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Protective effect of *Cinnamomum zeylanicum* aqueous extract against paracetamol induced nephrotoxicity in albino rats

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Abstract
In the current article, it is investigated the protective activity of *Cinnamomum zeylanicum* aqueous extract (CZAE) against paracetamol (PCM) induced nephrotoxicity in albino rats. Wistar albino rats (150-200 g) were divided into six groups and toxicity was induced by APAP (750 mg/kg, p.o, 10 days). CZAE (100, 200 mg/kg, p.o), silymarin (100 mg/kg, p.o) was administered to rats 2 h before PCM administration. Various biochemical parameters like serum urea, serum creatinine, uric acid and total protein levels were measured. Histopathological analyses of kidney injury were also studied. Treatment with CZAE (100, 200 mg/kg, bw) significantly (p<0.001, p<0.01) decreased serum urea and serum creatinine as compared with PCM rats. Decreased levels of uric acid and total protein were also significantly restored with CZAE treatment. Apart from these, CZAE treatment also reduced histopathological alteration induced by paracetamol in kidney. It was concluded that CZAE have a strong nephroprotective activity against PCM induced nephrotoxicity in albino rats.

Keywords: *Cinnamomum zeylanicum*, paracetamol, nephroprotective, silymarin

Introduction
PCM is most commonly used safe, non-prescription, over the counter, analgesic & antipyretic drug [1-3]. PCM at over dose can leads to potentially fatal hepatic and renal damage in human and experimental animals [4]. When PCM administered at therapeutic dose it metabolised by cytochrome P-450 and detoxified by glucuronidation as well as sulfation where as N-acetyl-p-benzoquinon-imine (NAPQI) with conjugated glutathione [5]. On the other hand high dose saturates the detoxification pathway of PCM due to glucuronidation and sulfation insufficiency, resulting in the depletion of cellular GSH, allowing NAPQI to bind to cellular protein which aggravate cellular oxidative stress, contribute to hepatic and renal damage[6]. The PCM induced kidney damage is acute tubular necrosis, increase creatinine levels, and decrease in glomerular filtration rate (GFR). Tubular cell injury is the main feature observed in PCM induced renal failure and the main functional evidence of proximal tubular injury is phosphaturia and low molecular-weight proteinuria [7]. A number of herbs are traditionally used in different country in response to drug induced liver and kidney disorder [8].
Cinnamomum zeylanicum (family Lauraceae) bark is tropical evergreen tree, native to Srilanka East and Middle Asia. It is also called differently in different languages such as dalchini in Hindi, carnelle in French, kaneel in German, canela in Spanish and yook gway in Chinese [9]. Cinnamon bark is a very common culinary spice and used in candy, toothpaste and perfumes. The cinnamon barks contain volatile oils (14%) of cinnamaldehyde (60%), euginol (10%) and trans-cinnamic acid (51%), phenolic compounds, tannin, catechins and proanthocyanidians: monoterpines and sesquiterpines, mucilage; starch, resin, sugar and trace of Coumadin [10]. The principal constituents are cinnamaldehyde [11]. In traditional medicine it has been used as analgesic [12], antimicrobial [13], antifungal [14], antioxidant [15], anti-inflammatory and antidiabetic [16]. In the present study we investigated the protective effect of aqueous extract of *Cinnamomum zeylanicum* bark on PCM induced nephrotoxicity in albino rats.

Materials and Methods
Chemical
PCM was obtained from gift sample from Arbro pharmaceutical company. Assay for kidney marker enzyme such as urea, creatinine, uric acid, and total protein were purchase from Erba

diagnostic Mannhein, Germany. All other reagents used in the experiment were of analytical grade.

Plant material

The *Cinnamomum zeylanicum* bark was procured from local market in old Delhi and authenticated and identified by Dr. Sunita Garg, CSIR-NISCAIR New Delhi. A voucher specimen (Ref. No. NISCAIR/RHMD/CONSULT/2018/3261-62) has been deposited in herbarium of CSIR-NISCAIR. New Delhi.

Preparation of extract

The *Cinnamomum zeylanicum* bark was dried and powder and extracted using water in Soxhlet apparatus for 8 hours. Then the extract was evaporated to dryness at 50 ^{0}C in a water bath and the final dry extract was stored in dark at -20 ^{0}C until used for the experiments. The percentage yield of extract was 12% w/w.

