Impact of Cinnamon Extract on Liver, Kidneys and Spleen of Diabetic Rats

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Abstract

The use of different plants and spices as herbal drugs for treating different diseases has been tested in many studies. Therefore, this study was designed to study the histological changes in liver, kidneys and spleen of male albino rats after treated with cinnamon extract in which, normal and diabetic rats were used. During this study, forty adult male rats weighting about 170-188g were randomly divided into four groups each with 10 rats; each group was designed as follows: The first group was used as a control and was fed on standard diet and tap water ad libitum. The second group was treated with alcoholic cinnamon extract (500 mg/kg.bw). Third group was group was injected with alloxan as a diabetic group. The fourth group was diabetic rats treated with alcoholic cinnamon extract (500 mg/kg. bw). After 30 days of experiment, rats were anaesthetized and the blood was taken by cardiac puncture for estimation biochemical parameters and samples of liver, kidneys and spleen were fixed in formalin 10% and processed for histological studies. During histological examination of liver, kidney and spleen, no structural changes were found in the tissue of the examined organs in normal rats treated with cinnamon. In the diabetic group, structural damages were observed in the liver and kidneys whereas, spleen showed no obvious changes in all animals, these damages were reduced after administration of cinnamon extract to diabetic rats. Conclusion: From the results of the present study, it can be concluded that ethanolic extract of cassia cinnamon bark has improving role against abnormal histological changes in kidneys and liver of diabetic rats.

Keywords

Cinnamon Extract, Diabetic Rats

1. Introduction

Plants have a long ancient history in the traditional medicine uses that are in a rising demand using their extracts and chemical bioactive compounds for producing drugs against many diseases (Nikbakht and Kafi, 2004). Cinnamon plant (family: Lauraceae) is a common food additive for it flavor and aromatic properties. Cassia cinnamon (Cinnamomum cassia) is one of the popular species of cinnamon, which is widely distributed in Asia especially in China (Jayaprakasha, et al., 2003). In spite of using inner bark of cinnamon as a food additive in cooking or to treat digestive system and urinary problems, fight bad breath, stave off common cold and promotion of wound healing for many years (Archer, 1998), but recently it has become increasingly popular for its beneficial role in glucose metabolism (Preuss, et al., 2006). Some study were mentioned that the active compounds of cinnamon (such as cinnamaldehyde, eugenol and other compounds) possess wide ranges of pharmacological effects that seems to be highly bioactive against diabetes by its effect on insulin secretion and stimulate glucose uptake by hepatocytes and adipocytes (Qin, et al., 2003 and Vangalapati, et al., 2012). Therefore, in many studies, experimental animals rats or mice were subjected to diabetes by alloxan or other drugs (Jarvill-Tavilor, et al., 2001) to study the role of cinnamon on blood glucose and its uptake by tissues with examining of histological changes in these
animals (Cao, et al. 2007 and Soliman, et al., 2012). Some studies found that the administration of cinnamon oil to alloxan induced diabetic rats for three months resulted in the reduction in kidney weight and glomerular histological changes that revealed features of normal renal tissues when compared with the renal tissues of untreated diabetic rats (Mahera, 2006 and Mishra, et al., 2010). Other found that the treating of cinnamon extract has effective role against tissues damage in the carbon tetra chloride (CCl4) treated animals through antioxidant roles of cinnamon (Nakamura, et al., 2001).

2. Materials and Methods

2.1. Animal and Housing

In this study, adult male albino rats (Rattus norvegicus) weighting 170-188g and 9-10 weeks of age were used. Rats were breed in the Animal House of the Department of Biology / Faculty of Science / University of Duhok and placed in ventilated polypropylene cages (30×25×17cm), four rats per cage with their access to standard diet and tap water ad libitum under standard laboratory conditions (12 h light: 12 h dark photoperiod, at 24 ± 2ºC) and acclimated to the laboratory conditions for 3 weeks then were mixed for breeding and adult males were used for experimental studies.

