Effect of Some Herbal Plants on Liver Function of Rats Treated with Trichloroethylene

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Abstract

**Background & Aims:** Curcumin (CCM), Cinnamon (CNN) and Ginger (GE) had been considered to possess antioxidant activities. This study aimed to investigate their protection effect against trichloroethylene (TCE)-induced hepatotoxicity and to demonstrate its possible mode of action.

**Methods:** Rats were fed CCM, CNN and GE singly with or without 5mg/l of TCE in drinking water. The rat were killed after treatment period of 8 weeks, and the serum levels of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), bilirubin, proteins, glucose, albumin and triglycerides were determined.

**Results:** TCE-receiving rat exhibited significant changes in biomarkers of liver function to indicate liver injury. The CCM containing diet significantly ameliorated the serum aminotransferases, especially ALAT, total protein and albumin. The CNN containing diet significantly ameliorated the total protein and albumin. The GE containing diet significantly ameliorated the serum aminotransferases, especially ALAT.

In conclusion, this work indicated that CCM is the most herbal plant which can protected and alleviated the liver function.

**Key Words:** Trichloroethylene - Curcumin - Cinnamon - Ginger - Rats

Introduction

Nowadays, there is an increasing interest in the protective function of dietary antioxidants, which play an important role in the protection of liver. CCM (diferuloyl methane) is a phenolic compound present in large quantities in the root of plant *curcuma longa*. It has been widely used as a spice and coloring agent in food. Recently, CCM has been considered to possess anti-inflammatory and antioxidant activities (Anto et al., 2000). The ability of CCM to prevent tumor formation in the skin, forestomach, duodenum, and colon of mice and in the tongue, colon, mammary glands, and sebaceous glands of rats has been well documented (sharma et al., 2001). CCM has been also shown to inhibit lipid peroxidation caused by many toxic agents in hepatocytes either in vitro or in vivo (Ramirez Bosca et al.,1995; Devasena et al., 2002). On the other hand, no treatment-related toxicity was observed up to an oral dose of 8000 mg/day for 3 month in mice (Chuang et al., 2000). This non toxic nature of CCM, as well as its multiple beneficial clinical effects, has made it one of the most attractive antioxidants.

CNN (Cinnamon), also known by Cassia, Sweet Wood, and Gui Zhi, is traditionally harvested in Asian
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countries (Leung, 1996; Toriizuka, 1998). Many studies have shown the diverse biological functions of cinnamon including anti-diabetic effects (Qin et al., 2003), anti-inflammatory (Lee et al., 2005), An antitumor effect of cinnamon was previously suggested in vitro (Schoene et al., 2005) without in vivo evidence or a working mechanism, and anti-microbial (Matan et al., 2006) and anti-oxidant (Singh et al., 2007).

In addition, Phenol compounds present in (cinnamon) spices that show natural anti-oxidant properties have been studied for substitution of synthetic anti-oxidants, due to possible side effects of synthetic anti-oxidants which may in some circumstances act deleterious to animal organisms (Pratt, 1992).

GE (Ginger), is one of the most widely used spices around the world and is a common condiment for a variety of compounded foods and beverages (Gupta, 2008). Ginger (Zingiber officinale Roscoe) has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). Recently, ginger has received increasing attention because of its pronounced anti-oxidant (El-Ghorab et al., 2010), anti-inflammatory (Minghetti et al., 2007), anti-diabetic (Afshari et al., 2007) anticancer activities (Shukla and Singh, 2007), anti-emetic, anxyolitic, anti-thrombotic, anti-pyretic, analgesic, and a treatment for toothache, insomnia, baldness, urinary tract infections, and as therapy for various gastrointestinal disorders (Balch, 1996), arthritis, rheumatism (Dedov et al., 2002; Wang and Wang, 2005; Tapsell et al., 2006), sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis (Ali et al., 2008), and cardiovascular effects (Kaul and Joshi, 2001; Afzal et al., 2001).

The present study was thus designed to investigate the protective activity of CCM, CNN and GE against TCE-induced liver injury. Selected routine work biochemical markers of liver function tests were chosen to express the effect.

Material and Methods

Chemicals: Pure TCE was purchased from Sigma Chem. Co.(St. Louis, MO, USA). Powdered CCM, CNN and GE was purchased from Libya spice market. Kits for biochemical measurements were supplied from Randox Labratories Ltd., UK.

