



Cinnamon Extract Ameliorates Liver Damage And Oxidative Stress Induced By Paracetamol In Male Rats

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ABSTRACT

Paracetamol is a harmless antipyretic and analgesic at the therapeutic dose, but when used by overdose cause hepatic damage. The study was planned to evaluate the effect of cinnamon extract on paracetamol-induced liver injury in rats. Thirty male rats were allocated into six equal groups, control group, silymarin group cinnamon group, paracetamol group, cinnamon + paracetamol group and silymarin + paracetamol group. At the end of experiment, blood and liver tissue samples were collected. Paracetamol caused rise in liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), with changes in protein and lipid profiles. It also caused hepatic lipid peroxidation with decreasing the activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). Pretreatment with cinnamon extract for 30 days improves the adverse effects of paracetamol evidenced by biochemical and histopathological findings. In conclusion, cinnamon extract can serve as a hepatoprotective agent against paracetamol-induced liver damage.

Keywords: Paracetamol; Hepatic damage; Cinnamon extract; Silymarin; Rats

1. Introduction

The liver is a vital organ for the regulation of body homeostasis. It keeps up different biochemical pathways controlling body development, protects from certain disorders and support production of energy (Rajkiran et al. 2015). Discovering and evaluating different hepatoprotective agents have recently attracted much attention (Shanmugasundaram and Venkataraman, 2006). There has been a growing interest in herbal medicines as alternative medicines useful for treating or preventing lifestyle related disorders. Silymarin, a flavanolignan, is obtained from both seeds and fruits of the plant *Silybum marianum* Gaertn. (Compositae). Silibin, silydianin, isosilybin, and silychristin are considered the essential flavanolignans in silymarin (Pradeep et al. 2007). Silymarin can be utilized as a hepatoprotective/hepatic regenerator, immunomodulator, antioxidant and anti-inflammatory (Elmowafy et al. 2013). It is proved to be effective in hepatic disease occurred by various drugs (Sherif and Al-Gayyar, 2013). Silymarin treats liver diseases via protecting the liver cells directly by stabilizing the membrane permeability and inhibiting lipid peroxidation (Mira et al. 1994). The great potential of plant-derived natural antioxidants in health promotion, disease hindering and improved consumer safety and acceptance make it better than synthetic antioxidants (Gulcin, 2006).

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Cinnamon (*Cinnamomum zeylanicum*) is utilized widely as a herbal medicine with its biologically active ingredient (Morgana et al. 2014). Cinnamon is involved in the treatment of inflammatory disorders and intestinal infections (Shen et al. 2012). Phytochemical investigation of cinnamon bark has detected a lot of flavonoids, coumarins, glycosides, alkaloids anthraquinone, steroids, tannins and terpenoids (Shihabudeen et al. 2011). Many studies have recommended that flavonoids are commonly used as antioxidants and may protect plants from oxidative stress obtained by unfavorable environmental conditions (Gould and Lister, 2006). The hepatoprotective effect of cinnamon has been reported by (Eidi et al. 2012).

Paracetamol (acetaminophen, 4-hydroxy acetanilide) induces large number of deaths, either alone or mixed with other drugs (Mowry et al. 2015). A low dose of paracetamol is generally utilized as antipyretic and analgesic agent however a higher dosage can produce hepatic damage in humans and also in rodents. The hepatotoxicity effect of paracetamol was not significantly recognized until 1980 despite other recorded side effects (Tittarelli et al. 2017) as acute liver failure, necrosis of renal tubules and hypoglycaemic coma (Lancaster et al. 2015). Paracetamol hepatotoxicity results by the reaction of metabolite N acetyl-p-benzoquinoneime (NAPQI), which induces oxidative stress and glutathione reduction (Mandade, 2011). The present study aimed to explore the potential effects of cinnamon on paracetamol-induced hepatotoxicity in male albino rats. **2.**

2. Material and methods

2.1. Preparation of plant extract

Dried cinnamon bark was processed and extracted using ethanol %80 in Soxhlet device for 8 h. The extract was then concentrated by evaporation. The final concentrated extract was stored in dark at 20- °C until it is used for the experiments (Eidi et al. 2012).

