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Comparative characterization of cinnamon, cinnamaldehyde and kaempferol for phytochemical, antioxidant and pharmacological properties using acetaminophen-induced oxidative stress mouse model

[Caracterización comparativa de canela, cinamaldehído y kaempferol para propiedades fitoquímicas, antioxidantes y farmacológicas utilizando el modelo de ratón con estrés oxidativo inducido por acetaminofeno]

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Abstract: This study was aimed to explore the comparative efficacy of cinnamon bark extract, cinnamaldehyde and kaempferol against acetaminophen (APAP)-induced oxidative stress. Cinnamon bark extract, cinnamaldehyde and kaempferol were utilized for *in-vivo* analysis. From the results of *in-vitro* screening tests, cinnamon ethanolic extract was selected for *in-vivo* study in mouse model. For this, Balb/c albino mice were treated with cinnamon ethanolic extract (200 mg/kg), cinnamaldehyde (10 mg/kg) and kaempferol (10 mg/kg) orally for 14 days followed by single intraperitoneal administration of APAP during 8 hours. Blood and organ samples were collected for biochemical and histopathological analysis. The results showed that cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol ameliorated APAP-induced oxidative stress and organ toxicity in mice. In conclusion, cinnamaldehyde and kaempferol possess comparable antioxidant potential even at 20-times less dose as compared to cinnamon bark ethanolic extract suggesting therapeutic potential in oxidative stress-related disorders.

Keywords: Phytochemical analysis; Cinnamon; Cinnamaldehyde; Kaempferol; Histopathology

Resumen: Este estudio tuvo como objetivo explorar la eficacia comparativa del extracto de corteza de canela, cinamaldehído y kaempferol contra el estrés oxidativo inducido por acetaminofén (APAP). Se utilizaron extracto de corteza de canela, cinamaldehído y kaempferol para el análisis *in vivo*. De los resultados de las pruebas de detección *in vitro*, se seleccionó el extracto etanólico de canela para estudio *in vivo* en modelo de ratón. Para ello, los ratones albinos Balb/c fueron tratados con extracto etanólico de canela (200 mg/kg), cinamaldehído (10 mg/kg) y kaempferol (10 mg/kg) por vía oral durante 14 días, seguido de la administración intraperitoneal única de APAP durante 8 horas. Se recogieron muestras de sangre y órganos para análisis bioquímicos e histopatológicos. Los resultados mostraron que el extracto etanólico de la corteza de canela, el cinamaldehído y el kaempferol mejoraron el estrés oxidativo inducido por APAP y la toxicidad orgánica en ratones. En conclusión, el cinamaldehído y el kaempferol poseen un potencial antioxidante comparable, incluso a una dosis 20 veces menor en comparación con el extracto etanólico de la corteza de canela, lo que sugiere un potencial terapéutico en los trastornos relacionados con el estrés oxidativo.

Palabras clave: Análisis fitoquímico; Canela; Cinamaldehído; Kaempferol; Histopatología.

INTRODUCTION

Drug-induced oxidative stress and toxicity is highly variable ranging from asymptomatic elevation of toxicity-biomarkers to organ failure (Guengerich, 2011). There are still many discrepancies in the effects of chemicals on experimental animals and humans. More than 900 drugs are described to cause liver injury and certified medications represent around > 50% of cases of acute liver failure whereas, the incidence of drug-related acute kidney injury may be as high as 60% (Ghane-Shahrbaf & Assadi, 2015). It is estimated that drugs cause roughly 20% of hospital- and community-acquired incidents of acute renal failure (Naughton, 2008). Acetaminophen (APAP), the commonly used analgesic and antipyretic drug, is safe at prescribed dosage but an overdose of APAP may cause severe liver and kidney damage. APAP is widely used in animal model to induce liver and kidney inflammation (Mossanen & Tacke, 2015; Yan *et al*., 2018).

A number of studies have authenticated the usage of traditional therapies to treat various diseases including intestinal, liver and kidney ailments (Rao & Gan, 2014; Mahmood *et al*., 2018; Shahid *et al*., 2018; Abbas *et al*., 2019; Hussain *et al*., 2019a; Khater *et al*., 2020). Cinnamon (*Cinnamomum zeylanicum*, family Lauraceae) also known as 'darchini', is widely used in tropical medicine. It mainly consists of essential oils, cinnamic acid, ethyl cinnamate, cinnamaldehyde and kaempferol (Rao & Gan, 2014). Several studies have shown preventive and therapeutic properties of cinnamon (Ranasinghe *et al*., 2013; Shahid *et al*., 2018; Hussain *et al*., 2019b; Sadeghi *et al*., 2019).

Despite beneficial effects of cinnamon, it is also reported that coumarin, one of the components of cinnamon, can cause liver toxicity (Eidi *et al*., 2012) underscoring the importance of isolation and characterization of active constituents of cinnamon having hepato-protective activity. Cinnamaldehyde, the active constituent mostly isolated from cinnamon bark oil (60-75% present) is known by its anticancer, anti-inflammatory, immunomodulatory and anti-diabetic activities (Subash Babu *et al*., 2007). Chao *et al*. (2008), had reported that cinnamaldehyde, even at minor concentrations, has potential to inhibit the secretion of tumor necrosis factor-α, interleukin-1β and reduce the excessive production of oxygen species. Another cinnamon-derived compound, kaempferol, possess anti-inflammatory, antioxidant and anticancer activities (Wang *et al*., 2018). Such beneficial effects involve in the inhibition of inflammatory cytokines in the liver through suppressing NF-kB activation (Luedde & Schwabe, 2011).