Diets: Standard diet was prepared from Casein (20%), Starch (32%), Sucrose(33%), Cellulose(5%) , Corn oil(5%) and Vitamin / Mineral(5%). (Abd-Allah, 2003).

Animals and Treatments:

Twenty five male albino Wistar rats (weight range 58 – 160 g ) were used for the experimental study. Animals were obtained from hellwan animal station, Ministry of Heath, Egypt . The experimental rats were housed in the animal house in zoology Department, Faculty of Science, Damietta Branch, Mansoura University, New Damietta, Egypt. They were divided into 8 groups of 5 rats each.

Group 1: (Normal) rats were given basal diet and water for 8 weeks.

Group 2: (Trichloroethylene) rats were given 5% TCE in drinking water for 8 Weeks.

Group 3: (Curcumin) rats were given Curcumin at 1g /kg diet for 8 weeks.

Group 4: (Trichloroethylene + Curcumin) rats were given 5% Trichloroethylene in drinking water and Curcumin at 1g /kg diet for 8 weeks.

Group 5: (Cinnamon) rats were given Cinnamon at 1g /kg diet for 8 weeks.

Group 6: (Trichloroethylene + Cinnamon) rats were given 5% Trichloroethylene in drinking water and Cinnamon at 1g /kg diet for 8 weeks.
Group 7: (Ginger) rats were given Ginger at 1g/kg diet for 8 weeks.

Group 8: (Trichloroethylene + Ginger) rats were given 5% Trichloroethylene in drinking water and Ginger at 1g/kg diet for 8 weeks.

**Sampling and Measurements:**
After 8 weeks of treatments, rats were killed by Anesthesia and the blood was withdrawn by syringe into blood centrifuge tubes. Blood collected was allowed to coagulate at 37°C for 30 minutes. Serum was separated by centrifugation at 4000 rpm and used for the estimations of ALAT (Reitman and Frankel 1957), ASAT (Reitman and Frankel 1957), total Bilirubin (Walloy and Gerade 1970), albumin (Doumas et al., 1971), triglycerides (Vassault(1986), total protein (Gornal et al.,1949) and Glucose (Trinder-reaction (1969)).

**Statistics:** Student’s *t* test was used for statistical analysis of the data. The significant levels were considered as: *p* ≤ 0.05, *p* ≤ 0.01 and *p* ≤ 0.001, (Millar 2001).

**Results**

**Serum Aminotransferases:**
Table (1) presents the results of serum ALAT and ASAT of rats treated with TCE with or without plant herbs. Means of serum ALAT and serum ASAT were significantly higher in rats treated with TCE than that of normal animals. On the other, serum ALAT and ASAT did not changed in animals fed on herbs alone than that of normal ones.

On the effect of herbs the mean of serum ALAT was completely improved in rats treated with TCE /CCM, but no changes were observed with CNN and GE. No alleviation was resulted in serum ASAT of rats fed on all experimental herbs.

**Liver function tests:**
Table (2) presents the results of serum concentrations of total protein, albumin and bilirubin of rats treated with TCE with or without plant herbs.

Means of serum total protein was significantly lower in rats treated with TCE than that of normal animals. On the other, serum total protein did not changed in animals fed on CCM and CNN alone than that of normal ones. On adding of Herbal plants to TCE treatment serum total protein was partially ameliorated with CCM and CNN .On the other hand, no significant changes were observed in rats treated with TCE/GE.

In similar, means of serum albumin was significantly lower in rats treated with TCE than that of normal animals. On the other, serum albumin did not changed in animals fed on CCM alone than that of normal ones. On adding of herbal plants to TCE treatment serum albumin was partially ameliorated with CCM and GE . On the other hand, no significant changes were observed in rats treated with TCE/CNN.

Means of serum bilirubin was significantly higher in rats treated with TCE than that of normal animals. On the other, serum bilirubin did not changed in animals fed on herbs alone than that of normal ones. On adding of herbal plants to TCE treatment serum bilirubin was partially ameliorated with CCM and CNN . On the contrary, no improvement was observed on adding GE to TCE treatment.