2.2. Chemicals

Silymarin was obtained from Sedico Pharmaceutical Company (Egypt). Paracetamol tablets 500 mg was purchased from El Nasr Pharmaceutical Company (Egypt).

2.3. Animals

Male albino rats weighing 150-170g were used for the present study. Rats were obtained from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were held in stainless-steel cages at 12 hr light-dark cycle and an ambient temperature of 21-24 °C, and were given standard diet and water ad libitum throughout the study. All experiments were approved by the institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

2.4. Experimental protocol

Thirty male albino rats were allocated into six equal groups (five rats/group).

Group I: Control group, orally administered distilled water for 30 successive days.

Group II: Silymarin group; orally administered silymarin (100 mg/kg b.wt) (Kolakota et al. 2017) for 30 successive days.

Group III: Cinnamon group; administered cinnamon extract orally (400 mg/kg b.wt) (Elkomy et al. 2016) for 30 successive days.

Group IV: Paracetamol group; administered orally a single dose of paracetamol (1 g/kg b.wt), two hours after the last dose of distilled water (Gad et al. 2013).

Group V: Cinnamon+ paracetamol group; orally administered cinnamon extract (400 mg/kg b.wt) for 30 successive days followed by a single dose of paracetamol (1 g/kg b.wt) two hours after the administration of the last cinnamon dose.

Group VI: Silymarin+ paracetamol group; orally administered silymarin (100 mg/kg b.wt) for 30 successive days followed by a single oral dose of paracetamol (1 g/kg b.wt) two hours after the last silymarin dose.

2.5. Sampling

2.5.1. Blood samples

48hrs after the administration of paracetamol rats were fasted and killed by decapitation Blood samples were collected in sterile tubes and serum was separated by centrifugation at 3000 rpm for ten minutes, and stored at -20°C for further biochemical analysis.

2.5.2. Liver samples

Liver was collected quickly after decapitation of the animals, washed with physiological saline and partitioned into two parts, a part was homogenized in 5 ml cold phosphate buffer pH (7.4) by an electrical homogenizer and further processed for the assessment of oxidative stress. The second part was fixed in 10% neutral buffered formalin for histopathological examination.

2.6. Biochemical analysis and liver function

Serum levels of aspartate amino aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), direct bilirubin, indirect bilirubin, total bilirubin, total protein, albumin, globulin, lipid profile, namely high-density lipoprotein (HDL-C) cholesterol, low-density lipoprotein (LDL-C) cholesterol, total cholesterol and triglycerides were estimated using commercially available kits (Biodiagnostic, Egypt) according to the manufacturer's instructions. Malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were measured in liver homogenate using available kits (Cusabio Biotech Co, Ltd., China) according to the manufacturer's protocol.

2.7. Histopathological examination

Liver specimens from control and treated groups were fixed in 10% neutral buffered formalin for 48 hr and then embedded in paraffin using conventional procedure) Bancroft and Gamble, 2007). 5 μm sections were stained with Hematoxylin and Eosin (H&E) and were examined under light microscope for histopathological changes in the liver architecture.

2.8. Statistical analysis

The results were represented as means \pm SE. Statistical significance was analyzed using Student's t test or ANOVA. Data were considered significant at $p \leq 0.05$.

3. Results

3.1. Effect of different treatments on liver markers

The activities of liver enzymes namely, AST, ALT and ALP are shown in figure 1. Oral administration of paracetamol (1 g/kg body weight) induced a marked elevation in all liver enzymes as compared to the control group ($p \leq 0.001$, Fig. 1 A, B, and C). Pretreatment with cinnamon extract before paracetamol administration (400 mg/kg body weight for 30 successive days) induced a significant reduction in both ALT and ALP as compared to the paracetamol group ($p \leq 0.05$ and $p \leq 0.001$ respectively, Fig. 1B and C), while no significant change was observed in AST activity (Fig. 1A). A statistically significant decrease in paracetamol-induced increase in AST, ALT and ALP was observed in silymarin pre-treated group (100 mg/kg body weight of silymarin for 30 days, $p \leq 0.001$).