Preliminary phytochemical study

A preliminary phytochemical analysis of *C. zeylanicum* extract was carried out to determine the phytochemical constituents using standard procedure to identify the constituent as described method [17-20].

Animals

Adult albino wistar rats (150-200 g) either sex were obtained from All India Institute of Medical Science (AIIMS), New Delhi, India. The animals were housed in cages and maintained at 24±2 ^{0}C with a relative humidity of 45-55% and 12:12 h light/dark cycle. The animals were providing with standard pellet feed with drinking water *ad libitum*. The experimental protocol was approved by the institutional animal ethical committee (IAEC) of HIMT College of Pharmacy (Reg. No. 1377/PO/Re/S/10/CPCSEA), Gautam Budh Nagar, Uttar Pradesh, India and performed in accordance with the guideline of committee for the purpose of control and supervision of animals (CPCSEA), New Delhi.

Acute oral toxicity

Acute toxicity study of extract of *Cinnamomum zeylanicum bark* was estimated in wistar albino rats (150-200 g) according to OECD guidelines (Organization for Economic Co-operation and development, guideline, No. 423[21]. The extract was administered in 1% carboxy methyl cellulose (CMC) at doses of 50, 300 and 2000 mg/kg body weight while the control received the CMC (1%) suspension only. Food and water withheld for 1-2 h after drug administration. Rat was observed for the initial 4 h after the administration of drugs, after that once daily during the following days. The behavioural changes observed for hyperactivity, ataxia tremors convulsion salivation, diarrhoea, sleep, and coma. The total observation period for eventually mortality was 14 days. No mortality was observed up 2000 mg/kg. One tenth and one twentieth of the maximum tolerated dose (2000mg/kg) of both plants extracts was selected for study. Therefore biological study was carried out at doses of 100mg and 200mg/kg.

Experimental Design

Animals were randomly divided into six groups of which six animals in each group.

Group I served as untreated control and fed with 1% CMC (1ml/kg, p.o) daily for 10 days. Group II served as toxic and treated with PCM (750 mg/kg, p.o) suspended in 1% CMC three alternative days for 10 days [22-4]. Group III and Group IV served as treatment group and were treated with *Cinnamomum zeylanicum* bark extract (100, 200 mg/kg, p.o) daily for 10 days. Group VI served as standard group and was treated as 100 mg/kg of silymarin [25] orally for 10 days. Group II, III, IV and V was administered with PCM suspension (750 mg/kg, p.o) for three alternative days for 10 days. At the end of treatment all animal were fasted for 12 h, blood sample were collected from all animals by puncturing retro orbital plexus under ether anaesthesia, later animals were sacrifice and kidney were collected for histopathological studies.

Biochemical Analysis

Blood was drawn by puncturing the retro-orbital plexus under diethyl ether anaesthesia using heparin coated capillaries. Serum was separated by centrifugation at 3000 rpm for 15 min, stored at -20 ^{0}C until analysis. Serum sample were used to determine urea, creatinine, uric acid, and total protein using commercially available assay kits (Erba diagnostic Mannheim, Germany).

Histopathological studies

After experimental period animals were sacrificed, kidney removed immediately, sliced and washed in saline and transfer into 10% formalin solution, after one week tissue were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 µm section, stained with haematoxylin-eosin dye and then observed under microscope.

Statistical analysis

All data are expressed as Mean± Standard Error of the mean ((SEM) and statistical analysis was performed using Graphpad prism-5 software (Graphpad Software). The statistical significance differences between groups were tested using one-way analysis of variance (ANOVA) followed by Tukey multiple compare tests. A significant difference was assumed for values of $p<0.05$ level.

Result

Preliminary Phytochemical analysis

Preliminary phytochemical studies revealed the presence of alkaloid, saponin, tannin, terpanoid, flavonoids and phenol.

Acute oral toxicity

The aqueous extract of *Cinnamomum zeylanicum* was subjected to acute toxicity testing in albino rats and was monitored for 24 h. The CZAE did not cause any mortality up to 2000 mg/kg and hence 1/10th and 1/20th of the maximum dose administered (100 and 200 mg/kg, p.o) were selected for the present study.

