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Hepatoprotective Impact of Cinnamon Aqueous Extract

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ABSTRACT

Cinnamon is used in the medical field for the treatment of many diseases, especially those related to liver disease. This study aimed to assess the effective compounds of cinnamon water extract and its role in protecting against liver disease in experimental rats. The liver cirrhosis was induced by carbon tetrachloride (CCl_4). In rats with liver cirrhosis caused by CCl_4 were observed to have elevated liver enzymatic activity in serum. The elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly enhanced to nearly normal after administering oral cinnamon aqueous extract at a dose of 0.6 ml / 100 g body weight/day for 12 consecutive weeks. There was a significant rise in the serum Malondialdehyde (MDA), while the activities of reduced glutathione (GSH), albumin and total proteins decreased significantly in rats treated with CCl_4. Cinnamon aqueous extract has a protective effect on the liver by lowering the level of MDA and elevating the activities of GSH, total proteins and serum albumin. The histological examination of the liver confirmed the enhancement effect in the studied liver biomarkers. In conclusion, the high content of phytochemicals in cinnamon aqueous extract may be considered as the main cause of the protective properties against liver disease.
Keywords: Cinnamon *(Cinnamomum zeylanicum L.,* Lauraceae*)*, Hepatoprotective activity.

INTRODUCTION

Cinnamon (*Cinnamomum zeylanicum L.*, Lauraceae) is a tropical plant grown wild in East Asia. The internal bark of this plant is used in folk medicine to treat a variety of health conditions (Bakkali *et al.,* 2008), in addition to its use as a spice in cooking processes (Gruenwald *et al.,* 2010). The health benefits effects of cinnamon have been discussed in previous literatures such as anti-inflammatory properties, anti-HIV activity, antimicrobial activity, strengthening cognitive activity, decreased cardiovascular disease, blood glucose , reducing the risk of colon cancer, anti-Alzheimer's, cholesterol-lowering effects, anti-yeast activity, anti-Parkinson antagonists, anti-platelet aggregation and improve blood circulation (Chung *et al.,* 2011, Khasnavis and Pahan 2012, Malik *et al.,* 2015, Hamidpour *et al.,* 2015, Mollazadeh and Hosseinzadeh 2016 and Connell *et al.,* 2016).

It is known that cirrhosis is caused by hepatic steatosis and liver fibrosis. Because of changing diet, lifestyle and oxidative stress caused by suboptimal environmental conditions. Liver cirrhosis has become one of the most serious diseases in the world. Several previous studies have attributed the appearance of cirrhosis symptoms to the effect of free radicals formed in the body and the low level of antioxidant defenses that limit their harm effect (Cichoż-Lach and Michalak 2014).

Decrease in membrane efficacy in terms of membrane safety and functions due to the formation of unsaturated fatty acid peroxide in biological membranes, leading to several serious diseases (Halliwell, 2006). Several internal protection mechanisms have been developed to reduce Reactive oxygen species (ROS) and damage caused by them (Alov *et al.,* 2015). However, this protection is incomplete because of the interaction between many factors, or when there are high levels of ROS, so the use of dietary antioxidants is an effective method of additional protection mechanisms.

Many phytochemicals have antioxidant properties to reduce or prevent liver disease caused by oxidative stress (Shahidi and Ambigaipalan 2015). Different types of non-alcoholic beverages are more common, including cinnamon containing a wide range of different natural antioxidants (Kawatra and Rajagopalan 2015).

The aim of this study is to select the optimum conditions for preparing water extract of cinnamon rich in bioactive substances. Also, the ability of this extract to improve the resistance of experimental rats to the symptoms of CCl_4 induced liver cirrhosis will be investigated.

MATERIALS AND METHODS

Materials

Cinnamon was purchased from the local market at Cairo city, Egypt. All chemicals were purchased from Sigma-Aldrich, Germany. The diagnostic biochemical kits were obtained from Bio Diagnostic Company, Al-Dokki, Giza, Egypt. Female albino rats (Sprague Dawley strain) were obtained from Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt).