We have recently reported that cinnamon prevents liver injury induced by acetaminophen in Balb/c mice (Hussain *et al*., 2019a). The objectives of the present study were to describe phytochemical profiling of cinnamon bark ethanolic extract by *in-vitro* qualitative and quantitative analysis, hepatorenal protective and antioxidant effects of cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol *in-vivo* against APAP-induced oxidative stress and organs injuries in Balb/c mouse model.

MATERIALS AND METHODS

Plant material

Barks of *Cinnamomum zeylanicum* were authenticated by Department of Botany, University of Agriculture, Faisalabad (UAF) Pakistan. The sample was placed in the Herbarium, Department of Botany, UAF with the reference number 244-1-18. Cinnamon barks were cleaned to eliminate dust particles and then dehydrated under shade and grinded into fine particles (Hussain *et al*., 2018; Hussain *et al*., 2019a; Bakr *et al*., 2019; Maslachah *et al*., 2019). Cinnamaldehyde and kaempferol (analytical grade) were purchased from Fluka Research Chemicals and Solarbio Life Sciences respectively.

In-vitro evaluation

Extracts preparation

For cinnamon extract preparation, 100 g of cinnamon was mixed in 1000 ml of solvent and placed in orbital shaker at 250 rpm for 5 hrs at 71ºC. The solution was sonicated (E30H Elmasonic, D-78224 Singen/Htw, Germany) and centrifuged (80–2 Centrifugal Machine, China) for half an hour to get supernatant extract.

Chemical properties determination

Free radical-scavenging activity was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Wiczkowski *et al*., 2013). Total phenolic contents (TPC) were by using Folin-Ciocalteu reagent (Ghasemzadeh & Jaafar, 2013). Total polyphenols were expressed as gallic acid equivalents (GAE) in mg/100 g of extract. To measure total flavonoid contents (TFC) in cinnamon extract, catechin was used as standard (Abdeltaif *et al*., 2018). Results were presented as catechin equivalents (CE) in mg/100 g of extract.

Proximate and mineral analysis

Proximate analysis of cinnamon was performed by following the method of Horwitz (2010). Moisture and dry matter contents were determined. After wet digestion of the cinnamon bark powder, 0.5 g of sample was analyzed for minerals by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman ZA3000 Series). Calcium, manganese, iron, copper, zinc, cobalt, cadmium and lead were analyzed by using the procedures described in Horwitz (2010).

Chromatographic analysis

Powdered plant material (100 g) was soaked in 1000 ml solution containing ethyl alcohol (Sigma Aldrich) and distilled water (80:20), for about 10 days. A precise and easy HPLC procedure was adopted with modifications for the simultaneous assessment of quercetin, chlorogenic acid, cinnamaldehyde and kaempferol in the plant extract (Seal, 2016). Briefly, the bark extract was characterized using HPLC on Shim-Pack CLC-ODS-2 (C-18 column, Shimadzu, Japan; maintained at 23-25°C) equipped with a UV-visible detector (SPD-10 AV, Shimadzu, Japan) and an HPLC pump (LC-10AT Shimadzu, Japan). The CSW32-Chromatography Station Data Apex Ltd. 2001 software were used for data acquisition. Percent area of the correlative sample was measured.

In-vivo evaluation

Reconstitution of Acetaminophen

APAP (Sigma Aldrich) was reconstituted in normal saline solution and homogenized using Vortex mixer. A dose equal to 200 mg/kg was administered intraperitoneally to Balb/c mice.

Study design and experimental protocol

The *in vivo*-evaluation was carried out on mice to test the protective potential of cinnamon bark extract in the management of APAP-induced acute liver and kidney injuries. Total, 48 healthy adult Balb/c mice, 4-6 weeks, weighing 29 ± 5 g were maintained at temperature 25 ± 4°C and relative humidity 55 ± 2 %. Animals received routine diet (mouse pelleted chow diet # 14) and water *ad libitum*. The pelleted diet contained crude protein (20%), crude fiber (4.5%), total ash (6%), fat (6%), nitrogen-free extract (63.5%), minerals and trace elements. After 2 weeks of acclimatization, mice were divided randomly into four main groups (n=12); Group I, control group, was maintained at routine diet (14 days), whereas Group II, III and IV were administered orally with cinnamon bark whole extract (200 mg/kg), cinnamaldehyde (10 mg/kg) and kaempferol (10 mg/kg) respectively for 14 days. The cinnamon extract dosage for mice was calculated based on previous literature (Khan *et al*., 2003). The daily cinnamon dose for mice was 200 mg/kg (Reagan-Shaw *et al*., 2008; Ranasinghe *et al*., 2012). At 15th day of the trial, each group was divided into two subgroups (n=6) and exposed to either vehicle (normal saline) or APAP (200 mg/kg) intraperitoneally for 8 hours. Then 8 hours after single dose administration, mice were sacrificed and blood and organ samples were collected for biochemical and histopathological studies.