Effect of CZAE on serum urea, creatinine, uric acid and total protein.

The level of serum blood urea and creatinine were significantly ($p<0.001$) increased in PCM treated rats as compare to normal control whereas serum uric acid and total protein were significantly ($p<0.001$) decreased in PCM treated rats as compare with normal control. The administration of CZAE (100, 200 mg/kg, bw, p.o) significantly ($p<0.01$, $p<0.001$) decreased serum urea, and creatinine levels whereas serum uric acid levels and total protein were significantly ($p<0.01$, $p<0.001$) decreased by

treatment of CZAE (100, 200 mg/kg b.w, p.o) as compared with PCM treated rats. When compared with PCM rats the serum blood urea and creatinine were significantly (p<0.001) decreased in silymarin (100 mg/kg, b.w, p.o) treated rats whereas the levels of serum uric acid and total protein significantly (p<0.001) increased show in Table1.

Table 1: Effect of CZAE on serum urea, creatinine, uric acid and total protein.

Goups	Urea(mg/dl)	Creatinine(mg/dl)	Uric acid(mg/dl)	Total Protein(mg/dl)
Normal control	34.1±1.43	0.79±0.01	3.01±0.02	7.79±0.01
PCM Toxic	78.5±0.96$^{\$\$}$	1.88±0.01$^{\$\$}$	1.04±0.02$^{\$\$}$	5.87±0.02$^{\$\$}$
CZAE(100 mg/kg)+PCM	71.9±1.4*	1.79±0.01*	1.12±0.01*	5.9±0.01*
CZAE(200 mg/kg)+PCM	64.00+1.8***	1.59±0.04***	1.25±0.01***	5.17±0.01***
Silymarin(100mg/kg)+PCM	51.1±1.04***	1.26±0.02***	2.34±0.02***	6.65±0.03***

All values were expressed as mean ± SEM for six rats in each group. $$ p < 0.001 as compared to control groups, ***p < 0.001, *p < 0.05, **p < 0.01 as compared to APAP groups

Histopathological studies

The Histopathological examination revealed that the normal control show normal renal tubule and glomeruli (figure: 1A). However the rats treated with PCM alone show severe dilation of renal tubule, infiltration of bowman space and damage of podocyte (figure: 1B). In contrast rat treated with PCM and CZAE (100, 200 mg/kg b.w) show neutrofil infiltration in glomeruli and less bowman space (figure: 1C), mild dilation, very less infiltration in bowman space and mild podocyte damage (figure: 1D) compared to PCM alone. The rats treated with silymarin show normal distal & proximal tubules, glomeruli and bowman space (figure: 1E).

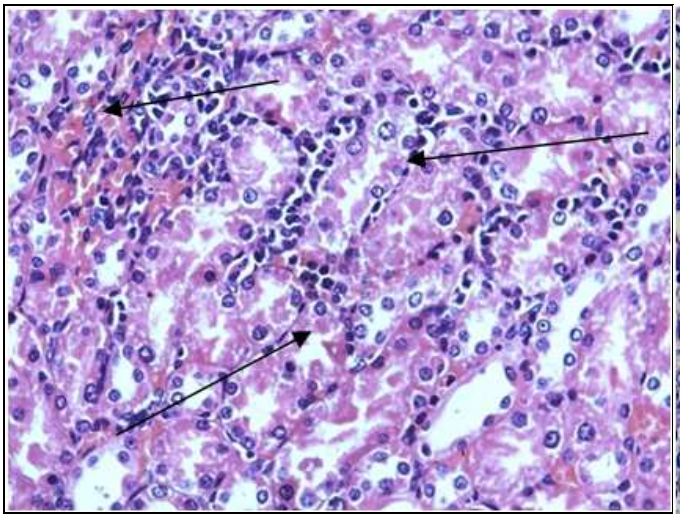

Fig 1A: (Control (1%CMC) 1ml/kg,bw)