Optimizing the preparation conditions of cinnamon water extract

The cinnamon extracts were prepared at five concentrations of 0.25, 0.5, 0.75, 1.0 and 1.25 % (w/v). For each concentration, cinnamon powder was weighted in beakers and then hot water was added at four different temperatures 40, 60, 80 and 100 °C. Beaker's contents were leaved until take the room temperature. The optimal extraction conditions were determined according to the results of antioxidant activity.

Total phenolics content

Spectrophotometer using the modified Folin-Ciocalteau colorimetric method was used to estimate total phenolics content (Eberhardt *et al*., 2000). The total phenolics content was expressed as a milligrams gallic acid equivalent/ ml extract (mg GAE/ ml) by reference to the gallic acid standard calibration curve.

Total flavonoids content

Total flavonoids content in the extracts was determine spectrophotometrically by Aluminum chloride complex forming assay (Piyanete *et al*., 2009). The total flavonoids content was expressed as a milligrams quercetin equivalent/ml extract (mg QE/ ml) by reference to the quercetin standard calibration curve.

DPPH$^{\bullet}$ radical scavenging activity

Spectrophotometric method was used to test the Radical-scavenging activity of the prepared cinnamon extracts reported by Brand-Williams *et al*., (1995).

Ferric reducing antioxidant power

Ferric Reducing Antioxidant Power (FRAP) was estimated by the method of Benzie and Strain (1996).

Reducing power

The reducing power of prepared cinnamon extracts was estimated according to the method reported by Oyaizu, (1986).

Animals experiment and diets

Cinnamon extract prepared at optimal conditions was biologically evaluated for its protective potential in female rats as model experimental animals. Twenty four adult rats were housed in screen-bottomed aluminum cages in room maintained at 25 ± 1°C with alternating cycles of light and dark of 12h. The basil diet used for feeding the experimental rats was consists of corn starch (60%) casein (20%) corn oil (10%) cellulose (5%) salt mixture (3.5%) vitamin mixture (1%). Salt and vitamin mixtures were presented in Table (1) according to AIN-93 guidelines (Reeves *et al*., 1993). All experiments were carried out according to the guidelines for the care and use of experimental animals .The rats were fed on the basil diet for 7 days, and then divided randomly into three groups (*n=8*). All group fed on the basil diet. The first group (G1) was identified as negative control group. Group 2 and 3 were treated by carbon tetra chloride (CCl_4) and identified as cirrhotic groups. Group 2 was considered as a positive control group (G2). Group 3 was treated by cinnamon extract (G3). The extract was given orally for the rats daily in a dose of 0.6 ml/100 g body weight for twelve weeks. Liver toxicity was induced by a weekly dose of CCl_4 (1 ml/kg body weight) diluted with corn oil at ratio was 1:1 (Ehrinpreis *et al*., 1980). The carbon tetra chloride given intraperitoneally injection to all rats except that normal control group was given corn oil. The dose calculated based on a consumption of 275 ml/day for a 70 kg human as reported by Rouanet *et al*., (2010). Blood samples also obtained from the retro-orbital plexus of the eyes from all animals of each group on 0, 30, 60 and 90 days according to the procedure of Schermer (1967). After the end of the experiment, the rats were slaughtered and organs were excised, specimen for liver was obtained and preserved in formaldehyde (10%) for the histopathological examination. Serum was separated and the serum biochemical analyses were carried out.

Table 1. Compositions of the Salt mixture (g) and vitamins of basil diet.

