± 0.22$^b$</td>
<td>3.30 ± 0.15$^b$</td>
<td>3.20 ± 0.36$^b$</td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

**Total bilirubin, direct bilirubin and indirect bilirubin**

The results indicated that mean values of total bilirubin, direct bilirubin and in direct bilirubin in the (+ve) control group were significantly increased as compared with (-ve) control group. All treated groups recorded significant decreased comparison with...
(+ve) control group. The best results in total bilirubin and indirect bilirubin found in Elettaria (200mg/kg), while groups treated with Laurus nobilis (200 mg /kg) and Elettaria (100mg/kg) recorded the best result in direct bilirubin.

Table (7): Protective effect of Laurus nobilis and Elettaria on Total bilirubin, direct bilirubin and indirect bilirubin in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T.BIL (mg /dl)</th>
<th>D.BIL (mg /dl)</th>
<th>IN.BIL (mg /dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(- ve) Control</td>
<td>0.50 ± 0.02c</td>
<td>0.13 ± 0.01d</td>
<td>0.37 ± 0.02d</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>1.42 ± 0.02a</td>
<td>0.23 ± 0.01a</td>
<td>1.19 ± 0.01a</td>
</tr>
<tr>
<td>Laurus nobilis (100 mg /kg)</td>
<td>0.76 ± 0.02b</td>
<td>0.17 ± 0.02b</td>
<td>0.59 ± 0.03b</td>
</tr>
<tr>
<td>Laurus nobilis (200 mg /kg)</td>
<td>0.61 ± 0.01c</td>
<td>0.14 ± 0.01cd</td>
<td>0.47 ± 0.02c</td>
</tr>
<tr>
<td>Elettaria (100 mg /kg)</td>
<td>0.63 ± 0.01c</td>
<td>0.14 ± 0.02cd</td>
<td>0.49 ± 0.03c</td>
</tr>
<tr>
<td>Elettaria (200 mg /kg)</td>
<td>0.55 ± 0.02d</td>
<td>0.15 ± 0.02c</td>
<td>0.40 ± 0.03d</td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

Serum lipid profile:

The mean values of cholesterol, triglyceride low-density lipoprotein and very low-density lipoprotein were significantly increased in the (+ve) control rats comparison with the (-ve) control group. All treated groups showed significant decrease (P<0.05) comparing with the (+ve) control rats. The best result was obtained in rats treated with Elettaria (200 mg /kg) in Parameter VLDL-c groups Treated with Elettaria (200 mg /kg) and Laurus nobilis (200 mg /kg) recorded the best result.

The mean values of HDL-c were low in (+ve) control comparison with the (-ve) control rats. All treated groups recorded significant increase (P<0.05) as compared with (+ve) control rats. The best result was obtained in rats treated with Elettaria (200 mg /kg).
Table (8): Protective effect of *Laurus nobilis* and *Elettaria* on lipid profile in rats (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-ve) Control</td>
<td>143.45 ± 1.55b</td>
<td>110.00 ± 1.20b</td>
<td>43.49 ± 1.39c</td>
<td>77.96 ± 0.85b</td>
<td>22.00 ± 0.31a</td>
<td></td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>143.45 ± 1.55b</td>
<td>110.00 ± 1.20b</td>
<td>43.49 ± 1.39c</td>
<td>77.96 ± 0.85b</td>
<td>22.00 ± 0.31a</td>
<td></td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (100 mg /kg)</td>
<td>123.00 ± 2.49c</td>
<td>102.00 ± 2.00c</td>
<td>45.31 ± 1.49d</td>
<td>57.29 ± 2.28c</td>
<td>20.40 ± 0.32b</td>
<td></td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (200 mg /kg)</td>
<td>100.75 ± 1.55b</td>
<td>97.00 ± 1.58b</td>
<td>48.37 ± 3.22b</td>
<td>97.00 ± 1.50d</td>
<td>19.40 ± 1.76c</td>
<td></td>
</tr>
<tr>
<td><em>Elettaria</em> (100 mg /kg)</td>
<td>143.45 ± 1.55b</td>
<td>109.00 ± 1.58b</td>
<td>41.28 ± 3.22b</td>
<td>80.37 ± 1.49d</td>
<td>21.80 ± 1.76c</td>
<td></td>
</tr>
<tr>
<td><em>Elettaria</em> (200 mg /kg)</td>
<td>100.75 ± 1.55b</td>
<td>97.00 ± 1.58b</td>
<td>48.37 ± 3.22b</td>
<td>97.00 ± 1.50d</td>
<td>19.40 ± 1.76c</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