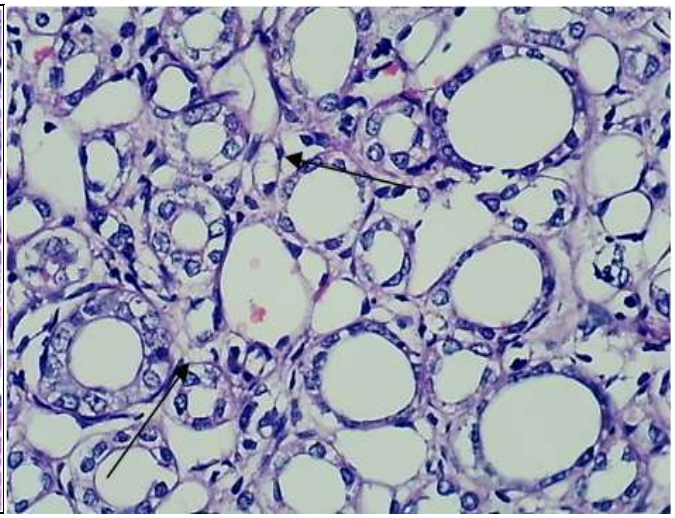

Fig 1B: (PCM 750 mg/kg, bw)

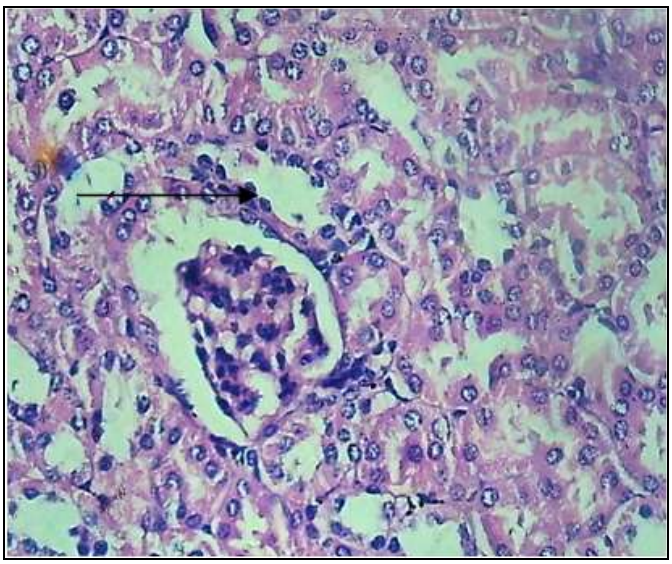

Fig 1C: (CZAE 100 mg/kg +PCM)

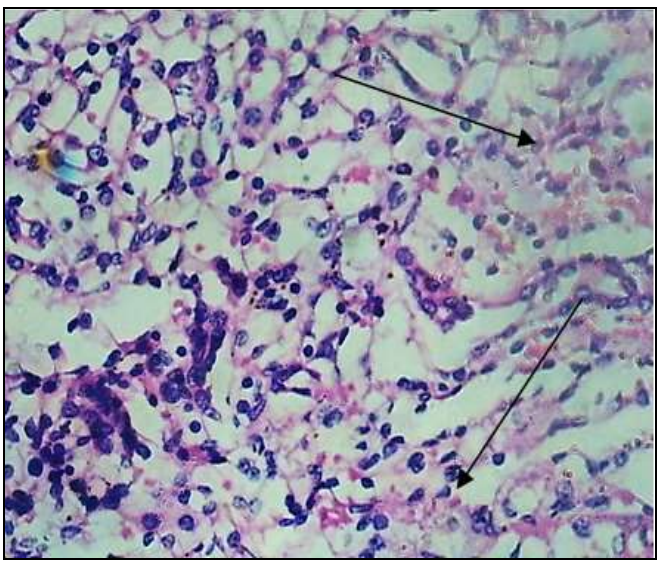

Fig 1D: (CZAE 200 mg/kg+PCM)