2.2. Preparation of Alcoholic Extract of Cassia Cinnamon

The stem barks of {Cinnamomum cassia (L.) Don.} Was purchased commercially from local markets in Duhok City in April 2013. The barks were authenticated by the Professor Dr. Saleem Esmael Shahbaz, Taxonomist at the Faculty of Agriculture and Forestry, Department of Forestry, University of Duhok.

Extraction of cassia cinnamon was prepared according to other previous studies by adding 100g dried powdered bark of cinnamon into 1000 ml of ethanol 70% (Scharlab S.L-Spain) in a dark bottle and mixed manually (4 times at least in a day) and kept in dark place at a room temperature for 2 days, then the mixture was filtrated with wattrman paper no.2 and evaporated to dryness under vacuum at 45°C by Rotary Evaporator (Eyela-United Kingdom) for removing ethanol. The residual extract was dissolved in a tap water whenever it used in the experiments (Soliman, et al., 2012 and Su-Chen, et al., 2013). This was repeated in each week to obtain a fresh cinnamon extract as a treatment for the experiment.

2.3. Induction of Diabetes

Twenty rats were fasted for overnight and treated with a single subcutaneously injection of alloxan monohydrate (150 mg/kg.bw) (Sigma - Alorich from United Kingdom), which was freshly dissolved in 0.1M citrate buffer (PH = 4.5) and injected immediately. Alloxan treated animals were allowed to drink 5% of D-Glucose (Scharlab S.L-Spain) for 2-4 h to prevent the potentially fatal hypoglycemia in injected rats. Symptoms of diabetes were appeared within 2 days after alloxan injection such as elevated blood glucose, polyuria, and glycosuria. Diabetes was confirmed in treated rats by testing blood glucose from the tail of animals by Acc-glucose check (Roche-Germany) and glucose in urine by using urine strips (Machinery-Nagal/ Germany), animals with fasting blood glucose more than 200mg/dl and glycosuria were considered as diabetes rats.

2.4. Standard Diet

Standard diet was prepared for 1kg as follows: wheat 665.5g, soya 256.2g, oil sun flower 43.5g, lime stone 14.9g, Ca3(PO4) 6.42, salt 6.34g, lysine 2.44g, methionine 1.56g, enzymes 0.8g, choline chloride 0.62g, vitamins 0.58g and trace elements 0.5g (National Research Council, 1995).

2.5. Histological Study

After dissection of animals (through longitudinally cutting in the abdominal region), the abdominal and thoracic cavity were opened immediately then the liver, kidney and spleen were removed and examined macroscopically. Tissues were cleaned several times with distilled water then fixed in formalin 10% (Scharlab S.L-Spain). The samples were preceded for histological section in the department of histopathology at the Medical Center Lab / Duhok City. The remaining steps were continued deepening on a study by (Bancroft and Gamble, 2002):

1. Dehydration of tissue was done by passing the tissue among ascending series of ethanol 85% for 15 min, ethanol 95% for 1 hour and ethanol 100% in two stages 30 min for each stage.

2. Clearing (de-alcoholization) of sectioned tissues was by immersing them in a xylene (Scharlab S. L-Spain) +100% alcohol for 30min, then another time in xylene for 30min.

3. Infiltration: The cleared tissue were infiltrated with xylene then placed in melted paraffin (Kaltek srl-Italy) at 58-60°C in two stages 1hour for each stage to evaporate the solvent (xylene) and paraffin let to enter the spaces of the tissue to allow the tissue to be filled with paraffin.

- Dehydration, clearing and infiltration stages were done by Auto Processer (Leica/Germany).

1. Embedding: The tissues were embedded in fresh and clean paraffin by Therm Embedding Paraffin (Thermo-Canada). The embedded tissue with melted paraffin was allowed to solidify and then it was ready for sectioning.
2. **Sectioning:** Blocks of solidified paraffin were sectioned by Rotary Microtome (Leica\ Germany) with 5µm thick and mounted on clean microscope slides.

