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Research Article

Effects of *Andrographis paniculata* on Carbon Tetrachloride (CCl_4)-Mediated Renal Oxidative Damage in Rats

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ABSTRACT

A herbal medicinal plant known as *Andrographis paniculata*, or "*hempedu bumi*," is recognised for its numerous medicinal properties and role in promoting community health. Despite its widespread use, the potential nephroprotective effects and underlying mechanism of action of *Andrographis paniculata* remain unexplored. To address this gap, the present study aimed to investigate the nephroprotective effects of *Andrographis paniculata* against renal oxidative damage induced by carbon tetrachloride (CCl_4) in rats. Sprague-Dawley rats were pre-treated with *Andrographis paniculata* extract via gavage (100, 200, and 300 mg/kg b.w., respectively) once daily for 14 days, followed by two doses of CCl_4 (1.2 ml/kg b.w.) on the 13th and 14th days. After two weeks, rats were sacrificed, and a nephroprotective analysis was performed. CCl_4 administration at a dose of 1.2 ml/kg body weight resulted in oxidative stress in the renal system, as evidenced by elevated lipid peroxidation levels (TBARS). This oxidative stress was accompanied by a significant decrease in the activities of antioxidant enzymes and a depletion in the levels of reduced glutathione ($p<0.05$). Histopathological examination confirmed the impairment of renal function. *Andrographis paniculata* significantly mitigated the majority of these alterations. Based on our research, the nephroprotective advantages of *Andrographis paniculata* can be attributed to its ability to act as an antioxidant and scavenge free radicals.

Keywords: Oxidative stress; *A. paniculate*; nephrotoxicity; carbon tetrachloride

Introduction

Reactive oxygen species (ROS), including O2•-, H2O2, and •OH, are continuously produced within the human body due to exposure to exogenous environmental chemicals and various endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer. Under normal circumstances, the body's antioxidant defence system efficiently detoxifies these ROS. However, when

there is excessive production of ROS or an inadequate antioxidant defence, oxidative stress occurs. Oxidative stress is a condition that occurs when there is an imbalance between the production of free radicals and the ability of the body to neutralize them with antioxidants. Free radicals are unstable molecules that can cause damage to the cells and tissues of the body (Aranda-Rivera et al., 2022). This oxidative stress leads to the attack of ROS on various biomolecules such as proteins, lipids, mitochondria, lipoproteins, and DNA, ultimately resulting in oxidative damage (Hajam et al., 2022). Oxidative damage is a term that refers to the harmful effects of free radicals on the cells and tissues of the body. Oxidative damage can affect various components of the cell, such as DNA, proteins, lipids, and membranes. Oxidative damage can lead to mutations, inflammation, cell death, and tissue dysfunction. Oxidative damage can be prevented or reduced by antioxidants, which are substances that can donate an electron to free radicals and neutralize them. The body can produce antioxidants or obtain them from dietary sources, such as fruits and vegetables. Antioxidants can also repair some of the damage caused by free radicals (Sies, 2020; Aranda-Rivera et al., 2022). Oxidative stress contributes to the development of several chronic human diseases, including diabetes mellitus (Zhang et al., 2020; Darenskaya et al., 2021), cancer (Klaunig, 2018; Jelic et al., 2021), atherosclerosis (Poznyak et al., 2020; Batty et al., 2022), arthritis (Phull et al., 2018; Kaur et al., 2021), neurodegenerative diseases (Singh et al., 2019; Teleanu et al., 2022), and the ageing process (Romano et al., 2010; Martemucci et al., 2022).

Carbon tetrachloride (CCl_4) is a well-established hepatotoxicity model that has been extensively studied (Clemens et al., 2019). However, it is important to note that CCl_4 can also induce toxicity in other organs, such as the kidney, heart, and brain (Unsal et al., 2020). Due to its toxicity, CCl_4 is commonly employed by researchers as a screening model to evaluate the protective effects of natural products or compounds against tissue damage caused by CCl_4. In the liver, CCl_4 is metabolised by the P450 enzyme system, forming a highly reactive trichloromethyl radical (CCl3•). This radical initiate lipid peroxidation of the cytoplasmic membrane phospholipids leads to physiological and morphological changes in hepatocytes, ultimately causing liver injury (Arroyave-Ospina et al., 2021; Vun-Sang et al., 2022).

