AMELIORATIVE EFFECT OF CINNAMON AQUEOUS EXTRACT AGAINST HYPERCHOLESTEROLEMIA INDUCED HEPATIC ALTERATIONS IN RATS

Mona Farouk MAHMOUD
Department of Pathology, Faculty of Veterinary Medicine, Benha University

Abstract

This study was done on four groups of male wistar rats. Control group, cinnamon aqueous group which fed on 200 mg/kg/day cinnamon aqueous extract orally, control positive group which was fed on a high cholesterol diet and treated group and was fed a high cholesterol diet and given cinnamon aqueous extract orally at 200 mg/kg/day for two months to evaluate the ameliorative effect of cinnamon aqueous extract on the alterations in liver induced by hypercholesterolemia. The histopathological findings showed vacuolar degeneration in some areas of the hepatic tissues. Diffuse fatty degeneration was noticed in most of the hepatocytes. Thickening in the CT surround the central and portal vein. The hepatic cells showed faint positive immunostaining reaction forKi67. In the treated group regeneration was noticed in most of the hepatocytes. The cells showed cytoplasmic degranulation especially the cells in the triad areas. Proliferation of the vonkupher cells was noticed. The hepatocytes showed positive immunostaining for Ki67 which indicate cell viability.Also theaddition of cinnamon aqueous extract adjusts the highest level of cholesterol, triglycerides, and low-density lipoprotein.

Key words. *Liver-hypercholesterolemia- cinnamon aqueous extract-rats*

Introduction

Hypercholesterolemia is considered nowadays as one of the most familiar metabolic diseases. Obesity, diabetes, and metabolic syndrome are closely associated with hypercholesterolemia (Postic and Girard, 2008; Trauner et al., 2010). Hypercholesterolemia can eventually lead to Non-Alcoholic Fatty Liver Disease (NAFLD), which is known to be a common cause of chronic liver disease in adults and children in many world regions. It is usually progress to cirrhosis or even Hepato Cellular Carcinoma (HCC) (Kim et al., 2012). Reports showed that 34% of the general population and over 75% of obese and extremely obese individuals are suffering from have fatty liver (Browning and Horton, 2004). In addition, deposition of lipids and triglycerides in liver of experimental animals was reported following high cholesterol diet supplementation (Lee et al., 2007). Experimentally induced hypercholesterolemia can impairs lipid metabolism leading to elevation of both blood and tissue lipid profile (Vasu et al., 2005). Moreover, studies demonstrated that even short exposure to HCD is capable of inducing hypercholesterolemia (Tomofuji et al., 2006).Hypercholesterolemia has been recognized as a risk factor for atherosclerosis, and is now emerging as a contributing factor for the progression of renal disease. A high cholesterol diet (HCD) and inadequate physical activity that characterize our modern lifestyle contribute to the development of hypercholesterolemia.

Plant products have been the basis for many medicinal therapies. Cinnamon (Cinnamomumvernum) leaves and bark are used extensively as spices in food or to produce essential oils in many countries. Cinnamon was used as medicinally and as flavoring for beverages, it was also used in embalming, where body cavities were filled with spiced preservatives. The plant has a hot taste and emits a spicy odor when crushed (Jayaprakashaet al.2003). Cinnamon is used to treat nausea and diarrhea and in wound healing (Skidmore, 2002, Kamathet al., 2003; Anderson et al., 2004; Shinget al., 2007) and it has anti-bacterial and anti-fungal properties (Nir et al. 2000). It also showed anti-inflammatory (Tung et al., 2008), antioxidant (Su et al., 2007) and hypotensive effect (Preuss et al., 2006). The hepatoprotective of cinnamon was reported by some investigators (Eidi et al. 2012, Sakr and Al-barakati,2014).

Cinnamon plant belongs to Luaraceae family, which has many therapeutic effects. The most important components in cinnamon are cinnamomin and cinna-maldehyde. In recent years, extensive researches have been made on cinnamon and its components on various organs.

Cinnamon can be used to treat reduced cholesterol and low density lipoprotein (LDL) (Khan et al., 2003), reduce the release of free radicals in the body (Shagauo and Davidson, 2006).