3. **Staining:** Slides were stained with haematoxylin (H) and eosin (E) stains (Atom Scientific\England) using Auto Staining Apparatus (Leica \ Germany) that was designed to stain the slides by processing them in the following stages: xylene in two stages 20 min for each stage. Ascending stages of ethanol series (100%, 95% and 70% in 2 min for each stage). Haematoxylin stain was used for 5 min, washed using tap water for 1 min, eosin stain for 2 min, descending ethanol stages (70%, 95% and 100% 2 min for each stage) and again xylene was used in two stages 2 min for each stage. Slides were mounted in Canada balsam (Scharlab S.L-Spain) then covered.

4. **Identification:** The mounted slides were identified by light microscope (Motic/China) for the detection of histological changes in each slide depending on comparing them with normal tissue in control group, then the pointed field was taken by specialized Camera (Anmo/Taiwan) connected with microscope.

### 2.6. Statistical Analysis

Data were analyzed statistically by using excel and GraphPad Prism 5 using analysis of variance (ANOVA) followed by Dunnet’s test. Firstly all groups were compared with control group (pointed as *), Diabetic groups were compared with each one (pointed as small letters; a, b). Results were expressed as mean ± standard error and P-values < 0.05 were considered statistical significant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>(Standard diet) Control</th>
<th>(Standard diet+Cinn. extract)</th>
<th>Diabetic group</th>
<th>Diabetic+Cinn. extract</th>
<th>Diabetic+Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg/dl</td>
<td>107.2±2.7</td>
<td>94.56±2.8*</td>
<td>401.8±3.5***a</td>
<td>221.1±3.5***b</td>
<td>161.56±2.2***c</td>
<td></td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>40.20±1.9</td>
<td>35.40±1.7</td>
<td>97.25±2.2***a</td>
<td>66.33±4.7***b</td>
<td>53.78±2.7***c</td>
<td></td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.38±0.019</td>
<td>0.33±0.017</td>
<td>0.61±0.026***a</td>
<td>0.51±0.028***b</td>
<td>0.42±0.023c</td>
<td></td>
</tr>
<tr>
<td>AST IU/L</td>
<td>125.0±1.8</td>
<td>124.0±3.2</td>
<td>178.3±4.4***a</td>
<td>152.8±3.1***b</td>
<td>139.7±4.8***c</td>
<td></td>
</tr>
<tr>
<td>ALT IU/L</td>
<td>58.30±3.2</td>
<td>55.11±1.8</td>
<td>128.2±3.9***a</td>
<td>99.11±4.6***b</td>
<td>66.44±2.9c</td>
<td></td>
</tr>
<tr>
<td>ALP IU/L</td>
<td>155.4±8.6</td>
<td>156.6±9.8</td>
<td>172.1±5.9a</td>
<td>156.3±7.3a</td>
<td>150.9±9.8a</td>
<td></td>
</tr>
<tr>
<td>TSB mg/dl</td>
<td>0.114±0.007</td>
<td>0.108±0.004</td>
<td>0.207±0.008***a</td>
<td>0.173±0.012***b</td>
<td>0.136±0.007b</td>
<td></td>
</tr>
<tr>
<td>Total Protein g/dl</td>
<td>6.53±0.14</td>
<td>6.63±0.17</td>
<td>5.22±0.28***a</td>
<td>5.79±0.15**b</td>
<td>6.18±0.13b</td>
<td></td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.85±0.15</td>
<td>3.90±0.13</td>
<td>3.21±0.19**a</td>
<td>3.40±0.11*a</td>
<td>3.75±0.17a</td>
<td></td>
</tr>
<tr>
<td>Globulin g/dl</td>
<td>2.55±0.13</td>
<td>2.52±0.17</td>
<td>1.90±0.13*a</td>
<td>2.26±0.12b</td>
<td>2.41±0.19b</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE, *= (P< 0.05), **= (P< 0.01), ***= (P< 0.001) for comparison all groups with control group. Small letters (a, b and c) represent comparison between diabetic groups.