**Antioxidant enzymes (GPx, SOD, CAT, Lipid peroxidation parameter (MDA) and tumor necrosis factor -α (α –TNF) in liver tissue.**

Table illustrated activity of liver glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were significantly decrease in positive control group when comparison with negative control group while increased in all treatment groups when comparison with (+ve ) control and best results found in groups treated with *Laurus nobilis* (200 mg /kg) and *Elettaria* (200 mg /kg).

Table (9) also showed that positive control group recorded a significant increase in the mean value of MDA and TNF-α compared with the negative control group while all treatment groups showed significant decrease compared with the (+ve) control rats and the best results found in groups treated with *Laurus nobilis* (200 mg /kg)and *Elettaria* (200 mg /kg).
Table (9): Protective effect of *Laurus nobilis* and *Elettaria* on GPx, SOD lipids peroxidation MDA, CAT and tumor necrosis factor -α in liver tissue in rats (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GPx  (U/mg)</th>
<th>SOD  (U/mg)</th>
<th>MDA  (nmol/mg)</th>
<th>CAT  (ng/mg)</th>
<th>α -TNF (Pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(- ve) Control</td>
<td>200.00 ±</td>
<td>205.33 ±</td>
<td>2.50 ±</td>
<td>15.00 ±</td>
<td>30.0 ±</td>
</tr>
<tr>
<td></td>
<td>2.00a</td>
<td>2.51a</td>
<td>0.100d</td>
<td>1.00a</td>
<td>2.00d</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td>70.00 ±</td>
<td>45.00 ±</td>
<td>44.00 ±</td>
<td>3.06 ±</td>
<td>150.0 ±</td>
</tr>
<tr>
<td></td>
<td>2.00c</td>
<td>1.00d</td>
<td>1.00a</td>
<td>1.00d</td>
<td>2.00a</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (100 mg /kg)</td>
<td>174.00 ±</td>
<td>170.00 ±</td>
<td>10.86 ±</td>
<td>6.00±</td>
<td>53.0 ±</td>
</tr>
<tr>
<td></td>
<td>2.00d</td>
<td>3.00c</td>
<td>1.00b</td>
<td>2.00c</td>
<td>3.00b</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> (200 mg /kg)</td>
<td>190.00 ±</td>
<td>190.00 ±</td>
<td>6.71 ±</td>
<td>10.00 ±</td>
<td>44.0 ±</td>
</tr>
<tr>
<td></td>
<td>1.00b</td>
<td>2.00b</td>
<td>2.00c</td>
<td>2.00b</td>
<td>2.00c</td>
</tr>
<tr>
<td><em>Elettaria</em> (100 mg /kg)</td>
<td>180.00 ±</td>
<td>173.00 ±</td>
<td>9.37 ±</td>
<td>7.00 ±</td>
<td>50.0 ±</td>
</tr>
<tr>
<td></td>
<td>2.00c</td>
<td>3.00c</td>
<td>1.00b</td>
<td>1.00c</td>
<td>1.00b</td>
</tr>
<tr>
<td><em>Elettaria</em> (200 mg /kg)</td>
<td>192.00 ±</td>
<td>194.0 ±</td>
<td>5.00 ±</td>
<td>12.00 ±</td>
<td>40.0 ±</td>
</tr>
<tr>
<td></td>
<td>2.00b</td>
<td>2.00b</td>
<td>1.00c</td>
<td>2.00b</td>
<td>4.00c</td>
</tr>
</tbody>
</table>

Means in the same column with completely different letters are significantly different at p<0.05.

**Histological Results:**

The results obtained from histological liver sections stained with H&EX 400 are illustrated in Fig.1 Liver section show no normal histological structure of hepatic lobules in (- ve) Control group (A). However, liver from paracetamol (+ ve) control group showed congestion of central vein, Kupffer cells activation , vacuolar degeneration of hepatocytes and dilatation with congestion of hepatic sinusoids as well as focal hepatocellular necrosis associated with inflammatory cells infiltration (B),while congestion of central vein and Kupffer cells activation observed in paracetamol + *Laurus nobilis* (100 mg /kg) group (C) when
compared to no histopathological changes except Kupffer cells activation in paracetamol + *Laurus nobilis* (200 mg /kg) group (D). However, exhibited Kupffer cells activation and microvesicular steatosis observed in paracetamol + *Elettaria* (100 mg /kg) group (E). No histopathological changes except Kupffer cells activation observed in *Elettaria* (200 mg /kg) group (F).