Blood sample collection for biochemical parameters analysis

Blood samples were collected in Clot Activator tubes (Gel & Clot Activator, Xinle, China) and centrifuged (80–2 Centrifugal Machine, China) at 1010 x g for 15 minutes to separate the serum. Serum samples were stored in the biomedical freezer (Sanyo Biomedical Freezer, MDF-U333, Japan) at -31°C till analysis.

Liver and kidney function tests

By following the protocol of Agrawal *et al.* (2016) the liver function tests i.e., alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed. Alkaline phosphatase (ALP) level was measured according to the method described by Park *et al*. (2013). These liver function tests were performed by using biochemical kits (DiaSys GmbH, Germany). Moreover, kidney function tests including serum creatinine and urea levels were measured by using biochemical kits (Innoline®, Merck pvt. Limited, Pakistan). All parameters were measured by using either automated biochemistry analyzer (BioLab-310) or microplate spectrophotometer (Thermo Scientific Multiskan GO™ equipped with SkanIt software 4.1) (Hassan & Hassan, 2018; Khalid *et al*., 2018).

Organ sample collection for histopathological analysis

Liver and kidney samples were collected and then observed for any macroscopic lesions and color changes. Samples were washed with normal saline solution and preserved in 10% neutral buffered formalin. Then fixed tissues were embedded in paraffin, sectioned (5 μm) and stained with hematoxylin and eosin (H & E) dyes. Samples sections were histologically evaluated with a light microscope (IM-910 IRMECO Gmbh & Co; Germany). Drug-induced organ damage was assessed on the basis of previous methods (Mann *et al*., 2012;

Alturkistani *et al*., 2015; Hussain *et al*., 2018; Bakr *et al*., 2019; Maslachah *et al*., 2019).

Statistical analysis

The significant effects of drug treatments were assessed statistically by applying one way analysis of variance followed by Duncan´s multiple range tests at 5% level of significance.

Ethics Approval

This study was approved by Institutional Biosafety and Bioethics Committee (IBC) by Permission No. 3456/ORIC and Synopsis Scrutiny Committee by Permission No. DGS/10969-72, University of Agriculture, Faisalabad, Pakistan. The animals in the present study were handled and cared for in accordance with the Guidelines of National Biosafety Committee 2005 and Punjab Biosafety Rules 2014.

ARRIVE guidelines

We ensure that our manuscript reporting follows the ARRIVE guidelines for animal experiments (Kilkenny *et al*., 2010).

RESULTS

In-vitro evaluation results

Antioxidant potential based screening of different cinnamon bark extracts

Antioxidant ability of the extracts, as determined by DPPH-free radical scavenging activity, was expressed as IC_{50} (µg/mL). Results showed lesser IC_{50} values for ethanolic extract (89.03) as compared to that of aqueous (173.26) or methanolic extract (158.08). Total phenolic and total flavonoid contents (mg/100g of extract) were higher in ethanolic extract (157.85 ± 3.08, 42.83 ± 0.53 respectively) as compared to that of aqueous (80.45 ± 6.87, 27.98 ± 2.83 respectively) or methanolic extract (145.14 ± 4.99, 39.49 ± 0.86 respectively). From results of *in-vitro* antioxidant assays, it was found that cinnamon ethanolic extract presented the maximum antioxidant potential when compared to that of cinnamon aqueous or methanolic extract.

Proximate and mineral analysis results

Ethanolic extract of cinnamon bark contained flavonoids, phenols, saponins, fats and fixed oils, carbohydrates and terpenoids, while alkaloids, tannins and glycosides were absent. Results of proximate analysis of cinnamon bark revealed the presence of dry matter (93.5%), moisture content (6.5%), crude protein (8.75%), crude fat (3.5%), crude fiber (33%) and total ash (3%). Mineral analysis showed the presence of calcium (34 mg/100 g), manganese (8.8 mg/100 g), iron (4.4 mg/100 g), copper (1.6 mg/100 g), zinc (1.3 mg/100 g) and cobalt (0.3 mg/100 g).

Characterization of cinnamon extract through HPLC

HPLC chromatogram showed the presence of quercetin, chlorogenic acid, cinnamaldehyde and kaempferol in cinnamon extract as shown in Figure No. 1. Constituents were identified by comparing their retention time with standard/reference compounds and their concentrations were expressed as ppm (mg/L). Results showed that kaempferol was present in highest concentration while cinnamaldehyde was present in lowest concentrations as shown in Table No. 1.

In-vivo evaluation results cinnamon, cinnamaldehyde and kaempferol protect mice from APAP-induced acute injury

Results of liver function biomarkers showed significant increase ($p<0.05$) in serum ALT and AST levels while significant decrease ($p<0.05$) in ALP after 8 hr of APAP exposure. The pre-treatment of mice with cinnamon extract, cinnamaldehyde and kaempferol restored APAP-dependent alterations in ALT and AST and ALP (Figure No. 2A-C). Kidney function test showed significantly increased ($p<0.05$) creatinine, urea and BUN levels after 8 hr of APAP exposure as compared to the control (vehicle) group. Mice pretreated with cinnamon bark extract, cinnamaldehyde or kaempferol did not show significant rise in creatinine, urea and BUN levels following APAP exposure (Figure No. 2D-F).