### 3. Results

#### 3.1. Effect of Cinnamon Extract on Kidney Structure

In the control group treated with cinnamon extract alone (fig. 2), kidney sections showed normal histological appearance of glomeruli and renal tubules when compared to control group (fig. 1). In the diabetic group (fig. 3), kidney sections showed variable damage through degeneration in glomeruli, dilatation in Bowman’s capsule, necrosis in renal tubules, congestion of blood vessels and hemorrhage in interstitial matrix. In diabetic rats treated with cinnamon extract (fig. 4), kidney sections showed reduction in kidney damage through decreasing congestion, hemorrhage, necrosis in glomeruli and renal tubules.
congestion, hemorrhage, necrosis in the central vein, necrosis and increased vacuolated hepatocytes, and sinusoidal dilation with variable degrees of inflammatory cells infiltrations. In the diabetic rats treated with cinnamon extract (fig. 8), liver sections showed decrease in liver damage through decreasing hemorrhage, congestion, necrosis and all related complications of diabetes on liver damage mentioned above during 30 days of treatment.

3.2. Effect of Cinnamon Extract on Liver Structure

In normal rats treated with cinnamon extract alone (fig. 5), liver sections showed normal histological appearance of hepatocytes and central vein with no pathological changes in comparison with control group (fig. 6). In the diabetic group (fig. 7), liver sections showed liver damage through variable congestion, hemorrhage, necrosis in the central vein, necrosis and increased vacuolated hepatocytes, and sinusoidal dilation with variable degrees of inflammatory cells infiltrations. In the diabetic rats treated with cinnamon extract (fig. 8), liver sections showed decrease in liver damage through decreasing hemorrhage, congestion, necrosis and all related complications of diabetes on liver damage mentioned above during 30 days of treatment.
3.3. Effect of Cinnamon Extract on Spleen Structure

In all groups of the current study (fig. 9 and 10), spleen sections were showed normal histological appearance of white pulp, red pulp, germinal center and central arteriole with no obvious pathological changes.

4. Discussion

4.1. Effect of Cinnamon Extract on Kidney Histology

In the histological examinations of kidney, normal structures were observed in rats treated with cinnamon extract alone at dose of 500mg/kg.bw. This effect was further supported by normal serum level of the related parameters to kidney dysfunction such as urea and creatinine in this group; this has been also reported by Khan, et al., (2012).

In diabetic rats as result of acute hyperglycemia, kidney damage was observed through various degrees of congestions, hemorrhage, and necrosis in glomeruli, tubules and interstitial matrix with dilatation of Bowman’s capsules because several metabolic, functional and structural changes of kidneys in alloxan induced-diabetic rats were found to have many fundamental similarities to those occurring during human diabetic nephropathy and this model has been used extensively in diabetes research to evaluate the pathogenesis of kidney in diabetic (Gaed, et al., 2004). These structural changes in kidneys of diabetic rats were supported by others studies, they observed that the first histopathological changes of kidneys during acute hyperglycemia are characterized by enlargement of glomerular mesangium due to increase of extracellular matrix synthesis that leads to glomerulosclerosis and tubule-interstitial fibrosis, basement membrane thickening, increasing endothelial cell permeability to albumin and increasing hyper-filtration that cause damage to tissues and blood vessels as end stage in diabetic renal disease (Masons and Wahab, 2003).