**Serum Metabolites:**
Table (3) presents the results of serum concentrations of Triglycerides and Glucose of rats treated with TCE with or without plant herbs. Means of serum triglycerides and serum glucose were significantly higher in rats treated with TCE than that of normal animals. On the other, serum triglycerides and glucose did not changed in animals fed on herbs alone than that of normal ones.
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On adding herbs to diet the mean of serum triglycerides was partially ameliorated in rats treated with TCE /CCM, but no changes were observed with CNN and GE. No alleviation was resulted in serum Glucose of rats fed on all tested herbs.

Table (1): Activities of serum aminotransferases in rats treated with CCM, CNN and GE with TCE.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>TCE</th>
<th>CCM</th>
<th>TCE+CCM</th>
<th>CNN</th>
<th>TCE+CNN</th>
<th>±GE</th>
<th>TCE+GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase (IU/l)</td>
<td>16.0±</td>
<td>28.6±</td>
<td>15.4±</td>
<td>16.6± 1.34</td>
<td>15.4±</td>
<td>29.4± 2.19</td>
<td>15.4±</td>
<td>23.8± 1.78</td>
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<tr>
<td>Mean ± SD</td>
<td>2.12</td>
<td>2.19</td>
<td>1.34</td>
<td>NS</td>
<td>1.34</td>
<td>NS</td>
<td>2.50</td>
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<td>*P-value vs. control</td>
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<td>*P-value vs. TCE</td>
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<tr>
<td>Aspartate aminotransferase (IU/l)</td>
<td>16.0±</td>
<td>32.2±</td>
<td>15.4±</td>
<td>32.2± 3.83</td>
<td>17.2±</td>
<td>34.0± 2.74</td>
<td>19.4±</td>
<td>29.4± 2.19</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.12</td>
<td>3.83</td>
<td>1.34</td>
<td>NS</td>
<td>1.64</td>
<td>NS</td>
<td>3.51</td>
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<td>*P-value vs. control</td>
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</table>

Number of animals per group=5  *= significant , **and***= significant higher , ****= significant very higher , NS = Non- significant value

Table (2): Serum Concentrations of serum metabolites in rats treated with CCM, CNN and GE with TCE.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>TCE</th>
<th>CCM</th>
<th>TCE+CCM</th>
<th>CNN</th>
<th>TCE+CNN</th>
<th>GE</th>
<th>TCE+GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>5.82±0.26</td>
<td>5.46±0.15</td>
<td>7.01±0.65</td>
<td>6.23±0.18</td>
<td>6.21±0.43</td>
<td>6.68±0.66</td>
<td>5.36±0.70</td>
<td>5.42±0.49</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.26</td>
<td>0.15</td>
<td>0.65</td>
<td>0.18</td>
<td>0.43</td>
<td>0.66</td>
<td>0.70</td>
<td>0.49</td>
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<td>*P-value vs. control</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>3.75±0.01</td>
<td>3.13±0.02</td>
<td>3.83±0.03</td>
<td>3.59±0.03</td>
<td>3.38±0.10</td>
<td>3.18±0.03</td>
<td>3.17±0.02</td>
<td>3.38±0.02</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.10</td>
<td>0.03</td>
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<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.22±0.06</td>
<td>0.65±0.11</td>
<td>0.26±0.02</td>
<td>0.63±0.03</td>
<td>0.31±0.05</td>
<td>0.58±0.04</td>
<td>0.25±0.04</td>
<td>0.74±0.14</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.06</td>
<td>0.11</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
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<th>GE</th>
<th>TCE+GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (g/dl)</td>
<td>70.5±4.66</td>
<td>180.4±18.48</td>
<td>65.6±7.05</td>
<td>159.1±11.96</td>
<td>73.6±8.96</td>
<td>184.4±10.56</td>
<td>70.9±9.37</td>
<td>178.5±3.12</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>56.07±0.56</td>
<td>102.79±2.77</td>
<td>52.41±0.68</td>
<td>107.24±1.40</td>
<td>55.66±1.09</td>
<td>104.24±2.43</td>
<td>55.24±0.69</td>
<td>105.08±1.29</td>
</tr>
</tbody>
</table>

Number of animals per group=5  *= significant , **and***= significant higher , ****= significant very higher , NS = Non- significant value

Discussion

The toxicity of TCE is dependent on its metabolism, which occurs by either cytochrome p-450 ( p-450) –dependent oxidation or glutathione ( GSH) conjugation. Metabolites derived from p-450 metabolism, including trichloroacetate ( TCA) and dichloracetate ( DCA), have been associated with the hepatotoxicity ( Davidson and Beliles, 1991; Lash et al., 1999).