Effects of treatments on liver histopathological alterations induced by APAP

Gross examination of liver from APAP-administered group showed reddish appearance as compared to other groups. Similarly, cinnamaldehyde and kaempferol pretreatment group represented normal liver. To determine the severity of hepatic damage by APAP, histopathological examination of the liver was performed that showed degenerative parenchymal structure of the liver at 8 hours following APAP exposure. These changes were identified with cellular infiltration and pyknotic nuclei and attenuated by pretreatment of cinnamon extract, cinnamaldehyde and kaempferol (Figure No. 3).

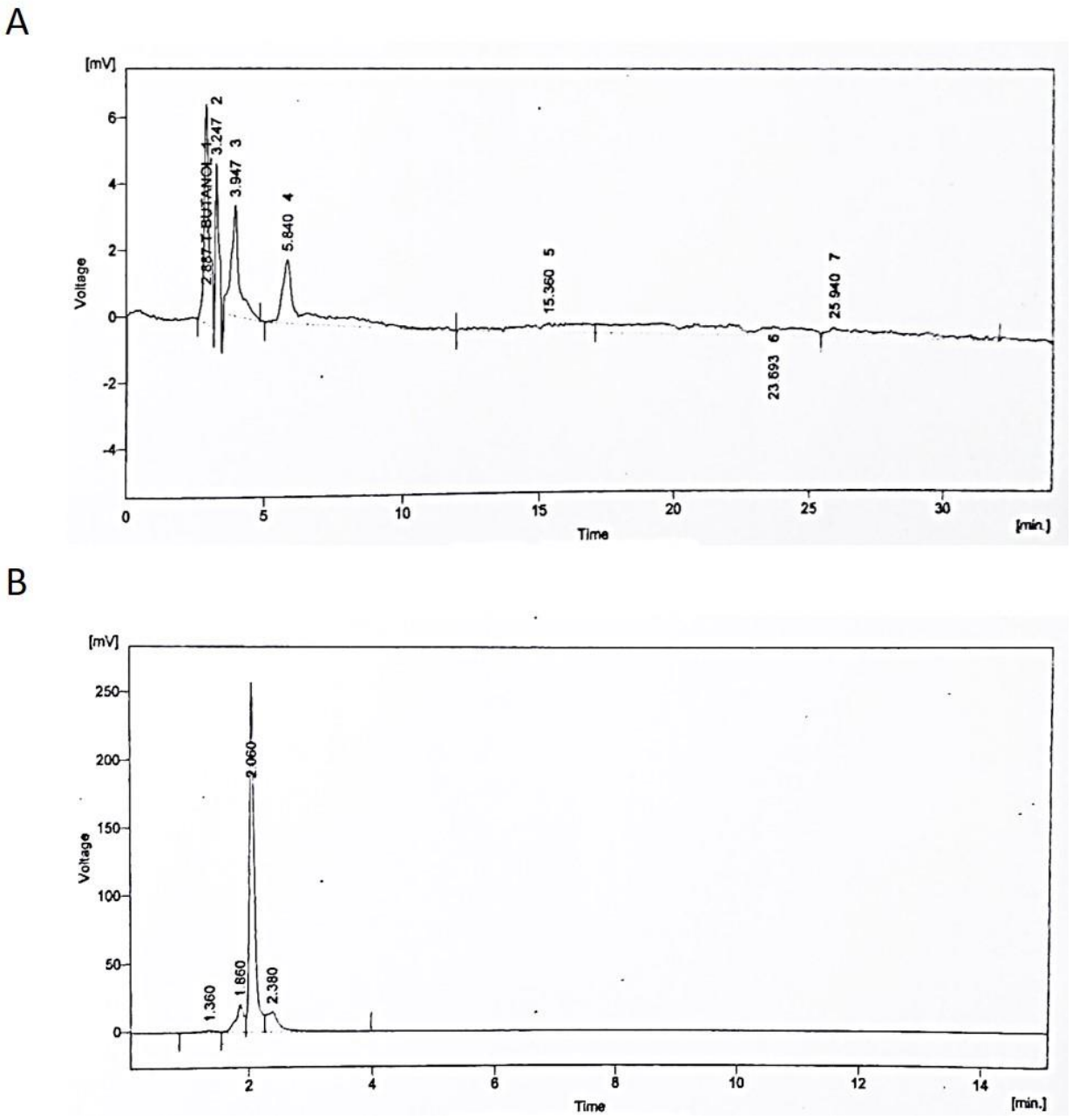
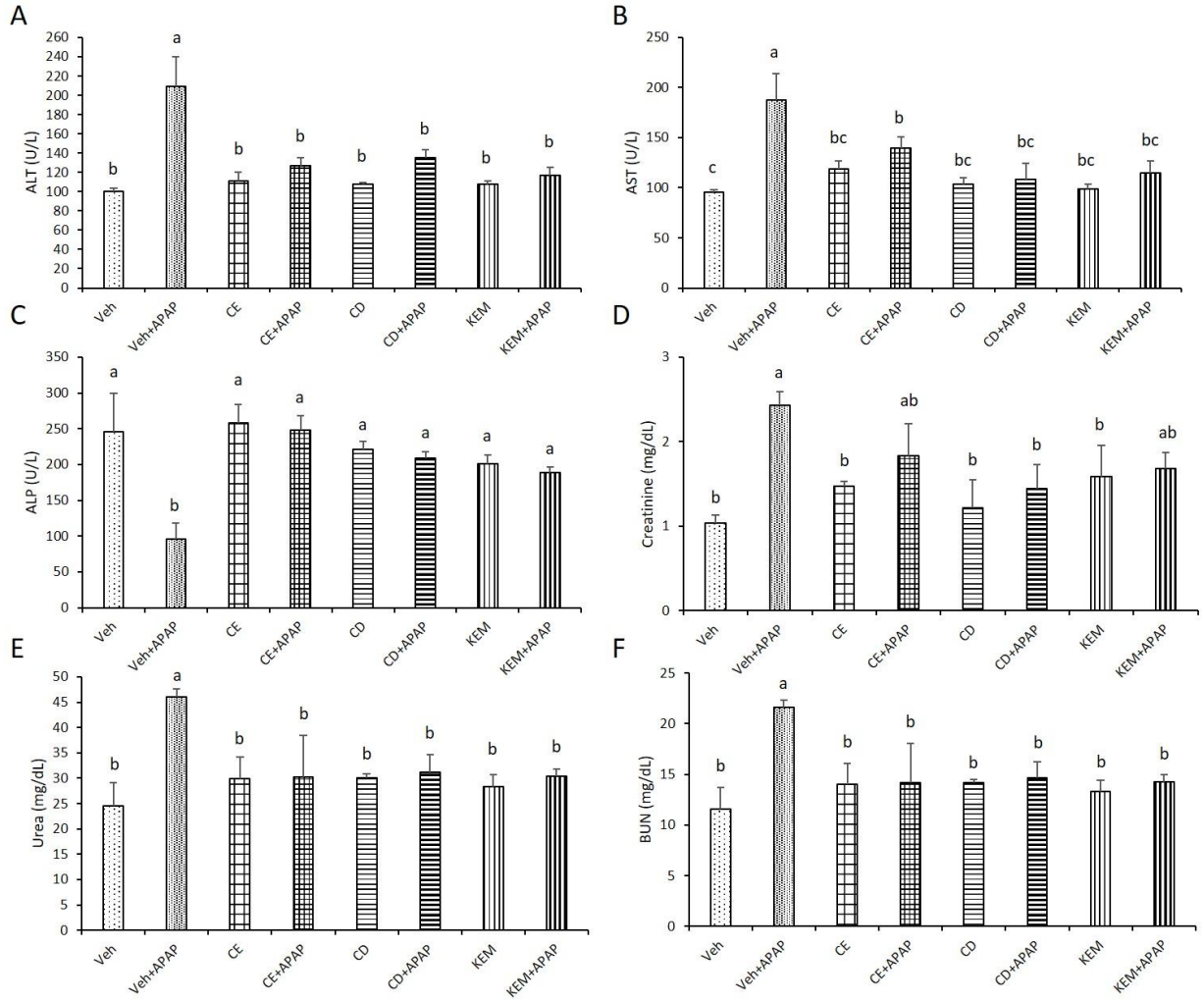