Andrographis paniculata, a member of the Acanthaceae family, is commonly referred to as the "King of the Bitters" or "*hempedu bumi*." It has been traditionally used to treat various ailments, including respiratory infections, fever, digestive disorders, and is a general immune booster. It is often used in

traditional medicine systems like Ayurveda and Traditional Chinese Medicine (TCM). The active compounds in *A. paniculata* are diterpene lactones known as andrographolides (Akowuah et al., 2008, 2009). This medicinal herb possesses substantial therapeutic potential for managing a range of illnesses and promoting general well-being, as indicated by multiple studies (Zaridah et al., 2001; Singha et al., 2003, 2007; Reyes et al., 2006; Sheeja et al., 2007; Hossain et al., 2007; Neogy et al., 2008; Akowuah et al., 2008, 2009). In traditional medicine, it is used for treating infectious fevers, colic pain, and liver diseases (Singha et al., 2007; Akowuah et al., 2008, 2009). Numerous studies have explored the biological and pharmacological effects of *A. paniculata*, including antimicrobial properties (Singha et al., 2003), anti-filarial activity (Zaridah et al., 2001), antiangiogenic effects (Sheeja et al., 2007), antidiabetic potential (Reyes et al., 2006; Hossain et al., 2007; Dandu & Inamdar, 2009), antioxidant properties (Neogy et al., 2008; Akowuah et al., 2008, 2009), as well as anticancer and antitumor activities (Trivedi & Rawal, 1998; Kumar et al., 2004; Verma & Vinayak, 2007). However, to date, no investigations have been conducted to explore the nephroprotective activity of *A. paniculata* and its underlying mechanism of action. Therefore, this study aimed to assess the nephroprotective effects of *A. paniculata* and elucidate its mechanism of action against CCl_4-induced oxidative renal damage.

Materials and Methods

Chemicals and Reagents

Oxidizied glutathione, thiobarbituric acid, tricholoacetic acid, dithionitrobenzoic acid, 1-chloro 2,4 dinitrobenzene, hydrogen peroxide, folin-Ciocalteu reagent (FCR), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sodium nitrate ($NaNO_3$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), 2, 2-diphenyl-2-picrylhadrazyl (DPPH), gallic acid, catechol, and ferric chloride (FeCl3) were purchased from Sigma Aldrich (St. Louis, MO, USA). Alcohol, acid alcohol, blue buffer, eosin, haema-toxylin, xylene and DPX mounting medium for histological assessment were purchased from Leica Biosystem (Wetzlar, Germany).

Plant Material

A. paniculata was obtained from Papar (5.48943°N, 115.80992°E), Sabah, and its identification and authentication were conducted by Mr Jonny Gisil, a botanist from the Institute for Tropical Biology and Conservation (ITBC) at Universiti Malaysia Sabah (UMS). The plant sample (voucher no: KPH003) was deposited at ITBC Herbarium, UMS, for future reference.

Preparation of Plant Extract

The aerial parts of *A. paniculata* were thoroughly rinsed with tap water before being dried in an oven at 35°C to 40°C. Once dried, the plants were finely ground using a blender to obtain a powdered form. For the extraction process, 5.0 g of the dried powder was mixed with 100 ml of 80% ethanol and allowed to extract for 4 hours at 40°C. The resulting mixture was then centrifugated and filtered through Whatman paper No. 1 (Whatman, Maidstone, Kent, UK). The filtrate was concentrated using Rotavapour® and then lyophilised. To ensure preservation, the lyophilised powder was stored in a dark bottle at a temperature of -80°C until it was ready for use.

Total Phenolic Content

The Folin-Ciocalteau method (Velioglu et al., 1998) was used to determine total phenolic content. A mixture consisting of Folin-Ciocalteau's reagent, 1.5 ml (1:10), and plant extract 200 µl was prepared. The mixture was thoroughly mixed and wait for 5 minutes at room temperature. Next, sodium carbonate (Na_2CO_3) 1.5 ml (60 g/l) was added to the mixture. Following an incubation period of 90 minutes in the dark at room temperature, the absorbance was measured at 725 nm against a blank using a spectrophotometer. Gallic acid served as the standard for determining the phenol content via the Folin-Ciocalteu method (Velioglu et al., 1998). The results were expressed in gallic acid equivalents (GAE) per gram of the sample. All measurements were performed in triplicate, and the mean values and standard deviations were calculated.

2, 2-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Assay

The ability to scavenge the stable free radical, DPPH was assessed (Hatano et al., 1988). All extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/ml. Different concentrations of the plant extracts (0.3mL) were mixed with 2.7 mL of an ethanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was vigorously shaken and allowed to stand in the dark for 60 minutes. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm using a spectrophotometer (model 4001/4). The radical scavenging activity (RSA) was expressed as a percentage of DPPH decolourisation using the equation: % RSA = ((Acontrol × Asample)/Acontrol) × 100, where Acontrol is the absorbance of the solution without the extract and Asample is the absorbance of the solution with extract at different concentrations. The extract concentration that caused 50% inhibition (EC50) was calculated from the plot of RSA percentage versus extract

concentration. Ascorbic acid was used as the standard, and triplicate measurements were conducted.