The aim of the current work is to evaluate the ameliorative effect of CinnamonAqueous Extract against Hypercholesterolemia and the protective role against hepatic disorders.

Materials and Methods

Preparation of cinnamon aqueous extract

The C. cassia aqueous extract was prepared from the air dried powdered cinnamon bark according to Azabet al., (2011). The aqueous extract was freshly prepared by soaking 10 grams of the grinded bark in 100 ml distilled water at 90 °C for 2 hours followed by filtration. The filtrate was dehydrated in oven at 80 °C overnight. The resulting dark reddish brown dry extract was weighed and the dry yield was then calculated.

Experimental Design and Animal Groups:

The present study was performed on 60 male Wister rats weighing 200-250 grams. The rats were divided into four groups:

Group 1: classified as the control (n=15) and received a standard balanced diet and gained tape water for 2 months.

Group 2:givencinnamon aqueous extract orally at 200 mg/kg/day according to Kim et al., (2006) and Azabet al., (2011) for 2 months.

Group 3:classified as control positive group (n=15) and was fed a high cholesterol diet (rat chow supplemented with 4% cholesterol and 1% cholic acid) according to Thiruchenduranet al., (2011) for 2months.

Group 4: classified as the treated group and was fed a high cholesterol diet and given cinnamon aqueous extract orally at 200 mg/kg/day according to Kim et al., (2006) and Azabet al., (2011) for 2months.

At the end of the experiment, the rats were sacrificed and the blood samples were collected for biochemical analysis. Small parts of the liver tissues were collected for histopathology and immunohistochemistry.

Levels of lipid profile (triglycerides, cholesterol, low density lipoprotein and high density lipoprotein levels).

The assay kits for lipid profile were obtained from Randox Laboratories Ltd., Ardmore, Co. Antrim, UK and assessed according to Onyeikeet al. (2012).

Tissue processing for light microscopy:

Different parts of the liver were taken at the end of the experiment for histopathological examination and immunohistochemistry. The tissue was prepared through paraffin technique. 5um thick sections were stainedaccording to (Bancroft and steven,1994).

Sections of the liver were further incubated with the primary antibody against Ki-67 (Anti-ki67, DAKO Corp.) for 30 minutes. The sections were then stained using avidin-biotin complex (ABC) by immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA). For demonstration of binding sites using, ABC chromogen was applied. Phosphate buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer's hematoxylin(Kiernan, 2008).

Statistical analysis

Data were represented as means ± SD. The differences were compared for statistical significance by ANOVA and post hoc Tukey's tests. Difference between groups was considered significant at $p < 0.05$. The statistical analysis was performed using software (SPSS Inc., Chicago, Illinois, USA).

Results

The liver of the control group characterized by central vein and hepatocytes arranged in the form of hepatic cords (Figure.1). These cells were positive immunostaining for Ki67 which indicated cell viability (Figure.2).

The liver of the cinnamon group characterized by central vein and hepatocytes arranged in the form of hepatic cords (Figure.3). The number of the von kupher cells showed increase in comparison to the control liver (Figure.4).

The liver of the hypercholestermic group showed vacuolar degeneration in some areas of the hepatic tissue (Figure.5). The hepatic cells showed faint positive immunostaining reaction of Ki67 (Figure.6). While most of the liver showed diffuse fatty degeneration in most of the hepatocytes. Thickening in the CT surround the central vein and hepatic vein (Figure.7 and 8).

The liver of the treated group showed regeneration of most of the hepatocytes. Some cells still showed vacuolar and fatty degeneration (Figure.9). The cells showed cytoplasmic degranulation especially the cells in the triad areas. Proliferation of the vonkupher cells was noticed(Figure.10). The vonkupher cells showed numerous proliferations. The CT surround the central vein returned back to thin like the control hepatic tissue (Figure.11). The hepatocytes showed positive immunostaining for Ki67 which indicate cell viability (Figure.12).