Salt mixture		Vitamins mixture	
Salt	**Wight (g)**	**Vitamin**	**Wight**
$CaCO_3$	304.5	Vit. A	2000IU
KH_2PO_4	327.5	Vit. D	200IU
$CaHPO_4.2H_2O$	60.0	Methionine	0.5mg
$MgSO_4.7H_2O$	103.5	Inositol	10 mg
NaCl	170.0	Niacin	4 mg
$Fe(C_6H5O_7).6H_2O$	28.0	Ca-pa ntothenate	4 mg
KI	0.81	Riboflavin	0.8 mg
$MnSO_4$	5.12	Thiamine	0.5 mg
$ZnCl_2$	0.25	Pyridoxine	0.5 mg
$CuSO_4.5H_2O$	0.31	Folic acid	0.2 mg
		Cholic acid	0.2 g
		Biotin	0.4 mg
		Vit.B12	0.003 mg
		P-aminobenzoic acid	10 mg
		Glucose	1000 g

Biochemical analyses

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP) and albumin (ALB) were carried out according to the method illustrated by Young (1995).

Determination of oxidative stress parameters:

Malondialdehyde (MDA) was determined according to Ohkawa *et al*., (1979) and reduced glutathione (GSH) was determined as stated by the method of Beutler *et al*., (1963).

Histopathological examination

Anatomy samples were taken from rat livers and fixed in a 10% formol solution for 24 hours. The samples were then washed with tap water and diluted alcohol was used in the following sequence (methyl, ethyl and absolute ethyl) for dehydration. Specimens were clarified in xylene and firmed in paraffin for 24 h at 56ºC. The paraffin wax blocks containing the tissue were sliced using microtome to 4 micron thickness. The tissue slices were deparaffinized and stained with hematoxylin & eosin and then examined by an electron optical microscope. (Banchroft *et al*., 1996).

Statistical analysis

ANOVA analysis was achieved using the PROC ANOVA method of Statistical Analysis System (SAS, 2000). Duncan multiple ranges at 5 % significance was used as described by Duncan (1955) to compare between means. Results followed by different alphabetical letters significantly differed.

RESULTS AND DISCUSSION

Optimizing the preparation of cinnamon water extract

Radical scavenging activity, Ferric reducing antioxidant power and reducing power were used as antioxidant activities for selecting the optimal conditions of preparing cinnamon water extract.

DPPH• scavenging activity.

Scavenging free radicals is one of the main antioxidation systems to prevent lipid peroxidation. Radical scavenging activity (%) of prepared cinnamon aqueous extracts prepared using different concentrations and temperatures were shown in Tables (2). The radical scavenging activities significant ($P \leq 0.05$) increased from 13% to 41% with increasing the cinnamon concentration from 0.25 to 1.25 % at 40 °C. The same trend had observed with the extracts those prepared at the other tested temperatures. The radical scavenging activities increased gradually with increasing the temperature from 40 to 100 °C. The radical scavenging activity reached to the maximum value when the cinnamon water extract was prepared at cinnamon concentration of 1.25 % using water heated to 80 °C (Table 2). In accordance with the other previous studies, the antioxidant contents of cinnamon could be increased by increasing the extraction temperature (Dudonné *et al.,* 2009; Abu Samah *et al.,* 2014 and Amrani *et al.,* 2009). This may be due to improving diffusion coefficients and the solubility of polyphenols (Chimbetete *et al.,* 2019).

Table 2. Radical scavenging activity (%) of prepared cinnamon aqueous extract prepared using different concentration and temperatures.

Concentration g/100ml	0.25	0.50	0.75	1.0	1.25
Temperature °c	Radical scavenging activity (%)				
40	13^{De}	26^{Dd}	28^{Dc}	42^{Da}	41^{Db}
60	22^{Ce}	34^{Cd}	36^{Cc}	49^{Cb}	53^{Ca}
80	26^{Be}	47^{Ad}	55^{Bc}	66^{Bb}	81^{Aa}
100	32^{Ad}	45^{Bc}	60^{Ab}	69^{Aa}	70^{Ba}

Means with the same capital letter in the same column are not significantly differed (p>0.05)
Means with the same small letter in the same row are not significantly differed (p>0.05)