Animals

Animal experiments were conducted in compliance with standard ethical principles, adhering to university regulations and federal laws regarding animal experimentation (Animal Ethics Committee (AEC): UMS/IP7.5/M3/4-2012). Adult male Sprague-Dawley rats, aged 8-12 weeks and weighing 120-150 g were obtained from the Animal House facility at the Health Campus of Universiti Sains Malaysia. The rats were housed in a controlled environment with a temperature of 25±2°C, a 12-hour light-dark cycle, and allowed to acclimatise for one week before the experiments. Food and water were provided *ad libitum* throughout the study. The freeze-dried extract was dissolved in distilled water daily before oral administration using a gavage method.

Experimental Protocol

To investigate the effects of *A. paniculata* extract on CCl_4-induced oxidative renal damage, antioxidant enzymes, and histopathological changes, a total of thirty adult male Sprague-Dawley rats (8-12 weeks old) weighing 120-150 g were randomly divided into five groups, with six rats in each group. Group I served as the control and received saline. Group II was administered two doses of CCl_4 (1.2 ml/kg b.w.) dissolved in a 1:1 corn oil solution via oral gavage on the 13^{th} and 14^{th} days. Groups III, IV, and V were pre-treated with *A. paniculata* extract orally via gavage at selected doses of 100 mg/kg b.w., 200 mg/kg b.w., and 300 mg/kg b.w., respectively, for 14 days, followed by two doses of CCl_4 (1.2 ml/kg b.w.) on the 13^{th} and 14^{th} days. All animals were euthanised 24 hours after the last dose of CCl_4 or saline within one hour. Renal tissues were promptly collected, carefully cleaned to remove extraneous material, and immediately perfused with ice-cold saline solution (0.85% w/v sodium chloride). These samples were then used for biochemical and histopathological analyses to evaluate renal function impairment. Doses of *A. paniculata* were selected based on previously published data (Koh et al., 2011).

Preparation of Renal Post-Mitochondrial Supernatant (PMS)

We followed the standard procedure described by Mohandas et al. (1984) for all biochemical estimations to prepare tissue fractionations.

Determination of Renal Lipid Peroxidation

To assess lipid peroxidation in renal PMS, we employed a modified version of the method described by Buege & Aust (1978), wherein the rate of TBARS

(malondialdehyde equivalents) production was measured. The absorbance at 535 nm was measured using a spectrophotometer. The results were expressed as nmol MDA formed per gram of tissue, employing a molar extinction coefficient of 1.56×10^5 M-1cm-1.

Determination of Renal Glutathione Reduced

The method described by Jollow et al. (1974) was utilised to determine the concentration of reduced glutathione in the kidney. The development of yellow colour was observed, and the absorbance was immediately read at 412 nm using a spectrophotometer. The results were expressed as µmol of reduced GSH per gram of tissue, employing a molar extinction coefficient of 13.6×10^3 M-1cm-1.

Assays of Renal Antioxidant Enzymes

a) Determination of Catalase (CAT) activity

The method of Claiborne (1985) was used to measure the catalase (CAT) activity. The reaction mixture consisted of 0.01 ml PMS (10% w/v), 0.99 ml phosphate buffer (0.1 M, pH 7.4), and 1.0 ml H_2O_2 (0.019 M). The absorbance change was monitored at 240 nm every 30 seconds for 3 minutes. The result was expressed as the amount of H_2O_2 consumed per minute per mg protein, using the molar extinction coefficient of 6.4 × 10/M/cm.

b) Determination of glutathione peroxidase (GPx) activity

The activity of glutathione peroxidase (GPx) was determined by following the method of Mohandas et al. (1984). The reaction solution consisted of 0.01 ml PMS (10% w/v), 1.58 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml EDTA (0.5 mM, pH 8.0), 0.1 ml sodium azide (1.0 mM), 0.1 ml GSH (1.0 mM), 0.1 ml NADPH (0.1 mM), and 0.01 ml hydrogen peroxide (30%) in a total volume of 2.0 ml. The reduction of NADPH at 340 nm was measured for 3 minutes at 30 seconds interval. Enzyme activity was calculated as the amount of NADPH consumed per minute per mg protein, using the molar extinction coefficient 6.22×10^3 /M/cm.

c) Determination of glutathione reductase (GR) activity

The method of Carlberg & Mannervik (1975) was used to determine the activity of glutathione reductase (GR). The assay mixture contained 0.025 ml PMS (10% w/v), 1.725 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1.0 mM), and 0.1 ml NADPH (0.1 mM). The reduction of NADPH at 340 nm was measured for 3 minutes at 30 seconds interval using a spectrophotometer (model 4001/4) and calculated as the amount of NADPH consumed per minute per mg protein, using the molar extinction coefficient 6.22×10^3 /M/cm.