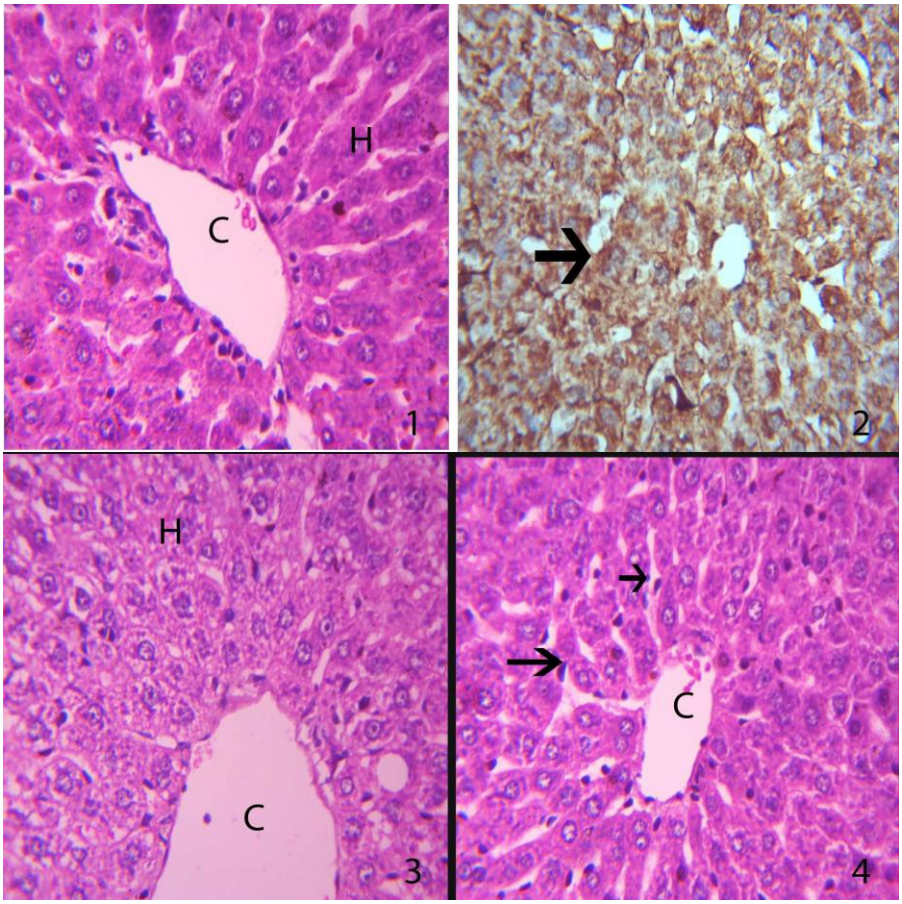

Fig.1. The liver of the control group, characterized by central vein and (c) and hepatic cords (H). H&E X20

Fig.2. The liver of the control group showed positive immunostaining for Ki67 (arrow) X20

Fig.3. The liver of the cinnamoun group, characterized by central vein and (c) and hepatic cords (h). H&E X20

Fig.4. The liver of the cinnamon group showed proliferation of the vonkupher cells (arrow) H&E X20