Figure No. 1

Representative HPLC chromatogram of cinnamon ethanolic extract. (A) HPLC chromatogram of quercetin, chlorogenic acid and cinnamaldehyde (B) HPLC chromatogram of kaempferol. The samples were characterized using HPLC, C-18 column with 25 cm × 4.6 mm, 5 µm particle size, maintained at 23-25°C. Injection volume was 5.0 µl and flow rate was set at 1 mL/min. The wavelength for absorbance was set at 280 nm.

Table No. 1
HPLC analysis of cinnamon ethanolic extract

Compound	Retention time (min)	Area (mV.s)	Area (%)	Concentration (ppm)
Quercetin	2.89	82.27	15.8	4.35
Chlorogenic acid	15.36	48.63	9.3	3.79
Cinnamaldehyde	25.94	54.76	10.5	1.17
Kaempferol	2.06	1413.31	76.3	438.13



CE, cinnamon bark ethanolic extract; CD, cinnamaldehyde; KEM, kaempferol.

Figure No. 2

Liver and kidney function markers in different treatment groups. Mice were pretreated with cinnamon bark ethanolic extract (200 mg/kg/day i.g.), cinnamaldehyde (10 mg/kg/day i.g.) or kaempferol (10 mg/kg/day i.g.) for 14 days. After 14 days, each of the pretreatment groups was further divided into two subgroups receiving either vehicle or APAP (200 mg/kg i.p.) during 8h. Liver and kidney function markers were to protects Balb/c mice from acute liver and kidney injury due to single toxic dose of APAP as assessed by measuring serum levels of (A) ALT (B) AST (C) ALP (D) creatinine (E) urea and (F) BUN. Results shown are the Mean ± SE of n=6 whereas, the groups with different alphabets are significantly different (p<0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

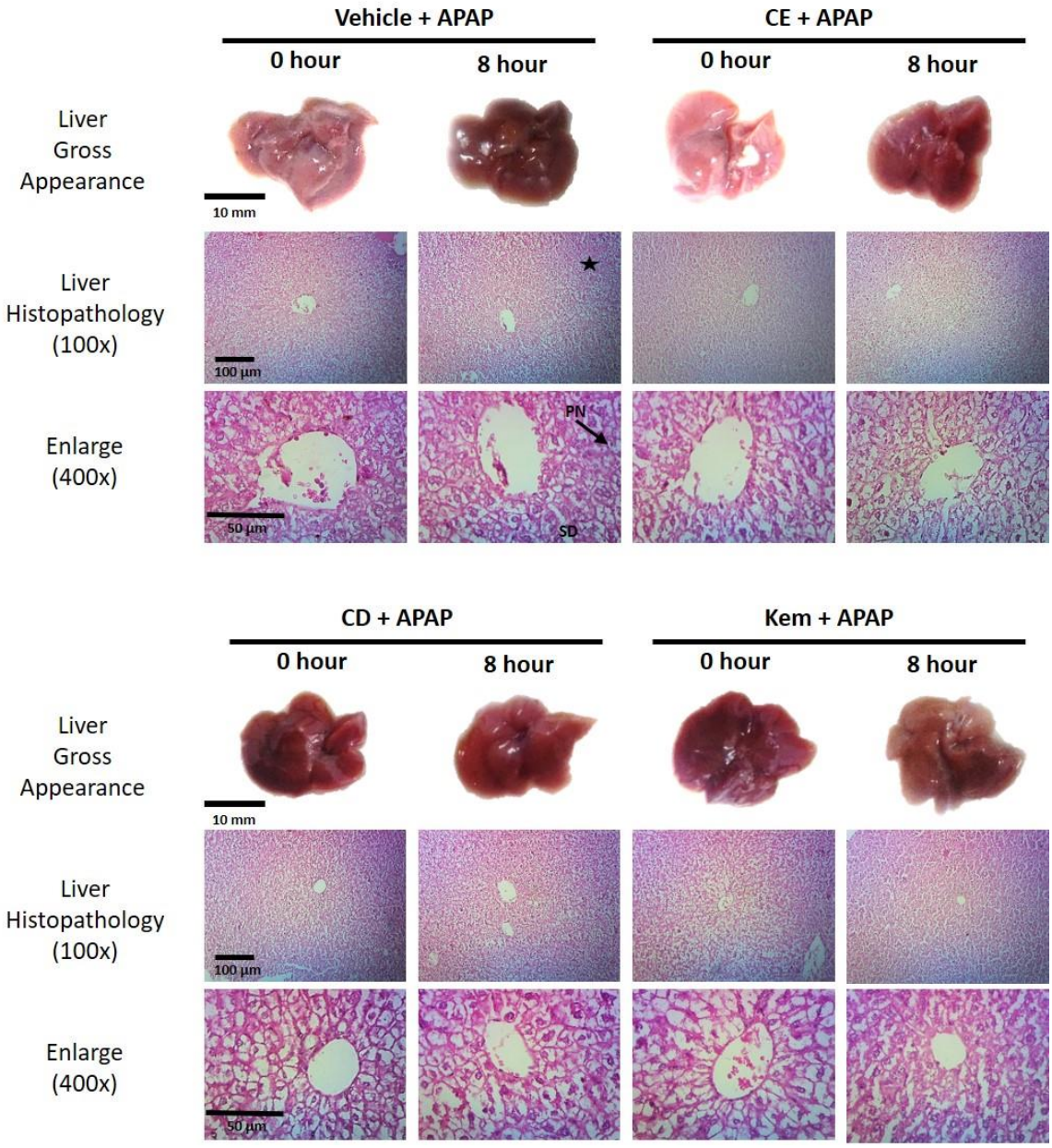


Figure No. 3

Effect of cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol on APAP-induced histopathological changes in liver. Pretreatment with cinnamon ethanolic extract (200 mg/kg/day i.g.), cinnamaldehyde (10 mg/kg/day i.g.) or kaempferol (10 mg/kg/day i.g.) for fourteen days prior to 0 and 8 h exposure with APAP (200 mg/kg i.p.) affects on gross and photomicrographs of liver tissue sections (H&E staining, 100x and 400x). APAP, acetaminophen; CD, cinnamaldehyde; CE, cinnamon ethanolic extract; H&E, hematoxylin and eosin; KEM, kaempferol; PN, pyknotic nuclei; SD, sinusoidal dilatation; Star★, cellular infiltration.