3.2. Effect of different treatments on protein profiles and bilirubin

Figure 2 shows serum levels of total proteins and their fractions (albumin and globulin) as indicator of protein metabolism. Paracetamol induced a marked decrease in the total protein and albumin as compared to the control group ($p \leq 0.001$, Fig. 2 A and B). In contrast, pretreatment with cinnamon extract (400 mg/kg) or silymarin (100 mg/kg) for 30 successive days causes an increase in paracetamol-induced decrease in the total protein and albumin ($p \leq 0.01$ and $p \leq 0.001$ respectively, Fig. 2 A

and B). However, no significant changes were observed in the level of globulin with different treatments (Fig. 2C).

Oral administration of paracetamol displayed a significant rise in the total bilirubin, direct bilirubin and indirect bilirubin comparing with the control group ($p \leq 0.001$, Fig. 3A, B and C). Cinnamon extract induced a marked reduction in paracetamol-induced elevation in total bilirubin, direct and indirect bilirubin ($p \leq 0.001$ and $p \leq 0.05$, Fig. 3A, B and C). Furthermore, pretreatment with silymarin (100 mg/kg) induced a significant decrease in paracetamol-induced increase in the total bilirubin, direct and indirect bilirubin ($p \leq 0.001$ and $p \leq 0.01$, Fig. 3A, B and C).

3.3. Effect of different treatments on lipid profiles

Total cholesterol, triglycerides, LDL-C and HDL-C are shown in figure 4. Administration of paracetamol induced a significant increase in the total cholesterol, triglycerides and LDL-C as compared to the control group ($p \leq 0.001$, Fig. 4A, B and C). Conversely, HDL-C significantly decreased after oral administration of paracetamol ($p \leq 0.001$, Fig. 4D). Pretreatment with cinnamon extract (400 mg/kg) or silymarin (100 mg/kg) for 30 successive days before the administration of paracetamol induced a significant decrease in the total cholesterol, triglycerides and LDL-C as compared to paracetamol group ($p \leq 0.001$, Fig. 4A, B and C). However, paracetamol-induced reduction in HDL-C was significantly elevated by pretreatment with cinnamon extract or silymarin ($p \leq 0.001$ vs paracetamol group, Fig. 4D).

Figure: 1

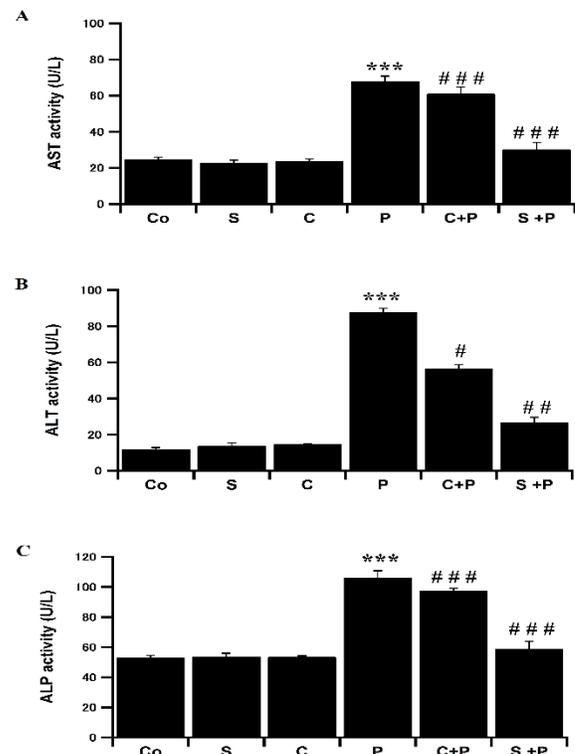


Fig 1. Effect of oral administration of silymarin or cinnamon on paracetamol-induced changes in serum levels of liver enzymes. A. Levels of AST in different treated groups. B. Serum ALT levels C. Changes in ALP enzyme activity. The values are represented as means \pm SE, (n=5/group). *** $p \leq 0.001$ vs control, # $p \leq 0.05$ and ### $p \leq 0.001$ vs paracetamol. Control (Co), paracetamol (P), silymarin (S), cinnamon (C), silymarin and paracetamol (S+P) and cinnamon and paracetamol (C+P).