The effect of TCE was agreed to that reported by (Maiti et al., 2004; Maiti et al., 2005 and Beppu et al., 2006). Many medicinal plants are used today in therapy of different diseases (Mascolo et al., 1989).

Curcumin Over the years, a number of studies have tried addressing the pharmacokinetics of that is poorly absorbed from intestine after oral administration of different doses of 3H-curcumin in rats (Ravindranath and Chandrasekhara, 1980, Ravindranath and Chandrasekhara, 1981 and Ravindranath and Chandrasekhara, 1982). It was shown that oral consumption of <curcumin> in rats resulted in approximately 75% being excreted in the feces and only traces appeared in the urine (Wahlstrom and Blenno, 1978), converted to monoglucuronide conjugates (Pan et al., 1999).

In the present work, adding CCM to TCE treated rats was completely improved the results of ALAT and albumin, but the other parameters, total protein, bilirubin and Triglycerides, were partially alleviated. These results are more or less agreed to that obtained by Abd-Allah (2003). Because the measurement of serum ALAT and albumin are more from the intestine. Numerous studies have suggested presence of different metabolites of <curcumin> It has been shown to be bio-transformed to 11% found in bile (Holder et al., 1978) ,suggesting poor absorption of <curcumin> of fecal excretion of <curcumin> with only dihydrocurcumin and tetrahydrocurcumin. the absolute abolishment of their increase closely indicate the ability of CCM to minimize the TCE-induced liver injury in the present study. It may be argued that the ability of CCM to scavenge free radicals
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might have been responsible for its antioxidative activity. Moreover, it is also with the hepatic metabolism of TCE though the modulation of liver drug metabolizing enzymes involved in its biotransformation, i.e cytochrome P450 and glutathione-s-transferase (Sharma et al., 2001; Thapliyal and Maru, 2001).

Similarly, the interaction between TCE and CCM effects on the metabolism of proteins, carbohydrates and lipid may be attributed to the same issue or to the lipid-lowering potent activity in vivo by the dietary curcuminoid (Asia and Miyazawa, 2001). However, this point is still unclear and needs further investigations to be resolved and cleared up.

CNN is a popular flavoring ingredient, widely used in food products. It has exhibited beneficial properties to health, such as antimicrobial activity, for controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and for treating the common cold (Anderson & Broadhurst, 2004; Murcia et al., 2004). CNN extracts can reduce lipid peroxidation in the (3-carotene-linoleic acid system (Mancini-Filho & Van-Koijj, 1998). CNN extracts exhibit a protective capacity against irradiation induced lipid peroxidation in liposomes, and quench hydroxyl radicals and hydrogen peroxide (Murcia et al., 2004). Extracts on lard and vegetable oils demonstrated that they could stabilize lard against oxidation and showed antioxidative properties when tested on vegetable oils during storage or frying conditions (Vekiari, & Thomopoulos, 1993).

In the present work, CNN was partially ameliorated the effect of TCE on the liver most selected biochemical markers, although total protein was showed completely improvement. Moselhy and Junbi (2010) observed that the elevated serum ASAT and ALAT enzymatic activities induced by CCl4 were restored possible that CCM might have interfered towards normalization significantly by orally administrated 200 mg/kg CNN of either extracts once daily for 7 days as compared to non treated rats. The results obtained indicated that CNN extract have potent hepatoprotective action against CCl4 by lowering the MDA level and elevate the antioxidants enzymes activities. GE (Zingiber officinale Roscoe) is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part (Mascolo et al., 1989). Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects demonstrated is antiplatelet, antioxidant, anti-tumour, antirhinoviral, antihepatotoxicity immunomodulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic anti-emic actions and anti arthritic effect (Fisher-Rasmussen et al., 1991; Sharma et al., 1994 and Kamtchouing et al., 2002). Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali et al., 2007).