d) Determination of glutathione S-transferase (GST) activity

The activity of glutathione S-transferase (GST) was measured following the method of Habig et al. (1974) using 1-chloro 2,4 dinitrobenzene as a substrate. The reaction mixture consisted of 0.1 ml PMS (10% w/v), 1.75 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml GSH (1.0 mM), and 0.05 ml 1-chloro-2,4-dinitrobenzene (CDNB) (1.0 mM) in a total volume of 2.0 ml. The absorbance change was monitored at 340 nm for 3 minutes at 30 second intervals. The enzyme activity was calculated as the amount of CDNB conjugate formed per minute per mg protein, using the molar extinction coefficient of 9.6×10^3 /M/cm.

e) Determination of NAD(P)H: quinone oxidoreductase (QR) activity

The activity of NAD(P)H: quinone oxidoreductase (QR) was determined by following the method of Benson et al. (1980) as described by Iqbal et al. (1999). The reaction solution contained 0.01 ml PMS (10% w/v), 1.1 ml Tris-HCl buffer (25 mM, pH 7.4), 0.7 ml bovine serum albumin (1 mg/ml), 0.02 ml Tween-20 (1% w/v), 0.1 ml FAD (0.15 mM), 0.02 ml NADPH (30 mM), and 0.05 ml 2,6-dichlorophenolindophenol (2.4 mM) in a total volume of 2.0 ml. The reduction of 2,6-dichlorophenolindophenol was measured at 600 nm for 3 minutes at 30 second intervals. The activity was expressed as the amount of 2,6-dichlorophenolindophenol consumed per minute per mg protein, using the molar extinction coefficient of 2.1×10^4 /M/cm.

f) Determination of glucose-6-phosphate dehydrogenase (G6PD) activity

The method of Zaheer et al. (1965) was used to measure the activity of glucose-6 phosphate dehydrogenase (G6PD). The total volume of 3.0 ml contained 0.1 ml PMS (10% w/v), 0.5 ml Tris-HCl buffer (0.2 M, pH 7.6), 0.05 ml NADP (0.1 mM), 0.05 ml glucose-6- phosphatase (0.8 mM), 0.25 ml MgCl2 (8 mM), and 2.05 ml distilled water. The absorbance change was monitored at 340 nm for 3 minutes at 30 second intervals. The activity was expressed as the amount of NADP consumed per minute per mg protein, using the molar extinction coefficient of 6.22×10^3 /M/cm.

g) Determination of gamma-glutamyl transpeptidase (γ-GGT) activity

The method of Orlowski & Meister (1973) was used to determine the activity of gamma-Glutamyl transpeptidase (γ-GGT). A total of 0.2 ml PMS (10% w/v) was added to 0.8 ml of substrate mixture containing glycyl glycine (40 mM), magnesium chloride (11 mM), and L-gamma-glutamyl-p-nitroanilide (4 mM) in

Tris-HCl buffer (185 mM, pH 8.25). The mixture was incubated for 10 minutes at 37°C in water bath. Then, 1.0 ml of trichloro acetic acid (25% w/v) was added to each tube and centrifuged at 8000 rpm for 20 minutes. The absorbance was measured at 405 nm by a spectrophotometer (model 4001/4). The activity was expressed as the amount of p-nitroanaline released per minute per mg protein, using the molar extinction coefficient of 1.74×10^3 / M/cm.

Renal Histopathological Examination

A section of the kidney was excised and immersed in a 10% phosphate-buffered formaldehyde solution for fixation. After fixation, the kidney tissues were embedded in paraffin wax, and thin sections measuring 4 µm thick were obtained. These sections were then stained with haematoxylin-eosin (H&E) using conventional methods. The stained kidney sections were converted into permanent slides and mounted. The slides were examined using a microscope equipped with photographic capabilities, and photomicrographs were captured.

Determination of Protein

Total protein content was determined using a protein assay kit (BCA1) based on the bicinchoninic acid method. Bovine serum albumin was employed as the standard for calibration purposes.

Statistical Analysis

The results were presented as mean ± SD. Statistical comparisons were conducted using one-way analysis of variance (ANOVA), and the homogeneity of variance was assessed using Levene's test. Data were analysed using the SPSS software (Release 17.0, SPSS). A significance level of $p < 0.05$ indicated a significant difference. The EC_{50} values were determined by employing non-linear regression analysis of the dose-response curve using GraphPad Prism 5.

Results

Total Phenolic

The total phenolic content of *A. paniculata* ethanolic extract was 72.39 ± 1.75 mg/g, expressed in gallic acid equivalent (GAE)/mg of extract.