Fig. 1: Microscopic images of hematoxylin and eosin (H & E X 400) stained liver sections showing (A) Normal (-ve) Control group. (B) Paracetamol (+ve) Control group. (C) Paracetamol + *Laurus nobilis* (100 mg /kg) group.
(D) Paracetamol + *Laurus nobilis* (200 mg /kg) group. (E) paracetamol+Elettaria (100 mg /kg) group. (F) Paracetamol+Elettaria (200 mg /kg) group.

**Discussion**

Muñiz-Márquez *et al.*, (2014) who found that the HPLC analysis showed that presence of *L. nobilis* extract of four phenolic compounds, which responsible for its antioxidant activities: resorcinol, pyrogallol, gallic acid and coumaric acid. Caffeic, vain, and ferulic acids in leaves extracts *L. nobilis* reported by Muchuweti, *et al.*, (2007). Nihal *et al.*, (2016) told about presence of gallic acid, tannic acid, 4, 5-Dicaffeoyl quinic acid and caffeic acid in HPLC extract analyses of cardamomum. Polyphenolic are natural antioxidants, as caffeic acid and gallic acids that decrease oxidation of vital biomolecules (Beltran-Ramirez *et al.*, 2008).

These findings are in agreement with Kanchana and Sadiq, (2011) who concluded that the body weight gain of the group of rats treated with paracetamol is lower than the normal control group. Dwivedi *et al.*, (2015) investigated that the bodyweight and feed consumption in the treated group with paracetamol decreased statistically significantly in comparison to normal group. Adil *et al.*, (2016) told that body weight of acetaminophene rats decreased significantly from normal rats. Al Chalabi *et al.*, (2020) who noticed that *Laurus nobilis* extract treated group achieved an amelioration in body weight gain comparison with rat suffering from diabetes. El-Segaeyn *et al.*, (2007) reported that *cardamomum* elements can improve nutrient status and appetite and enhance element metabolism in the *cardamomum* group.

In positive group, hepatic weight is increased and this effect may be caused by hepatic cell inflammatory. In the histopathetic studies of the group treated with paracetamol in the liver tissue, clearly the central vena was dilated with bleeding and the presence of inflammatory cells in hepatic cells, according to the Dwivedi *et al.*, (2015). Also, Kane *et al.*, (2016) investigated the increase in
mean liver weight in all acute acetaminophen-treated groups (as percentage of body weight). In acetaminophene rats, Adil et al., (2016) who told that relative liver weight was improved comparison with ordinary rats. Alam et al., (2017) mentioned that a significant increase in relative liver weight of the experimental groups of animals treated with paracetamol compared with normal group.

Those findings are line with Ahmed et al., (2001) who mentioned that acetaminophen induced hepatotoxicity caused over-production of AST and ALP due to liver parenchymal injuries and increased (ALP) synthesis, which indicated that bile ducts affected ALP in response to cholesterol and increased biliary pressure. In paracetamol administered rats Ravindran et al., (2013) found that elevated serum activity of SAT and SAT caused by an increased permeability, damage and/or hepatocytes necrosis. Sanchez-Valle et al., (2012) and Al Chalabi et al., (2020) who mentioned that statistical reduction of the ALT, AST and ALP liver function in diabetic group which gives Laurus nobilis alcoholic extract compared to normal control group. The possible mechanism of the results because of the antioxidant compounds in this extract is proof of the diabetes-associated phénomene of fat oxidation and of its effect on necrosis and liver cell damage, thereby regulating the function of the liver. Prevent diabetic liver damage by improving and increasing the activity of antioxidants, leading to a reduction in hepatic lesions markers such as ALT, AST and ALP (Alam et al., 2014). Casamassima et al., (2017) who told decrease of the liver enzymes of ALT and AST was the resulted of dried leaves Laurus nobilis meals. Girish et al., (2009) who told that the cardamomum activity of the ALT, AST, and ALP enzymes has decreased significantly. This may be because the extracts protect hepatic cells and preserve their functional integrity. Darwish and Abd El Azime, (2013) found that AST, ALT, and ALP levels were reduced, indicating hepatoprotective effects by treatment with extract of Elettaria cardamomum seeds.
This finding are emphasized by Elkomy et al., (2016) told that administration of paracetamol by oral caused significantly lower levels of albumin when comparison with the normal rats. Mohammed et al., (2021) mentioned that albumin and total protein in L. nobilis extract group are insignificantly increased comparison to diabetic group of rat. Aboubakr and Abdelazem, (2016) who revealed that aqueous extract of cardamomum increased the lowered serum level of albumin in hepatotoxicity rats induced by gentamicin.