In the present work, GE is partially ameliorated ALAT, ASAT, Triglycerides and albumin, but other biochemical markers did not showed changes.

There results are similar to the result of Sakr (2007), who observed that the Treating animals with ginger led to significant decrease in ALT and AST activity in albino rats. El-Sharaky et al., (2009) reported that the results showed significant lowering of serum ASAT, ALAT that Ginger extract was found to have a protective effect on CCl4 and acetaminophen-induced damage as confirmed by histopathological
examination of the liver in rats (Yemitan and Izeqbu, 2006).
In conclusion, the present results showed that   
skeleted plant herbs induced either complete improvement or partial amelioration on the effect of TCE on the liver . By comparison the CCM was the most potent whit normalize of most tested biochemical markers.

References


تأثير بعض النباتات العشبية على وظائف الكبد في الفأران المعالجة بتراي كلوروايثيلين
ليلي منصور عطية الغويج, د.أحمد مسعد غنيم, د.أشرف متولى السعيد, أ.د.جمال عبد الرحيم عبد الله
قسم الاحياء – كلية العلوم والتربيه. جامعة المرقب - مصري
قسم علم الحيوان – كلية العلوم بدمياط – جامعة المنصورة – دمياط الجديدة – مصري

الكركم والقرفة والزنجبيل هم مركبات فينولية من أصل نباتي. تستخدم بكثرة كتوابل الطعام. وقد وجد حديثا أن الكركم والقرفة والزنجبيل خصائص مضادة للأكسدة. وتهدف الدراسة الحالية لفحص ما إذا كان الكركم والقرفة والزنجبيل تقي من تلف الكبد المحدث بتراي كلورو ايثيلين, وكذلك لاظهار آلية نشاطه المحتملة.

الطريقة البحثية:

تم استخدام أبعون من ذكور الحيوانات البيضاء تتراوح أوزانهم بين 85 – 160 جم في الدراسة التجريبية. تم تقسيم حيوانات التجربة إلى ثمانية مجموعات خمس فئران في كل مجموعة. المجموعة الأولى (جرذان طبيعي) تناولت حمييم وماء حسب الرغبة, المجموعة الثانية (تراي كلوروايثيلين) تناولت 5% من تراي كلوروايثيلين مذابة في المجموعة الثالثة (الكركم) تناولت حيبي تحتوي على الكركم 1 جرام لكل كيلو جرام. المجموعة الرابعة (الكركم و تراي كلوروايثيلين) تناولت 5% من تراي كلوروايثيلين مذابة في مياه شرب متزامنة مع اعطاء وجبة تحتوي على الكركم 1 جرام لكل كيلو جرام. المجموعة الخامسة (القرفة) تناولت 5% من تراي كلوروايثيلين مذابة في مياه شرب متزامنة مع اعطاء وجبة تحتوي على القرفة 1 جرام لكل كيلو جرام. المجموعة السادسة (الزنجبيل) تناولت 5% من تراي كلوروايثيلين مذابة في مياه شرب متزامنة مع اعطاء وجبة تحتوي على الزنجبيل 1 جرام لكل كيلو جرام.

النتائج:

أظهرت مجموعة الفئران المعالمة بتراي كلوروايثيلين نموذج لوحة الأكسدة في الكبد (الإكسمة الفوقية في الدهون) والتي تمثلت في زيادة مستويات المصل من أنزيمات نقل الأمين. وقد أكتت الزيادة في مستويات الأنزيمات الدافعة للأمين. حدث تلف في خلايا الكبد أثناء وطأة الأكسدة المستحقة بتراي كلوروايثيلين. ولكن الوجبة المزودة بالكركم قد قمعت معنوي الزيادة في الأنزيمات الدافعة للأمين. والبروتين الكلي والأبيومين. والوجبة المزودة بالزنجبيل حسب الظاهر بالجزيئات الأذى. ويشكل كبير أنزيمات نقل الأمين.

الخلاصة:

أظهرت الدراسة الحالية أن الاعشاب المختبرة أدت ما إلى تحسين كامل أو جزئي على تأثير بتراي كلوروايثيلين على الكبد.

وعلل سبب المقارنة كان الكركم أكثر قوة في التحسين إلى الطبيعي من قبل الاعشاب المختبرة.