Figure: 2

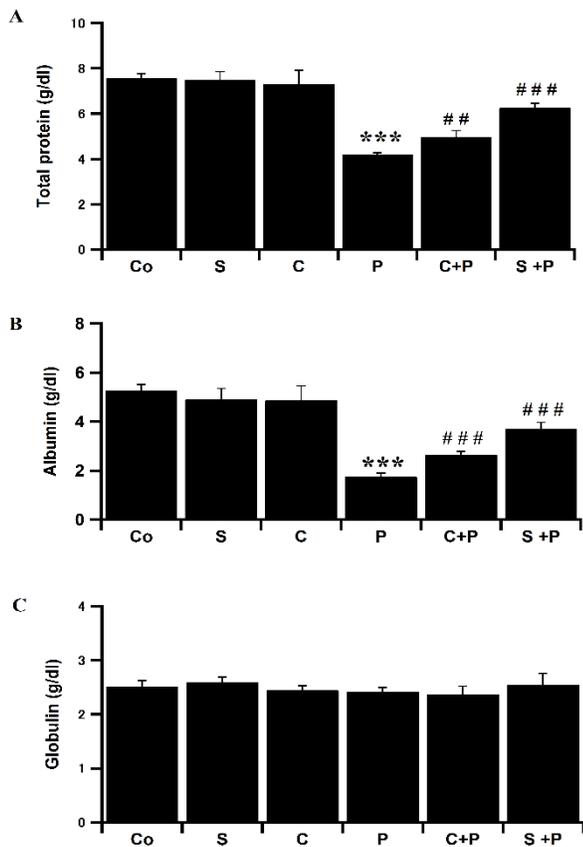


Fig 2. Effect of oral administration of silymarin or cinnamon on paracetamol-induced changes in serum protein profiles. Data are represented as means \pm SE (n=5/ group). Serum levels of A) total protein, B) albumin and C) globulin in different treated groups. ***p \leq 0.001 vs control, ##p \leq 0.01 and ###p \leq 0.001 vs paracetamol. Control (Co), Paracetamol (P), silymarin (S), cinnamon (C), silymarin and paracetamol (S+P) and cinnamon and paracetamol (C+P).

3.4. Effect of different treatments on oxidative enzymes and lipid peroxidation

Activities of SOD and CAT in addition to MDA (the lipid peroxidation marker) in the liver tissue are shown in figure 5. SOD and CAT activities were significantly lowered in paracetamol group as compared to the control group (p \leq 0.001, Fig. 5A and B). Cinnamon extract and silymarin pretreated groups expressed a significant increase in paracetamol-induced decrease in SOD and CAT activities (p \leq 0.001, Fig. 5A and B). The MDA level was markedly increased in paracetamol group as compared to the control group (p \leq 0.001, Fig. 5C). However, pretreatment with cinnamon extract or silymarin before the administration of paracetamol significantly decreased MDA level (p \leq 0.001, Fig. 5C).

3.5. Histopathological findings

The histological appearance of the liver sections from cinnamon treated groups was almost similar to the liver of the control group with normal central vein and hepatocyte appearance (Fig. 6C and 6A respectively). Some hypertrophied kupffer cells in were noticed in silymarin pretreated group (Fig. 6E). Liver sections of rats administered paracetamol showed dilated sinusoids with hydropic degenerated cells (Fig. 6B). In cinnamon pretreated group liver showed mild congestion with mild portal area fibrosis (Fig 6D). silymarin pretreated rats (Fig. 6F).