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of cinnamon water extracts was presented in Table (3). It was clearly noticed that the antioxidant power of different cinnamon extracts was significantly (*P≤0.05*) enhanced by increasing the cinnamon concentrations and elevating extraction temperatures. FRAP was gradually increased from 0.64 at concentration of 0.25% to be 2.08 at concentration of 1.25 % at 40 °C. The highest FRAP value was observed at concentration of 1.25 % and 100 °C as extraction temperature, with value of 2.58. In addition, the same tend was observed at all used concentrations. Concerning with the FRAP, the highest antioxidant activity could be obtained when cinnamon extract was prepared at concentration of 1.25% at 100 °C. The high antioxidant capacity of the extract may be referred to the highest hydrogen donate ability (Jun *et al.,* 2011 and Chimbetete *et al.,* 2019). Electron transfer based methods determine the capacity of an antioxidant in the reduction, which changes color when reduced. FRAP method is one of electron transfer assays, which based on different chromogenic redox reagents with different standard potentials (Mishra *et al.,* 2012 and Ioannou *et al.,* 2015).

Table 3. Ferric reducing antioxidant power (OD) of prepared cinnamon extract at different concentrations and temperatures.

Concentration g/100ml	0.25	0.50	0.75	1.0	1.25
Temperature °c	Ferric reducing antioxidant power (OD)				
40	0.64^{De}	1.16^{Cd}	1.17^{Dc}	1.87^{Db}	2.08^{Da}
60	0.77^{Ce}	1.13^{Dd}	1.81^{Cc}	2.14^{Cb}	2.28^{Ca}
80	0.85^{Be}	1.46^{Bd}	2.07^{Bc}	2.34^{Bb}	2.45^{Ba}
100	0.98^{Ae}	1.67^{Ad}	2.08^{Ac}	2.45^{Ab}	2.58^{Aa}

Means with the same capital letter in the same column are not significantly differed (p>0.05)
Means with the same small letter in the same row are not significantly differed (p>0.05)

Reducing power

The impact of the cinnamon extracts prepared using different cinnamon concentrations and temperatures on the reducing power (RP) was presented in Table (4). RP values were significantly *(P≤0.05)* increased from 0.08 to 0.61 with increasing the cinnamon concentration from 0.25 to 1.25 % at 40 °C. The same trend was observed with the extracts those prepared at 100 °C. The RP increased from 0.21 to 1.07 at concentrations 0.25 and 1.25 %, respectively, with significant differences. The RP value gradually increased with increasing the temperature from 40 to 100 °C reached up to 0.21 . Moreover, the RP reached to the maximum value with the extract that prepared using 1.25 % cinnamon and prepared at 100 °C. Generally, each of cinnamon concentration and preparation temperature had significant effects on the RP of prepared extract Chimbetete *et al.,* (2019).

Table 4. Reducing power (OD) of prepared cinnamon extract at different concentrations and temperatures

Concentration g/100ml	0.25	0.50	0.75	1.0	1.25
Temperature °c	Reducing power (OD)				
40	0.08^{Ce}	0.19^{Ad}	0.28^{Bc}	0.30^{Cb}	0.61^{CA}
60	0.14^{Ab}	0.29^{Bb}	0.36^{Bab}	0.58^{Bab}	0.88^{Ba}
80	0.18^{ABb}	0.29^{Bb}	0.358^{Bb}	0.58^{Bab}	0.92^{Ba}
100	0.21^{Ae}	0.41^{Ad}	0.52^{Ac}	0.77^{Ab}	1.07^{Aa}

Means with the same capital letter in the same column are not significantly differed (p>0.05)
Means with the same small letter in the same row are not significantly differed (p>0.05)

Total Phenolic and flavonoid compounds contents

Total phenolic and flavonoid compounds contents were determined in the cinnamon water extract prepared using the optimum conditions (concentration of 1.25% at 100 ° C). The total phenols and flavonoids in cinnamon extract were 3.703 mg GAE / ml and 0.483 mg QE / ml, respectively (Fig. 1). This finding demonstrated that the cinnamon water extract prepared in this study had high content of the natural phytochemicals. In this respect, phytochemical analysis of cinnamon bark showed that it contains steroids, tannins, flavonoids, glycosides, coumarins, alkalis and anthraquinone compounds (Shihabudeen *et al.,* 2011). Moreover, Ghobadi Pour *et al.,* (2019) revealed that the high phenolic content and antioxidant activity in cinnamon aqueous extract could be considered as biologically active compounds that increase the antioxidant activity.