Effect of A. paniculata on DPPH Radical Scavenging

As shown in Table 1, the 50 % scavenge of the DPPH radical (EC_{50} values) were as follows: ascorbic acid (10.06 ± 1.42) and *A. paniculata* (583.60 ± 4.25).

Table 1. DPPH free radical scavenging activity of ascorbic acid and *A. paniculata*.

Plant	EC_{50} (µg/ml)
Ascorbic acid	9.06 ± 1.89
A. paniculata	683.40 ± 4.55

Effects of A. paniculata Extract on Body Weight and Renal Index

The rats' body weight and renal index did not exhibit any notable alteration (Data not presented).

Effects of A. paniculata Extract on Renal Lipid Peroxidation and GSH

According to Table 2, renal malondialdehyde (MDA) levels showed a significant increase ($p< 0.05$) of 37% in CCl_4 challenge rats (39.85 ± 1.10 nmol MDA/g tissue) compared to control rats (29.15 ± 1.30 nmol MDA/g tissue). Additionally, renal GSH levels exhibited a significant decrease ($p< 0.05$) of 10% in CCl_4 challenge rats (7.32 ± 0.26 µmol reduced GSH/g tissue) compared to control rats (8.10 ± 0.34 µmol reduced GSH/g tissue). Pre-administration of *A. paniculata e*xtract showed a significant improvement in renal MDA and GSH levels.

Table 2. Effects of *Andrographis paniculata* extract on renal glutathione and lipid peroxidation of rats intoxicated with carbon tetrachloride.

Groups (n=6)	GSH (µmol reduced GSH/g tissue)	LPO (nmol MDA/g tissue)
Control	8.10 ± 0.34	29.15 ± 1.30
Carbon tetrachloride (CCl_4)	7.32 ± 0.26 *	39.85 ± 1.10 *
A. paniculata 100mg + CCl_4	7.46 ± 0.29	39.75 ± 1.77
A. paniculata 200mg + CCl_4	7.57 ± 0.22	39.34 ± 1.25
A. paniculata 300mg + CCl_4	7.54 ± 0.17	38.84 ± 1.30

Results are expressed as mean ± SD (n = 6).
* indicates significance at $p < 0.05$ compared to control group.
Abbreviation: CCl_4 (Carbon tetrachloride), GSH (Glutathione reduced), LPO (Lipid peroxidation), MDA (Malondialdehyde).

Effects of A. paniculata Extract on Renal Antioxidant Enzyme Activities

Table 3 displays the impact of *A. paniculata* extract on renal antioxidant enzyme activities. The administration of CCl_4 resulted in a significant decrease in the renal activities of CAT (31%), GPx (25%), QR (36%), GST (35%), GR (23%), and G6PD (33%). Notably, a significant increase in renal antioxidant enzyme activities was observed following a 14-day pretreatment of animals with *A. paniculata* extract. However, no significant changes in renal γ-GGT activity were observed with the administration of CCl_4 and pretreatment with *A. paniculata* extract.

Table 3. Effects of *Andrographis paniculata* extract on renal antioxidant profile of rats intoxicated with carbon tetrachloride.

Groups	CAT (µmol H_2O_2/min/mg protein)	GPx (nmol NADPH oxidised/min/ mg protein)	QR (nmol 2,6-DCP reduced/min/ mg protein)	GST (nmol CDNB conjugate formed/min/m g protein)	GR (nmol NADPH oxidised/min/ mg protein)	G6PD (nmol NADP reduced/min/ mg protein)	γ-GGT (nmol p-nitroanaline released/min/ mg protein)
Control	1.40 ± 0.04	94.93 ± 5.97	39.32 ± 2.95	56.46 ± 1.62	112.18 ± 4.30	4.67 ± 0.17	15.67 ± 0.70
Carbon tetrachloride (CCl_4)	0.97 ± 0.05 *	71.28 ± 1.74 *	25.30 ± 1.28 *	36.66 ± 1.85 *	85.82 ± 3.36 *	3.11 ± 0.15 *	16.00 ± 0.41
A. paniculata 100mg + CCl_4	0.97 ± 0.07	72.48 ± 4.15	25.69 ± 1.86	37.58 ± 1.34	86.76 ± 4.06	3.14 ± 0.11	15.47 ± 0.50
A. paniculata 200mg + CCl_4	1.00 ± 0.03	72.93 ± 3.87	27.58 ± 3.84	39.20 ± 1.22	87.87 ± 6.22	3.26 ± 0.08	15.52 ± 0.34
A. paniculata 300mg + CCl_4	1.03 ± 0.03	74.79 ± 6.23	27.80 ± 3.09	39.33 ± 2.27	91.87 ± 4.57	3.33 ± 0.21	16.10 ± 0.35

Results are expressed as mean ± SD (n = 6).
*indicates significance at $p < 0.05$ compared to control group.
Abbreviations: CAT (Catalase), GPx (Glutathione peroxidase), QR (Quinone oxidoreductase), GST (Glutathione S-transferase (GST), GR (Glutathione reductase), G6PD (Glucose-6 phosphate dehydrogenase), γ-GGT (Gamma-Glutamyl transpeptidase).