Figure: 3

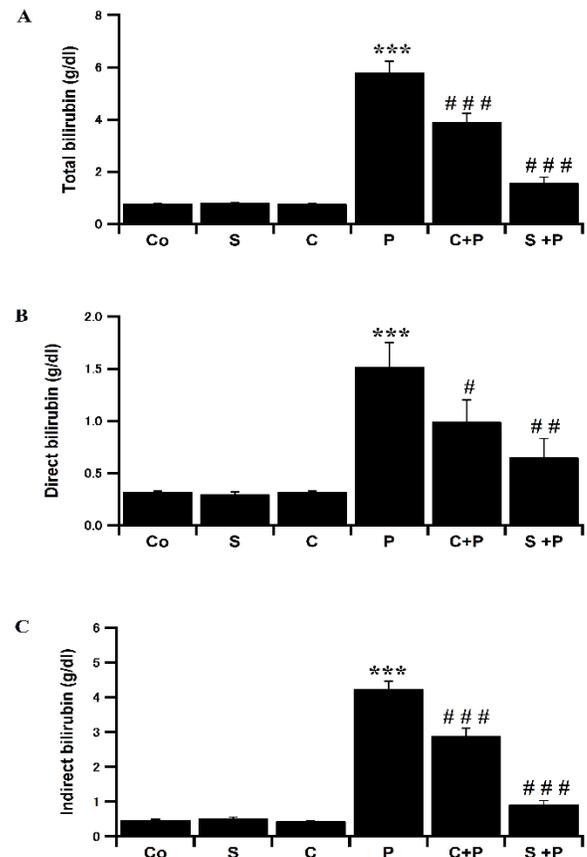


Fig.3. Effect of silymarin or cinnamon on paracetamol-induced changes in serum levels of A) total bilirubin, B) direct bilirubin and C) indirect bilirubin in different treated groups. Data are expressed as means \pm SE (n=5/ group). ***p \leq 0.001 vs control, #p \leq 0.05, ###p \leq 0.01 and ####p \leq 0.001 vs paracetamol. Control (Co), paracetamol (P), silymarin (S), cinnamon (C), silymarin and paracetamol (S+P), and cinnamon and paracetamol (C+P).

4. Discussion

The increased awareness of the herbal medicines is attributed to its rich content of phytochemicals which compete free radicals due to their antioxidant activity (Khalafalla et al. 2010) and also the simple availability without the need of difficult pharmaceutical synthesis (Stickel and Schuppan, 2007). Paracetamol, at high doses, could cause acute liver injury most probably via the formation of N-acetyl-p-benzoquinoneimine, a toxic metabolite, by cytochrome P4502E1 (CYP2E1). N-acetyl-p-benzoquinoneimine is usually inactivated by the hepatic glutathione, but when produced excessively, covalently binds to centrilobular hepatic proteins, contributing to hepatic toxicity (Gardner et al. 2002). In the present study, we used paracetamol to induce hepatotoxicity in rats and an attempt was made to relieve the adverse effects of paracetamol by using cinnamon as a medical therapeutic plant.

Administration of paracetamol revealed a critical increase in liver enzymes activities including AST, ALT and ALP (Fig. 1) which may be attributed to the damaged structural integrity of the liver by paracetamol (Ayaz et al. 2012). During hepatotoxicity, liver enzymes are structurally and functionally impaired by the free radicals resulting in liver damage (Nimila et al. 2016).