In diabetic rats treated with cinnamon, these damages in the kidney structures were reduced which has been supported by the reduction in the level of serum urea and creatinine and increased serum total protein, albumin and globulin that prevent further accumulation of proteins in the kidneys tissues resulted in the reduction of renal damage. Others found that progressive increasing of albuminuria in the kidneys is much more likely to be due to early stage in damage to the structural tissues in the kidney during diabetes that gradually proceeded to nephropathy, but reduction of albuminuria by cinnamon resulted in the improvement of renal function (Huang and Preisig, 2000) through prevention of further deposition of proteins in kidneys thereby decreased the enlarged glomeruli of diabetic rats with reduction in the necrosis, congestion and hemorrhage of whole kidney structures (Mahera, 2006 and Mishra, et al., 2010). As a support to above mentioned effects of cinnamon on histological examination of kidneys, Mahera, (2010) mentioned that cinnamon bark oil has effective role to prevent diabetic nephropathy. She observed after 12 weeks of cinnamon oil treatment in diabetic rats,
kidneys weight was decreased and glomerular and tubular histological changes were improved through reduction in the size of Bowman’s capsule and prevent mesangial increment. Therefore, treatment of diabetic rats with cinnamon extract indicates blood glucose lowering effects of this treatment was considered as the main reason for this improvement effect on kidney tissues (Khan, et al., 2012). As a result of the all motioned effects of cinnamon, the structural damages in the kidneys of diabetic rats that were treated with cinnamon seem to be prevented in most rats of the current study. Others reported that diabetes renal damage cannot be controlled entirely by traditional uses unless patients treated with hypoglycemic drugs (Kikkawa, et al., 2003).

4.2. Effect of Cinnamon Extract on Liver Histology
In control rats treated with cinnamon extract alone, liver showed no histological changes. Observation of the normal liver tissue treated with cinnamon alone indicated that cinnamon at this dose (500mg/kg.bw) has no effects on the histological functions of the liver. This effect was further supported by the normal serum activities of AST, ALT, ALP, TSB, and total protein as indicators of liver damage. Furthermore, Khan et al., (2012) observed similar effect of the cinnamon so its intake is almost safe and it can be used for long time.

In alloxan induced diabetic rats, damages in the liver were observed such as congestion, hemorrhage and necrosis in hepatocytes. Some evidences were observed that the development of chronic liver disease and progressive structural changes may occur after the diagnosis of DM which plays a role in the initiation and development of liver injury (Reaven, 1995) during deficits in the antioxidant defense enzymes and vitamins and increased lipid peroxidation (Laaksonen, et al., 1996 and West, 2000) resulted in the alteration in cell number, growth and cell death (Chatila and West, 1996).

Treatments of cinnamon extract for 30 days to diabetic rats resulted in the decrease of damaged parts in the liver with reduction in levels of AST, ALT, and total bilirubin as indicators for liver damage. Extracts of cinnamon have antioxidant properties by free radical-scavengering against liver injury (Moselhy, and Junbi, 2011). Mahera, (2010) reported that oral administrations of cinnamon oil produced a reduction in levels of AST, ALT, and total bilirubin as indicators for liver function and normalize the histopathological and biochemical abnormalities caused by the diabetes in alloxan induced diabetic rats. Decreased tissue damages in the liver through reduction in the congestion, hemorrhages, necrosis and inflammatory cells has been shown in some studies to be a clear signs for hepatoprotective effect of cinnamon extract (Vozarova, et al., 2002; Vijn, and Hayward, 2004 and Hala, et al., 2011). Similar results were described by (Friedman, et al., 2003; Mohamed, 2006 and Mona, 2011) they reported as a result of hyperglycemia, liver enzymes (such as ALT) increased that indicate hepatocytes damage, whereas decreasing them by cinnamon extract is an indicator of repair in the liver cells by cinnamon compounds. Khaki, et al., (2013) found that cinnamon acts as antioxidants against tissue damage in diabetic rats so it can be recommended for the diabetic patients.

4.3. Effect of Cinnamon Extract on Spleen Histology
Histological examinations of the spleen tissue in normal and diabetic rats showed no obvious structural changes during 30 days of the experiment in this study. Similar results have been obtained by (Youn, et al., 2008 and Yu, et al. 2012) they observed no structural changes in spleen of diabetic and treated animal with cinnamon; they further reported that during splenic disease, ethanolic extract of cinnamon cassia may have anti-inflammatory activity and improve the function of spleen. Studies about the effect of cinnamon on the histology of the spleen tissue during diabetes were limited therefore, few information of cinnamon effect on this organ are available. Mona, (2011) reported that spleen size and weight increased in diabetic rats (splenomegaly), but after feeding them on a diet supplied with cinnamon, size of spleen was decreased along with other internal organs, but she did not studied the histological changes of spleen during her study.

References