The results are supported by Hegde and Joshi, (2010) who indicated that the administration of acetaminophen caused hepatic abnormalities by significant increases in total bilirubin and direct bilirubin level hyperbilirubinemia caused by biliary tract obstruction and heme destruction. Ravindran et al., (2013) observed a substantial increase in serum bilirubin levels compared to all other treated groups with paracetamol intoxicated rats. Higher bilirubin levels are an indication of biliary obstruction and hemolysis a consequence of a reduced hepatocyte blood supply. Parekh and Klag, (2001) who told that L. nobilis methanol extract treatment significantly reduces increased serum liver enzymes and bilirubin levels to almost normal levels, which could be the result of membrane of plasma stabiliztion and preserving of functional state of liver of paracetamol toxicity. Mahmood et al., (2014) who reported that cardamomum extract has significantly decreased total bilirubin in serum towards normal which indicate that cardamomum has anti-hepatotoxic effect.

Results supported by Rajkapoor et al., (2008) & Elkomy et al., (2016) mentioned that comparison with normal control groups the oral administration of paracetamol has led to a considerable decrease in HDL-c. HDL-Cholesterol attributed to the overproduced of H2O2generated during microsomal metabolism of paracetamol generated during the cytochrome P450. Honmore et al., (2015) who cleared that administration of acetaminophen increases the total serum cholesterol, LDL and triglyceride and decreases the HDL level significantly. Because the lipoprotein and cholesterol metabolism have been impaired by
acetaminophen. Casamassima et al., (2017) mentioned that significant decrease in the lipid profile of the blood and increased HDL were the result of dried L. nobilis on meal. Al Chalabi et al., (2020) who indicated that decreased cholesterol concentration, TG, LDL and VLDL in diabetic groups treated with alcoholic extract of Laurus nobilis this is may be due to flavonoids found in Laurus nobilis which participated in lipid profile management, or could relate to the role of laurel leaves in decreasing the concentration of cholesterol. Chbili et al., (2020) recommended L. nobilis tea in healthy volunteer groups to improve blood lipid profile (increased HDL level and reduced triglycerid and LDL levels) and have possible positive effects on reducing risk for coronary heart disease (Musa et al., 2011 and Titilayo et al., 2018). Shobana and Naidu, (2000) and Sadeek and Abd el-Razek, (2010) indicated that cardamomum decreased in cholesterol and triglyceride concentrations has reflected its protective hepatocellular effects because cardamomum has phenolic compounds and essential oil which reduce the hyperlipidemia. Darwish and Abd El Azime, (2013) found that serum triglyceride and cholesterol levels in the diet of rats receiving cardamomum are attributed to inhibition of hepatic – HMG CoA reductase activity. In addition to its effectiveness in reducting LDL-c susceptibility to oxidation. Aboubakr and Abdelazem, (2016) who revealed that significant improvement in HDL-c, serum levels in cardamomum aqueous extract in hepatotoxicity rats induced by gentamicin