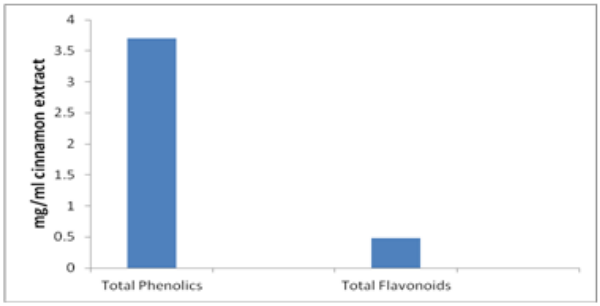

Figure 1. Total phenolics (mg GAE/ ml) and total flavonoids (mg QE/ ml) in cinnamon water extracts at optimal extraction conditions.

Biological assay and histopathology

The cinnamon aqueous extract prepared using the optimum conditions (concentration of 1.25% at 100 ° C) was biologically evaluated for its potential protective ability against liver cirrhosis induced by CCl_4. The obtained results indicated that injection of rats by CCl_4 led to elevate AST, ALT and MAD levels in blood serum (Fig. 2). The ALT and AST increased dramatically from 44 and 182 U/L at zero time to 73 and 221 U/L after twelve weeks, respectively. Significant (*P≤0.05*) decrease in total protein, albumin and the activities of GSH in the serum were observed in cirrhosis group (G2). The total proteins and albumin remarkable decreased to the minimum levels of 5.31 and 2.91 g/dl, after twelve weeks, respectively (Fig. 2). These finding indicated that serious liver damage has

been occurred due to treatment with CCl_4. On another hand, administration of aqueous extract of cinnamon (G3) improved total protein, albumin, ALT and AST activities to be near the normal values. This effect may be due to the presence of protective factors in cinnamon extract, which reduced lipid peroxidation resulting in significant decrease in MDA level simultaneously with a significant elevation in GSH activity. Non-significant ($P \geq 0.05$) difference was observed between the values those obtained from (G1 and G3).

The obtained results of the investigated hepatic biomarkers could be confirmed by the histological alteration showed in Fig. (3). Sections in livers for rats in negative group (G1), cirrhotic positive group (G2) and treated group by cinnamon extract (G3) shown in Figure 3. There was no histopathological change in G1. Moreover, the histopathological examination displayed normal histological structure of the central vein and surrounding hepatocytes in the parenchyma in G1 (Fig. 3, A). Thickening with collagen proliferation as well as inflammatory cells infiltration and degeneration in the underlying hepatocytes in the parenchyma were recorded in G2 (Fig. 3, B1). Fatty change was observed in diffuse manner all over the hepatocytes in the parenchyma (Fig. 3, B2). The portal area display inflammatory cells infiltration and few fibroblastic cells proliferation in G2 (Fig. 3, B3). Focal steatosis was detected in between the hepatic lobules of G2 (Fig. 3, B4). Thickening and inflammatory cells infiltrations were detected in the Glissons capsule while the underlying hepatocytes in the parenchyma showed degenerative changes in G3 (Fig. 3, C1). However, in G3, there was focal steatosis in the hepatic parenchyma with atrophy in the adjacent surrounding hepatocytes (Fig. 3, C2). In the same group, the portal area display congestion in the poirtal vein in addition to periductal inflammatory cells infiltration surrounding the bile ducts (Fig. 3, C3). Diffuse kupffer cells proliferation was detected in between the hepatocytes in G3 (Fig. 3, C4), moreover, the portal area display odema with the congestion in the portal vein (Fig. 3, C5) and congestion in the central vein in addition to sinusoids (G3 C6).