Figure: 4

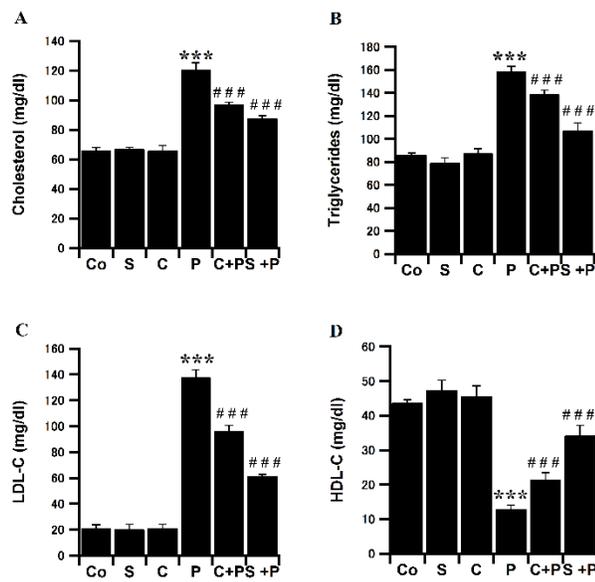


Fig 4. Effect of oral administration of silymarin or cinnamon on paracetamol-induced changes in serum lipid profiles. A) changes in total cholesterol . B) Triglycerides levels. C) HDL-C in different groups. D) Changes in LDL-C levels .values are expressed as means± SE (n=5/ group). ***p ≤ 0.001 vs control and ###p ≤ 0.001 vs paracetamol. Control (Co), paracetamol (P), silymarin (S), cinnamon (C), silymarin and paracetamol (S+P) and cinnamon and paracetamol (C+P).

We demonstrated that pretreatment with cinnamon extract effectively protects against paracetamol-induced liver damage whereas cinnamon extract significantly lowered the levels of liver enzymes that were elevated by paracetamol administration. It has been reported that the ethanolic extract of cinnamon prompted suppression of increased ALT and AST activities (Elkomy et al. 2016). Additionally, the increased levels of liver enzymes in paracetamol-treated rats were decreased by silymarin pretreatment (Fig. 1) indicating hepatoprotective effects. Silymarin prevents liver damage by keeping the integrity of the plasma membrane, and suppressing the drainage of liver enzymes has been reported (Pradeep et al. 2007).

Total protein assessment is considered a tool for examining the functional status of the liver as liver is responsible for synthesizing serum proteins excluding γ -globulins (El Faras and Elsawaf, 2017). Paracetamol induced a significant reduction in serum levels of total proteins, serum albumin with no significant change in globulin level (Fig. 2). Reducing the concentration of albumin occurs mainly due to increased of vascular permeability during acute inflammation and their release into intercellular spaces (Matić et al. 2016). Paracetamol-induced reduction in serum albumin level could be a result of a decline in the number of cells essential for albumin synthesis in the liver through necrosis (Goldwasser and Feldman, 1997). Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM (Kaplan and Pesce, 1996). Both cinnamon extract and silymarin reversed the effects of paracetamol on total proteins and albumin levels revealing a hepatoprotective effect. Stimulation of protein synthesis by the liver contributes the acceleration of recovery and the creation of liver cells (Tadeusz et al. 2001). Furthermore, silymarin alleviates liver damage by keeping the integrity of the plasma membrane (Pradeep et al. 2007) and aids in regenerating the injured liver cells, by stimulation of RNA polymerase I enzyme, which increases ribosomal protein synthesis and consequently helps to regenerate hepatocytes (Hamza and Al-Harbi, 2015).