Effects of A. paniculata Extract on Renal Histopathological Changes

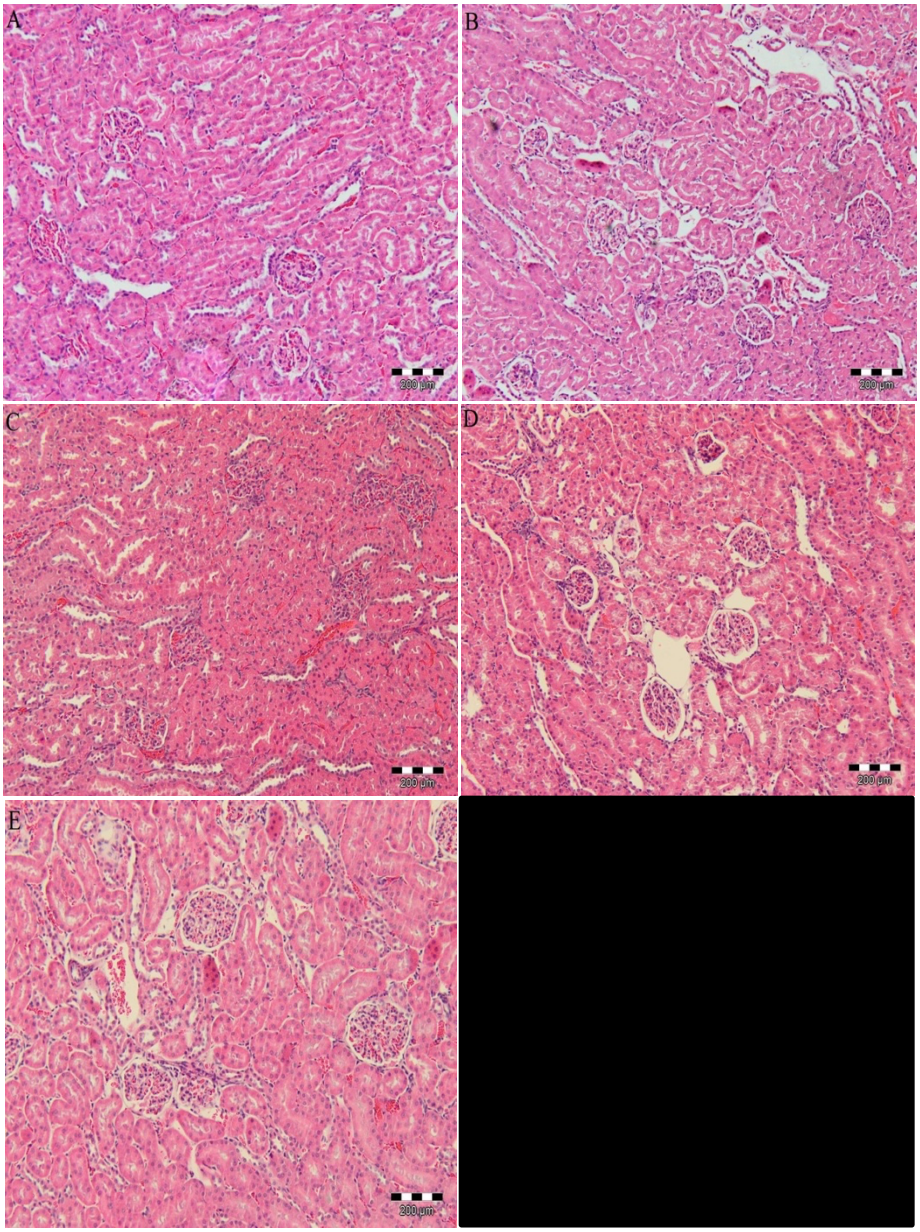

Figure 1. Histopathological sections of kidney. (A) Control; (B) Carbon tetrachloride, CCl_4; (C) *A. paniculata* 100 mg/kg b.w. and CCl_4; (D) *A. paniculata* 200 mg/kg b.w. and CCl_4; (E) *A. paniculata* 300 mg/kg b.w. and CCl_4. Sections stained with Hematoxylin and eosin. Magnifications 100X.