Several previous studies demonstrated the relationship between the biomarker assay and the changes in histology of the liver. Deterioration of liver cells leads to loss of function, and change the permeability of cell membranes resulted in leak of enzymes such as ALT and AST in the extracellular space followed by the emergence of odema and infiltration (Bellassoued *et al.*, 2019; Rasool *et al.*, 2019; El-Bahr, 2014; Al-Sultan and El-Bahr, 2015). Fat peroxide is shown when the amount of free radicals presented in high amount comparing to antioxidant level in the body, therefore, the MDA may be raised. These peroxides bind to sensitive body compounds such as double bonding of membranes and cause damage (Marimuthu *et al.*, 2013 and Im *et al.*, 2014).

The water extract of cinnamon raised the total protein levels toward the normal levels in the blood serum, thereby protecting the liver. This may be due to stimulating protein synthesis and accelerating the regeneration and production of liver cells (Hamidpour *et al.*, 2015 and Yashin *et al.*, 2017). Therefore, histopathological consequence are in harmony with Eidi *et al.*, (2012), who describe that, pretreatment of experimental rats with cinnamon extract significantly enhanced the construction of hepatic cells and moderated hepatotoxicity induced by the CCl_4.

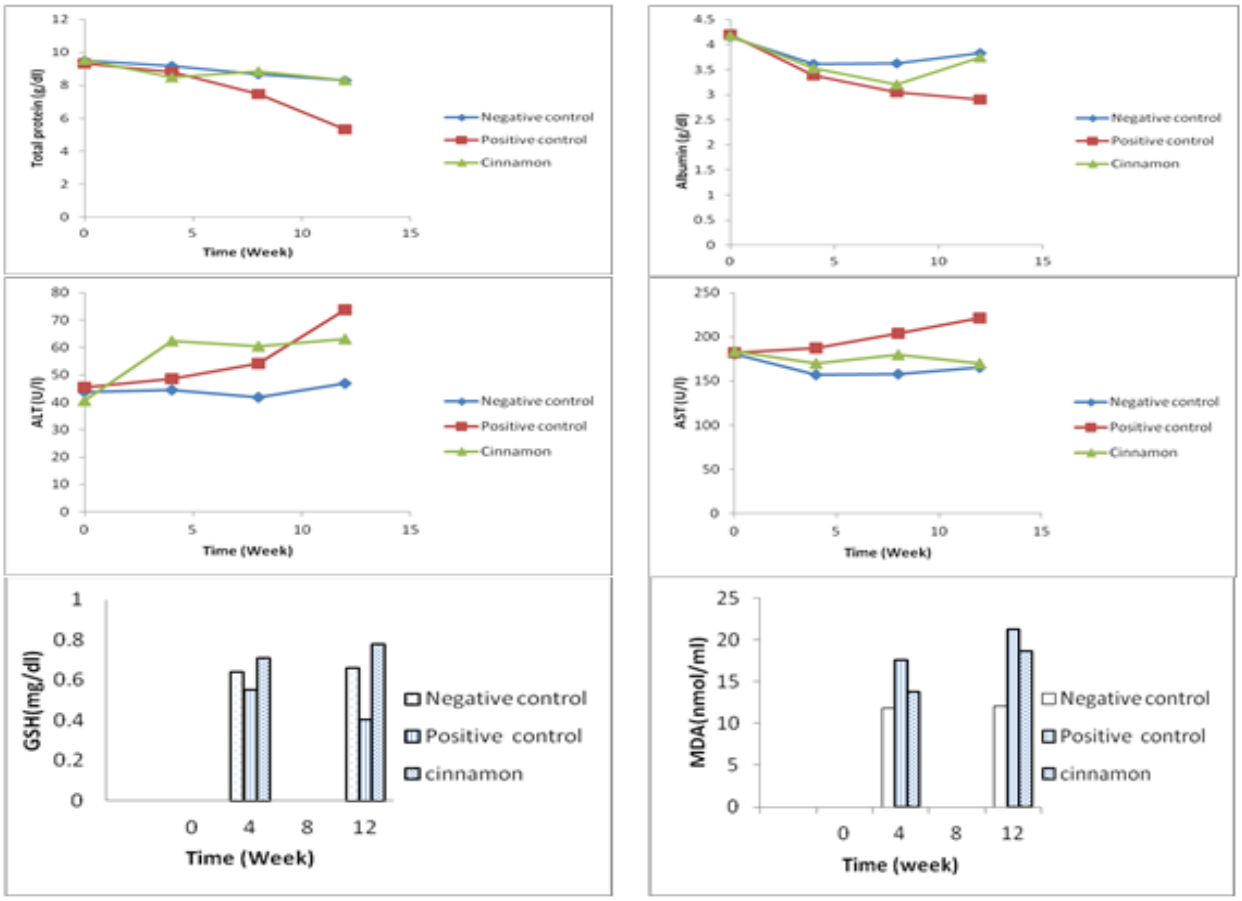

Figure 2. Effect of cinnamon extract prepared at optimal conditions on the total protein, albumin, ALT, AST, MAD and GSH in rates had chronic liver cirrhosis for twelve weeks