Figure: 5

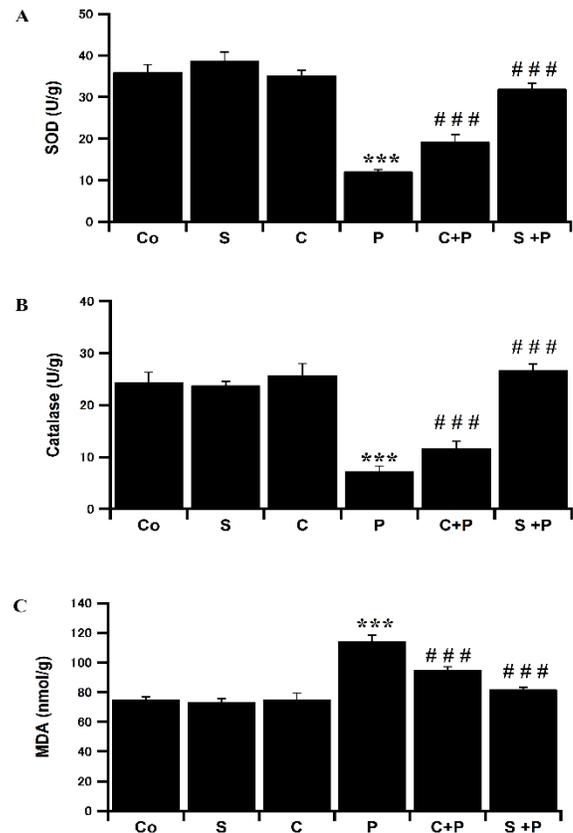


Fig 5: Effect of oral administration of silymarin or cinnamon on paracetamol-induced changes in the activity of antioxidant enzymes and lipid peroxidation. The activities of A) SOD, B) catalase and C) MDA in the liver of control and treated groups. The values are represented as means± SE (n=5/ group). ***p ≤ 0.001 vs control and ###p ≤ 0.001 vs paracetamol. Control (Co), paracetamol (P), silymarin (S), cinnamon (C), silymarin and paracetamol (S+P) and cinnamon and paracetamol (C+P).

Serum bilirubin is one of the real tests of liver function since it reflects the liver's ability to take bilirubin and process it into bile (Jaeschke et al. 2003). Hyperbilirubinemia was noticed in paracetamol treated rats (Fig. 3), that may results from the creation of more bilirubin than the liver can process, and liver damage may impair its ability to secrete normal amounts of bilirubin or block the liver's output channels (Sabina et al. 2013). Cinnamon extract or silymarin pretreatment lessens the paracetamol-induced hyperbilirubinemia, the same as reported by (Feher et al. 1987). The rise in total lipid, triglyceride, total cholesterol and LDL -C (Fig. 4) suggests a serious hepatocellular injury and affirming the hepatotoxic nature of paracetamol or may be due to the inhibition or obliteration of triglycerides secretory system by liver (El-Hadary, 2015). Hypercholesterolemia may result from the inhibition of bile acid synthesis (Yakubu et al. 2013). However, The decrease in HDL-C level may be due to the overproduction of H₂O₂ created during the cytochrome P450 mediated microsomal metabolism of paracetamol (Raj Kapoor et al. 2008). Silymarin or cinnamon extract decreased the total cholesterol, triglycerides and LDL-C levels with increase in HDL-C level. Silymarin inhibits 3 hydroxy 3- methyl glutaryl (HMG) coenzyme A reductase, the key enzyme involved in cholesterol synthesis (Nassuato et al. 1991) and improves the removal of LDL-C by the liver (Krecman et al. 1998). Silymarin reduces plasma level of cholesterol and LDL in hyperlipidaemic rat but not in normal rats

(Alkuraishy and Alwindy, 2012). It might be associated with bioavailability of silymarin. It is very low soluble in water, and this limits its bioavailability due to poor gastrointestinal absorption (Morazzoni et al. 1992). Furthermore, cinnamon has a strong lipolytic activity, it prevents hypercholesterolemia, hypertriglyceridemia and decreases free fatty acids (Rahman et al. 2013).