Effects of treatments on kidney histopathological alterations induced by APAP

Gross examination of kidney from APAP-exposure mice group showed abnormal rough surface of kidneys with dark reddish color as compared to other groups. Histopathological examination of the kidney was performed to determine the severity of renal damage by APAP. APAP induced mild inflammatory and vascular changes with tubular necrosis in the kidney tissues. Cinnamon extract pretreatment normalized the renal tissue architecture and only showed mild vascular changes in the kidneys, but no

signs of inflammatory changes were observed, comparable to those observed upon acute exposure of APAP. Similarly, cinnamaldehyde and kaempferol pretreatment group, the inflammatory changes were ameliorated (Figure No. 4).

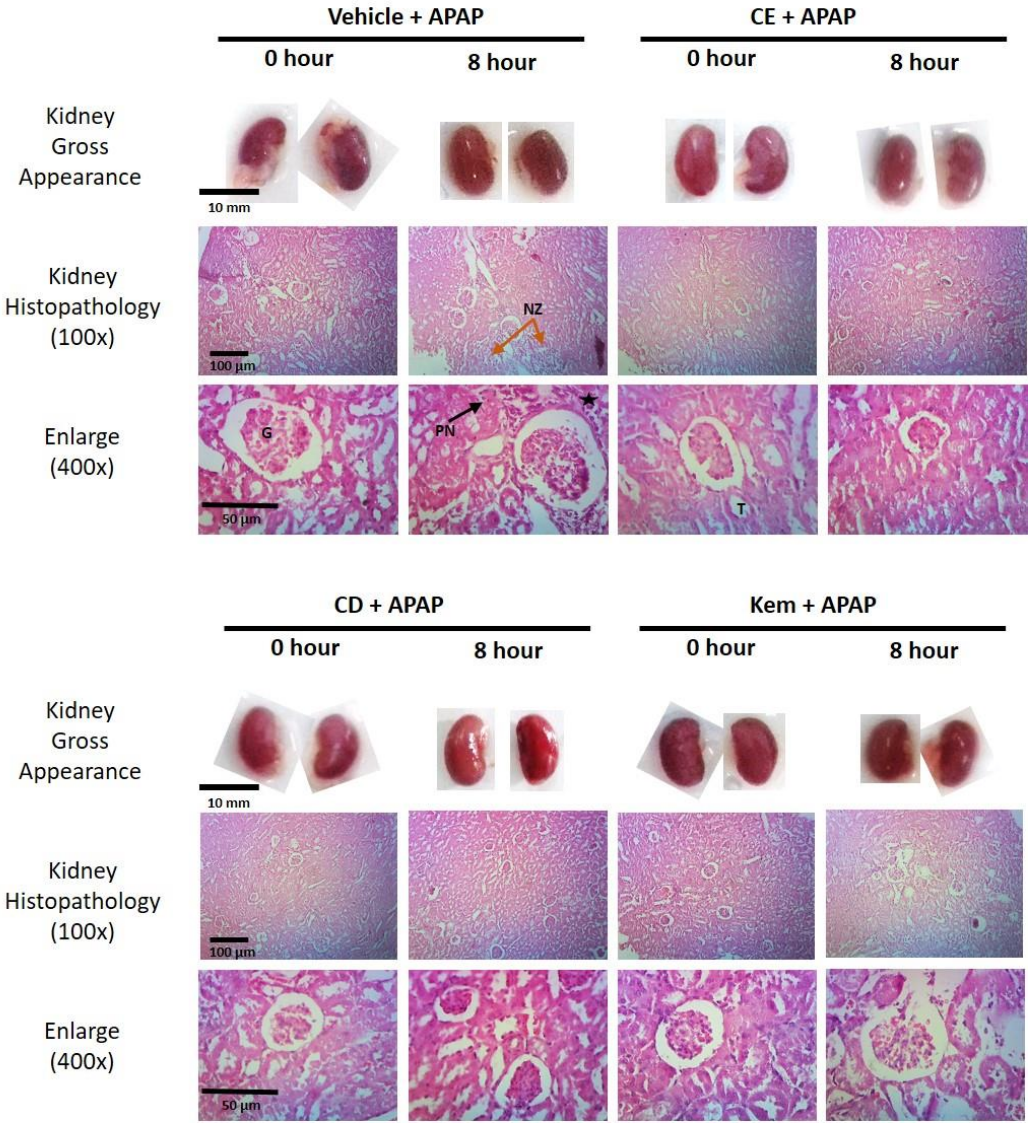


Figure No. 4

Effect of cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol on APAP-induced histopathological changes in kidney. Pretreatment with cinnamon ethanolic extract (200 mg/kg/day i.g.), cinnamaldehyde (10 mg/kg/day i.g.) or kaempferol (10 mg/kg/day i.g.) for fourteen days prior to 0 and 8 h exposure with APAP (200 mg/kg i.p.) effects on gross and photomicrographs of kidney tissue sections (H&E staining, 100x and 400x). APAP, acetaminophen; CD, cinnamaldehyde; CE, cinnamon ethanolic extract; G, Glomerular tuft; H&E, hematoxylin and eosin; KEM, kaempferol; NZ, necrotic zone; PN, pyknotic nuclei; Star★, cellular infiltration; T, renal tubule.