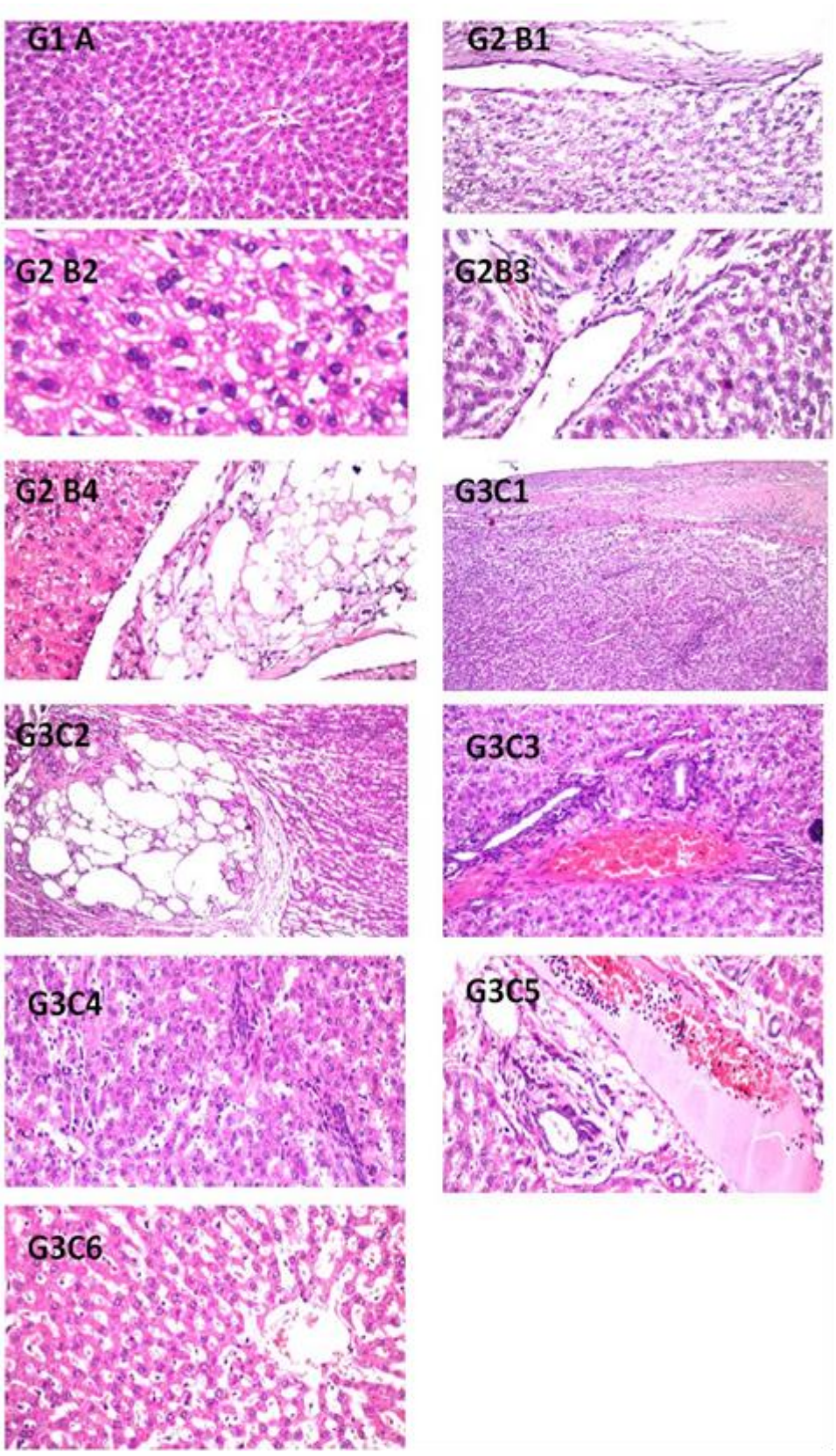

Figure 3. Sections in livers for rats in negative group (G1), cirrhotic positive group (G2) and treated group by cinnamon extract (G3).

CONCLUSION

In conclusion, the optimum conditions for preparing cinnamon aqueous extract were obtained using a concentration of 1.25% at extraction temperature of 100 °C. This extract was characterized by its high phenolic and flavonoid contents, which had an important role as antioxidants. The high content of these bioactive components may play a significant role as protective bio-ingredients against liver cirrhosis in rats treated with carbon tetrachloride.

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تأثير المستخلص المائى للقرفة على حماية الكبد

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تستخدم القرفة في المجال الطبي لعلاج العديد من الأمراض ، خاصة تلك المتعلقة بأمراض الكبد. تهدف هذه الدراسة إلى تقييم المركبات الفعالة لمستخلص القرفة المائي ودورها في الوقاية من أمراض الكبد في جرذان التجارب. تم