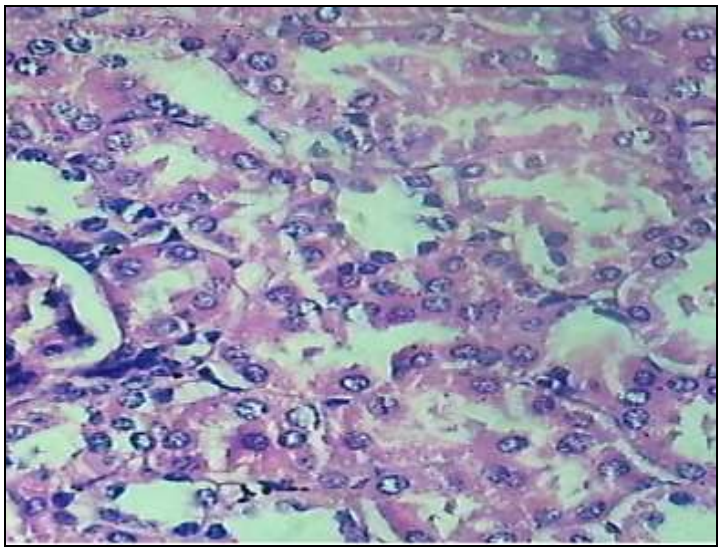

Fig 1E: (Silymarin+ PCM)

Fig 1: Histopathology of kidney showing normal PCT, DCT and Glumeruli (1A), PCM induced dilation of tubules , infiltration in bowman space , damage of podocytes, and infiltration of cells (1B), less neutrofil infiltration and less bowman space (1C), mild dilation, very less infiltration and mild podocyte damage (1D), Normal distal and proximal tubules, Bowman space, Glomeruli and infiltration of cells (1E)

Discussion

PCM is widely used analgesic and antipyretic medicine [26], large dose is generally related to hepatotoxicity and nephrotoxicity [27]. PCM is metabolized through glucuronidation and sulfation reaction primarily occurring in the liver which result in the water soluble metabolites that excreted via kidney [28]. Nephrotoxicity due to PCM result from the toxic effect of its highly reactive intermediate metabolite NAPQI. The protein (specifically selenium binding protein and glutamine synthetase) in the S3 segment of the proximal tubule are arylates by NAPQI, initiating cell death of renal tubules [26]. Glutathione depletion and lipid peroxidation also are considered as important factors of nephrotoxicity that causes due to PCM overdose. Increased concentration of serum urea and creatinine are consider for investigating drug induced nephrotoxicity in human and rats [29]. In present studies we demonstrate that administration of nephrotoxic dose of PCM to rats result in significant increased in urea and creatinine levesl where as significant decreased in uric acid and total protein in PCM treated rats as compared to normol control rats. Theses result are agreement with that was observed and reported that an increased in urea and creatinine in rats after PCM adminidtration [30]. Moreever Elkarib[2] reported that an increased in urea and creatinine levels after PCM administration in rats. However administration of CZAE significantly decreased in serun urea and creatinine levels where as significantly increased in uric acid and total protein as compared to PCM treated rats. When compare with PCM treated group the serum urea and creatinine significantly (p<0.001) decreased in silymarin treated group whereas uric acid and total protein were significantly (p<0.001) increased (Table:1). The significant decreases in serum protein levels in PCM treated rats could be explained by excess secretion in the urine. An earlier reported that in decreased in total protein after PCM administration[31]. The decrease in protein levels in the serum may be attributed to the dysfunction of the proximal convoluted tubule because proteins are completely absorbed from tubules under normal conditions. Total protein levels depressed in hepatotoxic condition due to faulty protein biosynthesis in liver, the PCM induced nephrotoxic condition in rats also causes similar situation and that the treatment of CZAE caused return to normal levels, which justifying its nephroprotective activity [32].

The biochemical results were also confirmed by the histopathological finding which showed damaging of the glomeruli and surrounding Bowman capsule and mild swollen tubules (Figure: 1B). Previous finding reported that administration of PCM (750 mg/kg) affect the proximal tubules, glomerulus or more distal part of the nephron [33, 34]. CZAE administration could protect the kidney injuries may be due to presence of active principal containing in it. The preliminary phytochemical studies showed the presence of different phytochemical such as alkaloid, flavonoids, saponin and triterpenoid which may be responsible for its protective activity. Several studies proved the nephroprotective effect of alkaloid, saponin and triterpenoids [24].

Conclusion

In conclusion the PCM treated resulted in impairment of renal functional maker and histopathological changes in kidney of rats. Treatment with CZAE lead to significant restored biochemical parameter in paracetamol treated rats. This beneficial effect of *Cinnamomum zeylanicum* bark may be atributed to the amelioration of the renal functional. Our finding suggest that *Cinnamomum zeylanicum* bark might be potential nephroprotective agent against acetaminophen induced nephrotoxicity.

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