Dietary cinnamon inhibits the hepatic HMG Co-A reductase activity resulting in lowering hepatic cholesterol content and suppressing lipid peroxidation via the enhancement of hepatic antioxidant enzyme activity (Lee et al. 2003). Another mechanism for lipid-lowering activity of cinnamon is the increase of LDL-R gene expression (Kassaei et al. 2016). Cinnamon did not change the rats' lipid profiles at a concentration proportional to what is used in human diets (Sambaiah and Srinivasan, 1991) but it increased adipose tissue lipolysis in high-fat diet-fed mice (Khare et al. 2016). Our results demonstrated that the administration of cinnamon extract inhibited the paracetamol-induced reduction in HDL-C level that may be attributed to the rise in the lecithin cholesterol acyl transferase activity which may contribute to the blood lipids regulation (Patil et al. 2004). The body has a protective mechanism to neutralize the free radical induced damage by a set of endogenous antioxidant enzymes, such as CAT and SOD (Venkumar and Latha, 2002). Production of reactive oxygen species (ROS) and glutathione exhaustion are key players in paracetamol-induced toxicity (Olaleye et al. 2010). We demonstrated a significant decline in the levels of SOD and CAT and a critical elevation in MDA in paracetamol managed rats (Fig. 5). Almajwal and Elsadek (2015) reported that the administration of paracetamol resulted in a significant increase in the hepatic tissue concentration of MDA, while antioxidant enzymes such as SOD, CAT and GSH have been significantly reduced. In the present study rats pretreated with silymarin showed a significant increase in CAT and SOD with decrease in MDA levels. The protective properties of silymarin might be attributed to their antioxidant flavonoids (Gazak et al. 2007). Silymarin can protect the liver against xenobiotic injury by controlling liver secretion and uptake of plasma lipoprotein (Toklu et al. 2008). Also, it increases the intercellular glutathione content with scavenging of free radicals (Toklu et al. 2008). Pretreatment with cinnamon extract showed a protective action against paracetamol-induced oxidative stress via increasing the antioxidant enzymes SOD and CAT, and decreasing MDA levels. The protective action of cinnamon on paracetamol-induced liver damage may be attributed to its suppressive effect on ROS generation, through its phenolic and flavonoids contents (Azab et al. 2011). The hepatoprotective effects of cinnamon extract were further documented by the microscopical examination of the liver specimens (Fig. 6) whereas; Pre-treatment with silymarin or cinnamon extract improved the hepatic lesions induced by paracetamol administration.

5. Conclusion

Pretreatment with cinnamon extract has hepatoprotective effects on the hepatic toxicity induced by the administration of paracetamol. Such effects may be attributed to the enhancement of the hepatic oxidative status via decreasing MDA and increasing SOD and CAT. Therefore, cinnamon extract can be used as a hepatoprotective agent in paracetamol treated animals. However, further studies are required to clarify the exact mechanism/s by which cinnamon induce its action.

Conflict of interest

The authors declare no conflict of interests, financial or otherwise.

Author contributions

All authors are contributed equally to this work.

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Figure: 6

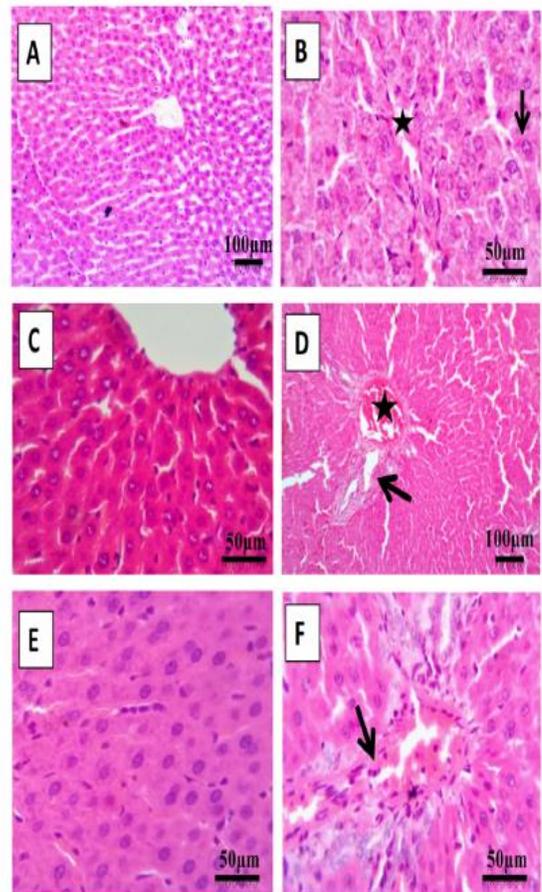


Fig. 6: Histopathological examination of liver sections from different treated groups. Liver sections of A) control group, B) paracetamol group C) cinnamon groups, D) cinnamon and paracetamol, E) silymarin, and F) silymarin and paracetamol. In (D) arrow indicates cholangitis and star indicates congested hepatic vein. In (F) arrow indicates infiltration of the portal area by mononuclear cells. (300x in slide A and D, and 1200x in the others, H&E).

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