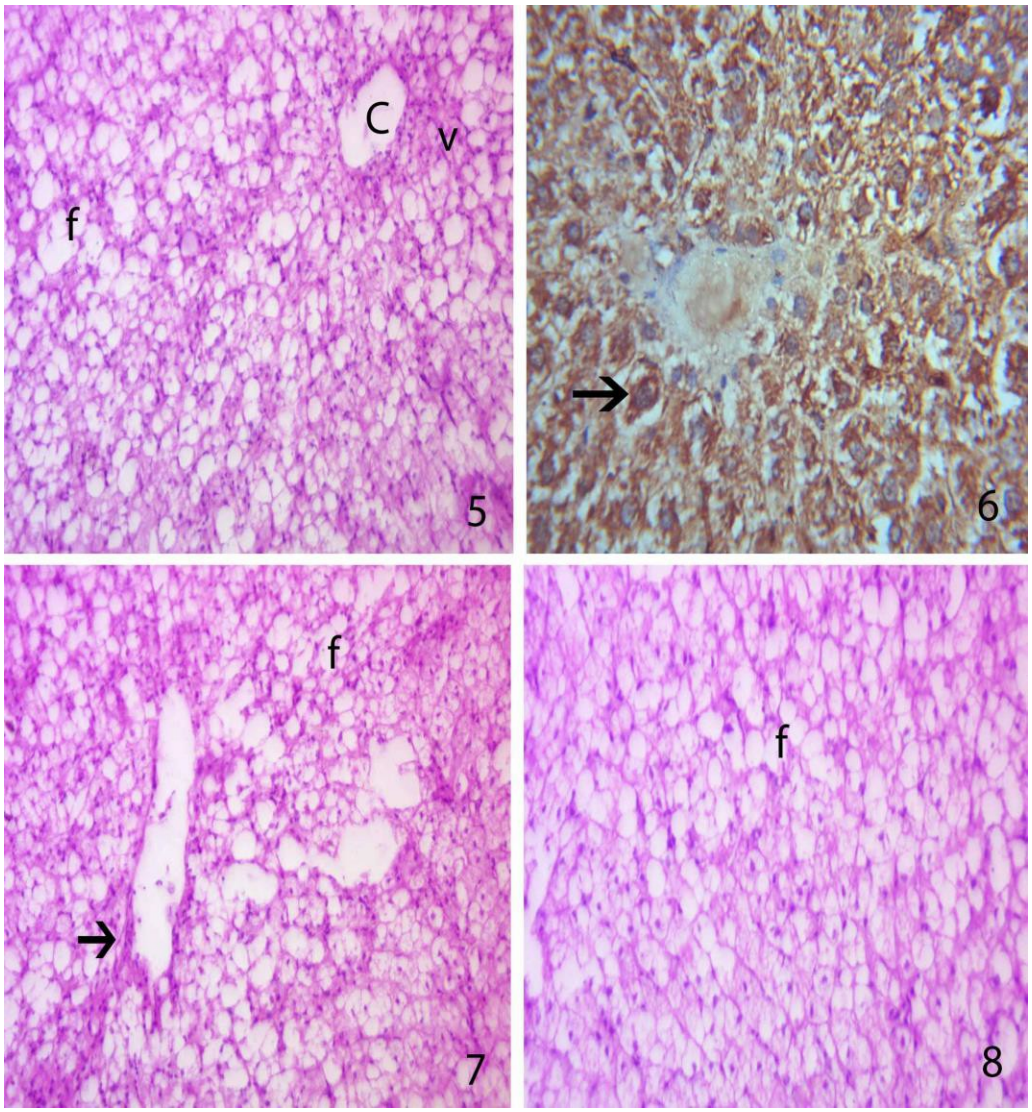

Fig.5. the liver in the hypercholestermic group showing; vacular degeneration (V) and diffuse fatty degeneration (f). H&E X10.
Fig.6. the liver in the hypercholestermic group showingfaint immunostaining for Ki67 (arrow) in fatty liver .X20.
Fig.7. the liver in the hypercholestermic group showing thickening in the wall of the hepatic vein (arrow) H&E X10.
Fig.8. the liver in the hypercholestermic group showing diffuse fatty degeneration (f). H&E X10.