The renal section showed normal architecture of the kidney section with a normal tubular brush border, glomerulus and Bowman's capsule (**Figure 1A**). In contrast, marked histological changes were observed in the CCl_4-treated group (**Figure 1B**). Some glomeruli showed dilatation of Bowman's space and glomerular atrophy. In addition, the tubular was dilated, and brush borders were lost in proximal convoluted tubules. Capillary congestion was also observed in the peritubular vessels. However, pretreatment with *A. paniculata* extract markedly ameliorated the kidney morphology and architecture (**Figures 1C, D and E**). The kidney lesions such as necrosis, glomerular atrophy, tubular dilatation, loss of brush border and congestion were reduced in the pretreatment of rats with *A. paniculata* extract.

Discussion

Our present study was conducted to evaluate the *in vivo* protection of *A. paniculata* against CCl_4- induced nephrotoxicity. Our study has revealed that CCl_4 caused toxicity in renal tissues. CCl_4-induced toxicity is a well-known experimental model. In addition to hepatotoxicity (Clemens et al., 2019; Baig & Ahmad Khan, 2023), it was also reported to cause toxicity in other tissues such as the kidney, heart, brain, and testis (Adewole et al., 2007; Jayakumar et al., 2008; Khan & Ahmed, 2009; Khan et al., 2009, 2010). Previous studies have reported abnormal renal function with increased blood urea nitrogen and creatinine (Adewole et al., 2007; Khan et al., 2009, 2010). The elevation of blood urea nitrogen and creatinine indicates the possible indicator of kidney damage. However, the creatinine level is not necessarily elevated until at least half of the nephrons are damaged (Khan et al., 2009). We failed to observe the elevation of blood urea nitrogen and creatinine in injecting CCl_4 rats (Data not shown). This observation is in good harmony with other studies where there were no alterations in blood urea nitrogen and creatinine levels (Tirkey et al., 2005; Ogeturk et al., 2005; Manna et al., 2006). Ogeturk et al. (2005) reported no significant increase in blood urea nitrogen and creatinine, while damage to renal cortical and subcortical areas was observed in the histopathological examination. This might be due to insufficient exposure to CCl_4. Eventually, it demonstrated no elevation of blood urea nitrogen and creatinine levels. Khan et al. (2009) reported the elevation of blood urea nitrogen and creatinine after exposure to CCl_4 for four months.

CCl_4 undergoes biotransformation by P450 enzymes such as cytochrome CYP2E1, CYP2B2, and possibly CYP3A, resulting in the formation of highly reactive metabolites, namely CCl3• and CCl3OO• (Recknagel et al., 1989; Weber et al.,

2003). These metabolites attack polyunsaturated fatty acids, triggering lipid peroxidation and the generation of malondialdehyde (MDA) within liver cells (Recknagel et al., 1989; Weber et al., 2003). MDA is known to play a significant role in the development of various diseases (Recknagel et al., 1989; Weber et al., 2003). It indicates tissue damage and is considered the end product of lipid peroxidation (Ohkawa et al., 1979; Vaca et al., 1988). Our study showed a significant increase in renal MDA levels in rats treated with CCl_4.

Additionally, GSH, an essential non-enzymatic antioxidant involved in detoxifying reactive, toxic metabolites within cells, plays a crucial role. Depletion of GSH can initiate necrosis (Yang et al., 2008). Renal GSH level was decreased in CCl_4-treated rats in the current study, parallel to previous studies (Tirkey et al., 2005; Ogeturk et al., 2005; Manna et al., 2006; Adewole et al., 2007; Jayakumar et al., 2008; Khan et al., 2009, 2010). The decline of renal GSH level might be due to the increased utilisation of cells to scavenge the free radicals caused by CCl_4 (Jollow et al., 1973). Renal toxicity was observed by the elevation of MDA and reduced GSH in the present study. Pretreatment of rats with *A. paniculata* extract revealed significant amelioration of renal MDA and reduced GSH levels, possibly due to its antioxidative activity (Neogy et al., 2008; Akowuah et al., 2008, 2009). The mechanism behind the antioxidative property involves the action of antioxidants, which are molecules capable of neutralizing free radicals and preventing or minimizing the damage they can cause.

Cellular antioxidant enzymes play a crucial role in defending against oxidative stress. CAT and GPx are responsible for breaking down harmful hydrogen peroxide (H_2O_2) into water. The catalytic action of GPx leads to the oxidation of GSH to GSSG, which is then reduced back to GSH by GR. As the primary phase II enzyme, GST plays a vital role in detoxification (Srivastava & Shivanandappa, 2010). Its function involves facilitating the conjugation of GSH with xenobiotics and their reactive metabolites, producing water-soluble compounds (Yang et al., 2008). G6PD, on the other hand, is an enzyme bound to the cell membrane and is involved in eliminating lipid peroxides and toxic oxygen radicals. It initiates the initial steps of the pentose phosphate metabolic pathway (Shreve & Levy, 1977). QR, a flavoprotein, utilises either NADH or NADPH as a reducing cofactor and plays a vital role in detoxifying reactive quinones into less toxic hydroquinones, as described by Iqbal et al. (1999). The reducing process by QR prevents the interaction between semiquinone and oxygen molecules, thus bypassing the production of semiquinone. Additionally, the formation of endogenous catechol estrogen quinones leads to the generation of O2•- and their covalent binding to DNA, resulting in the formation of depurination adducts