DISCUSSION

Cinnamon is an important spice and herb in modern system of medicine, having multipurpose medicinal properties. The antioxidant properties of this spice are accredited to the presence cinnamaldehyde and kaempferol. Even though this spice had received interest for the phytochemical investigations for many years, there is little evidence of the isolation and pharmacological characterization of its phytoconstituents. In this study, we found that protective effects of cinnamon bark extract, cinnamaldehyde and kaempferol against APAP-induced toxicity involve antioxidant mechanism.

With rapidly increasing use of herbal medicines, there is great concern about the quality and safety of medicinal plant materials. Although phytoconstituents obtained from same plant differ in structures but with similar antioxidant and curative potential act synergistically in detoxification (Kroes, 2014). In our study, proximate analysis of cinnamon powdered bark revealed the presence of moisture content 6.5% that was within acceptable limit of 6-15% for most of drug extracts (Kha & Chaudhry, 2010; Ereifej *et al.*, 2015). Mineral analysis determines the presence of different micro- and macronutrients in the herbal formulation. We performed mineral composition analysis of cinnamon powdered bark and the results were in accordance with the previous studies (Ereifej *et al*., 2015). Generally, the concentration of cinnamaldehyde determines the flavor quality of cinnamon that contains about 1-3.5% essential oil consisting primarily of cinnamaldehyde (60-75%) (Utchariyakiat *et al*., 2016). These results are in accordance with the previous research (Woehrlin *et al*., 2010; Kurniawati *et al*., 2017).

DPPH findings are in accordance with Abeysekera *et al.* (2017) who reported that ethanolic extract of cinnamon bark possess more (107.69 ± 2.01%) free radical scavenging activity (mg Trolox equivalents/g of cinnamon) while the methanolic extract showed less (60.49 ± 0.48%) free radical scavenging activity (mg Trolox equivalents/g of cinnamon). Results of total phenolic and flavonoid contents of cinnamon were comparable with previous studies (Prasad *et al*., 2009). We found that ethanolic extract contain more antioxidant potential, compared to the aqueous and methanolic extracts. Cinnamon aqueous extract presented the minimum antioxidant activity and methanolic extract may not be used *in vivo* due to alcohol toxicity (Singh *et al*., 2018).

In the current study, the hepatic and renal function biochemical parameters together with the histopathological alterations in the liver and kidneys were evaluated in pretreated and APAP-induced toxicity groups. Inside body, APAP is metabolized by enzymes CYP2E1 and 3A4 to form the toxic metabolite NAPQI that cause oxidative damage to hepatic and renal cells. In this way, the overdose of APAP elevates the risk of organ failure due to its potential hepato- and nephrotoxicity (Hussain *et al*., 2019a). The results of ALT, AST, creatinine and urea revealed that liver and renal functions were affected by overdose of APAP. A significant increase in serum ALT and AST levels was observed after IP administration of 200 mg/kg APAP dose. This finding is consistent with previous study (Hou *et al*., 2012). The gross appearance of liver showed degenerated pattern with dark reddish color, the characteristic mark of APAP-toxicity. Pretreatment with cinnamon extract prevented the extent of liver degeneration. The histopathological analysis of liver showed vascular and inflammatory changes and sinusoidal dilatation which were similar to previous studies (Lim *et al*., 2010; Hassan & Hassan, 2018; Hussain *et al*., 2018; Weng *et al*., 2019). In contrast, after pretreatment of cinnamon, cinnamaldehyde and kaempferol, the severity of liver damage was prevented.

The biochemical markers creatinine, urea and BUN were quantified to evaluate renal function. Intraperitoneal administration of 200 mg/kg APAP caused a significant increase in creatinine, urea and BUN levels which was consistent with other studies after oral (Ghosh *et al*., 2015) and intraperitoneal administration (Roomi *et al*., 2008) of APAP. The toxicity associated with APAP also resulted in injury to the tubules and glomerular damage. The protective effect of cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol against APAP toxicity was not as obvious in the kidneys as it was in the liver. This might be due to some additional mechanisms such as the presence of deacetylase and prostaglandin synthetase enzymes, different *in-situ* biotransformation or different sensitivity of the renal tissues (Sakuma *et al*., 2009). Overall, the histopathological examination indicated that pretreatment with cinnamon, cinnamaldehyde and kaempferol suppressed the acute liver and kidney injury as consistent with the improvement in serum biochemical parameters.

Our results show that cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol played an important role in ameliorating APAP-induced hepato- and nephrotoxicity possibly due to their antioxidant properties, yet more work is to be

done on isolation and molecular characterization of other cinnamon phytoconstituents possessing antioxidants properties.

CONCLUSION

In this study, *in-vitro* and *in-vivo* analysis of cinnamon showed antioxidant and protective potential against acetaminophen toxicity. Presence of cinnamaldehyde and kaempferol highlights the importance of cinnamon spice. Hepatic biomarkers (ALT, AST and ALP) and hepatic histopathological changes as well as renal biomarkers (creatinine, urea and BUN) and renal histopathological changes revealed acute toxicity patterns in mice after 8 hours of acetaminophen administration. Pretreatment with cinnamon bark ethanolic extract, cinnamaldehyde and kaempferol ameliorated APAP-dependent acute hepatic and renal toxicity.

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