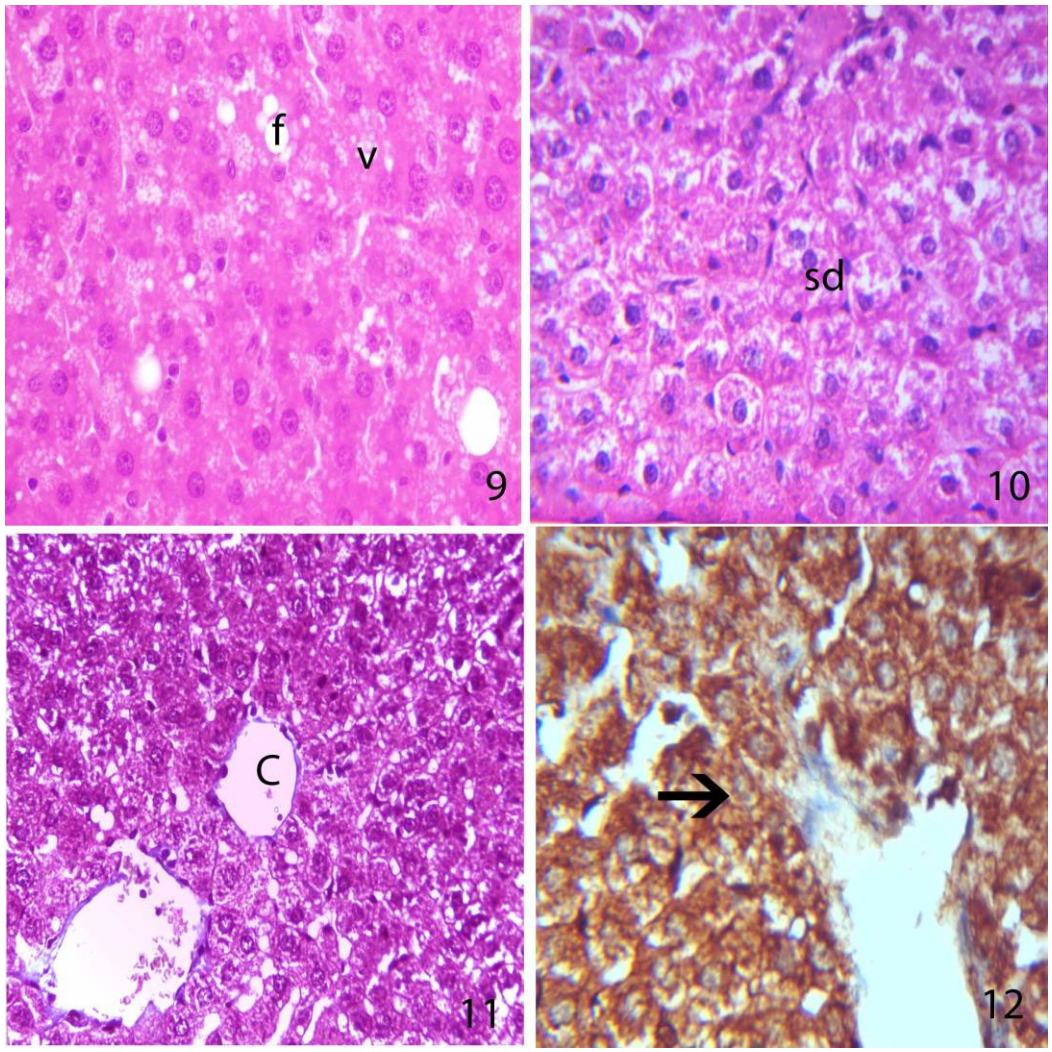

Fig.9. the liver in the treated group showing decrease the number of the fatty degeneration cells (f), while some cells showed vacuolar degeneration (v) H&E X10.
Fig.10. the liver in the treated group showed cytoplasmic degranulation of some hepatocytes. H&E X20.
Fig.11. the liver in the treated group showed thin CT around the central vein. H&E X10
Fig.12. the liver in treated group showed positive immunostaining for Ki67 (arrow).

Biochemical analysis

Rats that received the HCD showed a significant increase in the cholesterol, triglycerides, and low-density lipoprotein levels, and a significant decrease in the high-density lipoprotein level compared with the levels of the control group. These parameters significantly decreased, with the exception of high-density lipoprotein, which increased, in rats that received the HCD and cinnamon compared with those that received only the HCD (Table, 1). The lipid levels showed non-significant change in rats that received cinnamon compared with the control group.

Table 1

Effects of cinnamon, HCD and HCD+ Cinnamon on wistar rats cholesterol, triglyceride, LDL and HDL levels compared to negative control group.

Group	Triglyceride (mg/100ml)	Cholesterol (mg/100ml)	LDL (mg/100ml)	HDL (mg/100ml)
Group 1 (control)	37.92 ± 0.29	64.53 ± 1.12	37.8 ± 2.5	18.19 ± 0.20
Group2 (cinnamon)	33.11 ± 0.26	63.30 ± 1.44#	33.1± 2.01	17.28 ± 0.19
Group 3 (HCD)	71.13 ± 0.03*†	109.32 ± 1.02*†	42.29 ±0.91 *†	15.59 ± 0.36*†
Group4 (HCD+cinnamon)	38.59 ± 0.42#	65.13 ± 1.0#	35.9± 2.3#	20.19± 0.25#

Values are represented as mean ± SD (n=6)

Significance was considered $P<0.05$

* Significant change compared to control untreated (group 1)

Significant change compared to HCD group (group 3)

†Significant change compared to HCD treated with cinnamon group (group 4).