إحداث تليف الكبد باستخدام رابع كلوريد الكربون (CCl_4). في الجرذان المصابه بتليف الكبد الناجم عن CCl_4 لوحظ ارتفاع نشاط الأنزيمات الكبدية في مصل الدم. تم تحسين الأنزيمات الكبدية (AST) و (ALT) بشكل كبير إلى ما يقرب من المعدلات الطبيعية بعد المعاملة الفموية بالمستخلص المائي للقرفة بجرعة 0.6 مل / 100 جرام من وزن الجسم / يومياً لمدة 12 أسبوعًا. وقد وجد أن هناك ارتفاع كبير في بيروكسيدات الدهون فى مصل الدم ، في حين انخفض الجلوتاثيون المختزل، الزلال والبروتينات الكلية بشكل كبير في الجرذان التي تم معاملتها ب CCl_4. وقد ثبت أن مستخلص القرفة المائي له تأثير وقائي على الكبد عن طريق خفض بيروكسيدات الدهون وزيادة الجلوتاثيون المختزل ,البروتينات الكلية والزلال فى مصل الدم. كما أكد الفحص النسيجي للكبد التأثير الايجابي للمتسخلص المائي للقرفه على المؤشرات الحيوية التي تمت دراستها في الكبد. في الختام ، يمكن اعتبار المحتوى العالي من المواد الكيميائية النباتية في مستخلص القرفة المائي السبب الرئيسي لخصائص الحماية ضد أمراض الكبد.

الكلمات الدالة :القرفة (*Cinnamomum zeylanicum L. Lauraceae*) ، نشاط الكبد .