Ravindran et al., (2013) who observed that rats treated with paracetamol significant reduction in SOD activity in tissues and erythrocytes this decline could be due to inefficient ROS scavenging which could be involved in the in activation of oxidative enzymes. Sakran et al., (2014) told that liver and total plasma antioxidant GPx, CAT and SOD in the Paracetamol group decreased significantly from their normal control group and the concentration of MDA increased markedly comparison with the standard control group. Honmore et al.,(2015) studied that acetaminophen administered significantly decreased SOD and increased MDA in hepatic tissue due to toxicity induced by the
acetaminophene, increased peroxidation of free radicals and/or decreased antioxidant protection system activity. Rathee et al., (2018) have shown that a significant increase is observed in paracetamol group comparing with standard control group in oxidative stress malondialdehyde and inflammatory mediators. Also, Mallikarjuna et al., (2008) told that reduction in catalase activity during the ingestion of paracetamol which caused by inefficient scavenging of $\text{H}_2\text{O}_2$. Ravindran et al., (2013) who investigated that a significant modulation of antioxidant modulation by co-administration of methanolic extract of Laurus nobilis in erythrocytes, plasma, and tissue with paracetamol to suggest an enhanced effect of Laurus nobilis on cellular antioxidant defence. Elevation of antioxidant status presumably provides a protection against the peroxidation of lipids by quenching and detoxifying free radicals that almost normalize them. Yadav and Bhatnagar, (2007) reported cardamomum inhibits lipid peroxidation because it's strong reducing power and superoxide radical scavenging activity. Cardamomum has a significantly lower level of MDA in irradiated animals and increases the activity of antioxidant enzymes SOD & CAT. Nihal et al., (2016) reported that cardamomum treatment decreased liver damage and also significantly inhibited the formation of hepatic MDA as well as increased antioxidant enzyme activities in the liver such as SOD, GPx and catalase CAT in the hepatic of diethylnitrosamine treated rats due to antioxidant effect of cardamomum and has inhibit of inflammatory markers.

**Conclusion**

Paracetamol has toxic side effects on experimental animals proved by biochemical and histological results. The results concluded that using high doses of alcoholic extracts of Laurus nobilis and Elettaria cardamomum improved liver function and hematological parameters so we recommended people to use Laurus nobilis and Elettaria cardamomum as spices or tea to benefit from their health benefits.
References


التأثير الوقائي المحتمل للمستخلصات الكحولية لبعض الأعشاب
على التسمم الكبدي المحدث بالباراسيتامول في فئران التجارب.

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قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلي – جامعة الأزهر - مصر

المستخلص العربي

يوجد العديد من النباتات التي يستخدمها الكثيرون من الناس كتوابل مثل اللورا والحبيان، الـ (النميل)، أجريت الدراسة الحالية لمعرفة التأثير الوقائي للمستخلصات الكحولية لكلا من ورق اللورا والحبان بتركيز مختلفين (100 و 200 مجم / كجم من وزن الجسم) على السمية الكبدية التي يسببها الباراسيتامول (400 مجم / كجم من وزن الجسم) في فئران التجارب. تم استخدام 30 فأر تتراوح أوزانهم بين (150 ± 10 جم). تم تقسيم الفئران إلى 6 مجموعات متساوية. تركت أحد المجموعات كمجموعة ضابطة سالبة، المجموعة (0) تم تغذيتها على الغذاء القياسي كمجموعة ضابطة، جميع المجموعات الأخرى تم تغذيتها على الغذاء القياسي بالإضافة إلى جرعات من الأعشاب عن طريق الفم كالتالي: المجموعة (3 و 4) تم إعطائها ورق اللورا بتركيزين (100 و 200 مجم / كجم من وزن الجسم)، والمجموعة (0 و 6) تم إعطائها الحبان بتركيزين (100 و 200 مجم / كجم من وزن الجسم) وذلك يوميا لمدة 28 يوماً، وفي اليوم الحادي والعشرين بالإضافة لمستخلصات الأعشاب تم إعطاء المجموعات (2، 3، 4، 5، 6) الباراسيتامول بتركيز (400 مجم / كجم من وزن الجسم) عن طريق الفم لمدة أسبوع. في نهاية فترة التجربة تم حساب التقييم البيولوجي. كما تم تقدير مستوي إنزيمات الكبد والبيليروبين والبروتينات ودهون الدم في السير ومصادر الأكسدة وعامل النخر في أنسيجة الكبد. أظهرت النتائج أن المستخلص الكحولي لورق اللورا والحبان أدى إلى حدوث تحسن في التقييم البيولوجي ومستوي إنزيمات الكبد ومصادر الأكسدة مقارنة بالمجموعة الضابطة الموجبة. يمكن أن نستنتج أن نتناول مستخلصات أوراق اللورا والحبان يحميان الكبد بصورة ملحوظة من الآثار الضارة للباراسيتامول.

الكلمات المفتاحية: اللورا، الحبان، الباراسيتامول، وظائف الكبد، الإنزيمات المشاهدة للاكسدة

المجلد السابع - العدد السابع والعشرون والثلاثون - نوفمبر 2021

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