Discussion

Liver is a vital organ play a major role in metabolism and excretion of xenobiotic from the body. Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies (Jaya et al.,2014).

Fatty liver is a well-known metabolic disease. It is usually associated with other diseases such as obesity, diabetes, and metabolic syndrome (Trauner et al., 2010). Studies has indicated that individuals suffering from fatty liver represent 34% of the general population and over 75% of obese and extremely obese (Browning & Horton, 2004). Feeding of the experimental rodents with high cholesterol diet (HCD) is reported to cause hypercholesterolemia and deposit cholesterol in liver (Lee et al., 2007).

The two main consequences associated with fatty liver are resistance to insulin and Oxidative stress. Insulin signals more fat to be produced in the liver which ends up accumulating itself in the liver and due to this there would be extreme high levels of oxidative stress leading to inflammations. A world known fact is that Cinnamon is a good anti- oxidant. This can address the issue of oxidation stress imposed by the insulin. It makes the system more susceptible and sensitive to insulin thereby leading to fewer insulin secretions and lesser accumulation of fat. Cinnamon extracts contain anti-inflammatory property which helps to cure the inflammations of the liver due to excess alcohol consumption (cirrhosis of liver). This is because cinnamon inhibits the expressive of the factor MyD88 – myeloid differentiation primary response gene which causes the inflammation and cirrhosis (Soliman et al.,2012).

The liver is characterized by diffuse fatty degeneration due to hypercholestermic induced by high fat diet. The addition of cinnamon extract adjusts the cell activity and returned it back to normal histophysiological pattern. These results were supported by the findings of (Soliman et al.,2012).

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by Rerkasan et al. (2008).

The inner bark of cinnamon (Cinnamomum zeylanicum L.) is commonly used as a spice andhas also been widely employed in the treatment and prevention of disease (Akram et al.,2012).

Numerous studies have showed that cinnamon extracts commonly function as antioxidants. Murcia et al., (2004) reported that cinnamon extracts exhibit a protective capacity against irradiation induced lipid peroxidation in liposomes, and quench hydroxyl radicals and hydrogen peroxide. Ethanolic extract of cinnamon has potent hepatoprotective action against CCl4 by lowering the MDA level and elevating antioxidants enzymes activities (SOD and CAT)(Moselhy and Ali,2009). Extracts of cinnamon bark has revealed the presence of flavonoids, glycosides, coumarins, alkaloids, anthraquinone, steroids, tannins and terpenoids(Shihabudeen et al., 2011). It is suggested in this work that the hepatoprotective of cinnamon aqueous extract is attributed to its antioxidant effects of flavenoidscomoponents.

Cinnamon extracts were also reported to produce hepatoprotective(Moselhyand Ali, 2009], antioxidant (Roussel et al.,2009, Azab et al.,2011), anti-obesity (Couturier et al.,2010), hypolipidemic(Vafa et al.,2012 and Shatwan et al.,2013).

Results from serum lipid, cholesterol and lipoprotein status of HCD rats showed increased triglyceride, cholesterol serum, low-density lipoprotein (LDL), and decreased in the high-density lipoprotein concentration (HDL) compared to the three other groups these results are in accordance with Thiruchenduranet al. (2011) in rats, ShimamotoandSofikitis (1998) in rabbits.

Cinnamon extract has a protective effect on the liver. It changed the adverse effect produced by HCD by reducing the cholesterol transport. There are several investigations proposed that, the administration of cinnamon to mice positively affected the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased and plasma triglycerides were reduced (Kim et al., 2006). Another study by (Rahmanet al., 2013) found a reduction in the total cholesterol, triglycerides, and low-density lipoproteins in rats administered cinnamon powder (15%) for 35 days. Additionally, cinnamon oils reduced the cholesterol levels in broiler chickens (Ciftciet al., 2010). All those beneficial effects might due to antioxidant and cholesterol and lipid-lowering activity of cinnamon extract as previously described by Rao and Gan (2014), they reported that, cinnamon has an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering activities.

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