and the induction of cancer (Ohkawa et al., 1979). Our study observed a decrease in renal antioxidant enzyme levels (CAT, GPx, GR, GST, and G6PD) in the group administered with CCl_4. The findings of our study align with previous studies, which demonstrated a depletion of antioxidant enzymes in CCl_4-induced oxidative renal damage (Güven & Yilmaz, 2005; Tirkey et al., 2005; Ogeturk et al., 2005; Manna et al., 2006; Adewole et al., 2007; Khan et al., 2009, 2010). We found that rats pre-treated with *A. paniculata* extract for 14 days exhibited a significant elevation in antioxidant enzyme levels. Moreover, there were no notable changes in the activity of renal γ-GGT in animals treated with CCl_4 or pre-treated with *A. paniculata* extract, similar to previous studies (Idéo et al., 1972).

Furthermore, the biochemistry results were complemented by histopathological examination. CCl_4-treated rats showed abnormality in renal tissues such as atrophy of glomerulus, dilatation of Bowman's capsule, dilatation of tubule, loss of brush border and tubular necrosis. It is in good agreement with previous studies (Tirkey et al., 2005; Ogeturk et al., 2005; Manna et al., 2006; Adewole et al., 2007; Khan et al., 2009, 2010; Xu et al., 2010). Pretreatment with *A. paniculata* extract showed amelioration in renal histopathological changes. Histopathological examination revealed the same finding as the biochemistry results.

Overall, the nephroprotective effect of *A. paniculata* extract was observed in the present study. This might be due to the biodistribution of the active antioxidative compounds. Affinity to specific proteins in tissue and the amount and interaction of the antioxidant affect the compound biodistribution. Renal tissues might have a relatively low intake of active compounds compared to other tissue, like the liver, where the hepatoprotective effect has been reported. The lower retention of active compounds eventually causes the lesser absorption of active compounds into cells and acts as an antioxidant. Thus, the transient interaction with kidney cells disallows the active compounds to be taken by the cells (Stahl et al., 2002; Manach et al., 2004; Porrini & Riso, 2008; Xu et al., 2010). The study conducted by Kwon et al. 2001 highlighted an intriguing finding regarding the accumulation of polyphenol isoflavone aglycones in the prostate gland compared to other tissues. Polyphenol isoflavones are a subgroup of phytochemicals found in various plant-based foods, particularly in legumes like soybeans. They have garnered considerable attention for their potential health benefits, including their role in reducing the risk of certain chronic diseases (Kwon et al., 2001).

Bioavailability is also playing an important factor in affecting the mechanism of action of active compounds. Phytochemicals like andrographolide, which is the primary active compound in A. paniculata, significantly contribute to its bioactivity. A deeper exploration of the specific mechanisms by which these phytochemicals exert their medicinal effects, such as their anti-inflammatory and immunomodulatory properties, can provide a more comprehensive understanding of A. paniculata's bioactivity. The bioavailability of active compounds through the intravenous route is typically very high but not 100%, as there is usually a small, albeit possibly insignificant, amount of absorption involved. For the oral route, the bioavailability is generally lower, unless the bioavailability (BA) of these active compounds is known. By considering both bioavailability and biodistribution, the higher concentration of liver accumulation and retention of antioxidants in the liver through the oral route causes the effective protection of antioxidants in the liver. On the other hand, the lower active compound accumulation and retention in the kidney subsequently cause lesser protection. Furthermore, the plasma concentration of active compounds is much higher throughout the intravenous route than the oral route. Thus, the active compounds are transported by systemic circulation to the kidneys and excreted through urine. Given this, the intravenous routes transport the active compounds to the kidney without passing through the liver; hence it might be more effective than the oral route in protecting the kidney against CCl_4-induced oxidative damage (Xu et al., 2010).

Conclusions

In viewing all the observations, a significant nephroprotective effect was found in the present study. Further investigations of biodistribution and bioavailability of active compounds are essential to further evaluate the nephroprotective effects of *A. paniculata* extract. In addition, other studies on different administration routes are also necessary to be conducted.

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Authors Contributions

Senty Vun Sang and Pei Hoon Koh experimented. Dr Mohammad Iqbal helped design the experiments and prepare the manuscript.

Conflict of Interests

The authors declare no conflict of interest.

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