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Renoprotective effect of *Capsicum annum* against ethanol-induced oxidative stress and renal apoptosis

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Abstract

The present study explored the ameliorative potency of aqueous extract of *Capsicum annum* (*AqCA*), against oxidative imbalance and renal toxicity induced by ethanol. Randomly grouped male Wistar rats ($n = 6$), were marked as ethanol-treated (2 g/kg bw, i.p.), CA_{125} (125 mg/kg bw, i.p.), CA_{250} (250 mg/kg bw, i.p.), ethanol pre-treated with *CA* (similar doses), and control (0.5 ml normal saline, i.p.), and treated for 30 consecutive days. Biochemical analysis of tissue and serum parameters was performed, along with histopathological and histochemical studies. Also, we performed TUNEL assay and western blotting for our experimental groups. Statistical analysis revealed significant ($p \leq .001$) alteration in the levels of antioxidant enzymes, serum urea, creatinine, pro-inflammatory cytokines, and cleaved caspases, along with histopathological alterations in the ethanol-treated group. Prior treatment with *AqCA* prevented ethanol-induced alterations in tissue and serum parameters. These findings indicate that the extract of *CA* can protect renal cells from ethanol-induced damage by inhibiting oxidative stress, inflammatory response, and apoptosis.

Practical applications

Chronic alcohol consumption is a major public health concern that leads to various diseases and social problems as well. It affects both the affluent and non-affluent society equally. Alcohol (ethanol) is a renowned hepato-toxicant and a well-documented risk factor for oxidative stress, with less known effect on the kidney. Thus, it is essential to investigate the effect of alcohol metabolism on the kidney to find a remedy to prevent it. The present investigation depicts the anti-oxidative and anti-inflammatory role of *Capsicum annum* against ethanol-induced renal damage. The outcome of this study can be utilized in the future for phytotherapeutic herbal drug formulation. Besides, the bioactive components identified in the study can be further explored by researchers or pharmaceutical corporates for potential therapeutic purpose against renal impairment.

Abbreviations: AI, apoptotic index; BUN, blood urea nitrogen; *CA*, *Capsicum annum* L.; Caspase, cysteine aspartic acid-specific protease; Cont, control; Cu-Zn SOD, copper zinc superoxide dismutase; DAPI, 4, 6-diamidino-2-phenylindole; EtOH, ethanol; G6PD, glucose 6-phosphate dehydrogenase; GPx, gluthanione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-s-transferase; H-E, hematoxylin-eosin; IL-6, interleukin-6; MDA, malondialdehyde; Mn-SOD, manganese-superoxide dismutase; PBS, phosphate buffer saline; ROS, reactive oxygen species; SODs, superoxide dismutases; TBARS, thiobarbituric acid reactive substance; TNF-α, tumor necrosis factor-α; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

KEYWORDS
apoptosis, *Capsicum annum*, ethanol, oxidative stress, renal toxicity

1 | INTRODUCTION

A report by the World Health Organization (WHO) states that the detrimental use of alcohol ranks among the top five risk factors for disease, disability, and death throughout the world (Lim et al., 2012; WHO, 2011). According to WHO, about 3.0 million deaths, or 5.3% of all global deaths in 2016, were due to excess consumption of alcohol (WHO, 2018).

With respect to chronic alcohol abuse, most public attention is paid to the issues of addiction and liver disease. Though alcohol is primarily metabolized in the liver, a part of it is also metabolized in extra-hepatic tissues like kidney, owing to the presence of ethanol metabolizing enzymes viz., alcohol dehydrogenase and cytochrome P450 2E1(CYP2E1) (Epstein & Lieber, 1988; Latchoumycandane, Nagy, & McIntyre, 2014). Moreover, alcohol abuse is also associated with an impaired immune system, osteoporosis, as well as various forms of cancer (Bagnardi, Blangiardo, La Vecchia, & Corrao, 2001; Ratna & Mandrekar, 2017). Although the consumption of alcohol in a moderate amount is linked to a decreased risk of several cardiovascular diseases, heavy alcohol use increases the risk of developing cardiomyopathy, hypertension, and arrhythmias (Piano, 2017). There is an evidence that ethanol produces alterations in the mitochondrial structure and function of several organs (Manzo-Avalos & Saavedra-Molina, 2010), including liver (Klein & Harmjanz, 1975), Kidney (Leal, Jorge, Joana, Maria, & Isabel, 2017), and heart (Regan, 1990), both in laboratory animals as well as in humans (Pachinger, Mao, Fauvel, & Bing, 1975).

Nature has provided us with an immense source of pharmacologically active molecules, which has been used over the decades for the treatment of several diseases. Secondary metabolites present in plants such as phenols, flavonoids, steroid glycosides, terpenoids, anthocyanin, thiols, and carotenoids act as free radical scavengers and prevent renal cells against oxidative damage (Molina-Jijón et al., 2011; Sabiu, O'Neill, & Ashafa, 2016). Hence, in recent decades herbal alternatives have stimulated a new wave of interest in ethnomedicine that would be invaluable especially because of its cost-effectiveness and minimal side effects (Desai, Patel, Devkar, Patel, & Ramachandran, 2012). In different countries, many herbs are used in folk medicine to treat drug or toxin-induced renal damage (Palani, Raja, Kumar, Soumya, & Kumar, 2009), but they lack scientific support. Therefore, it is crucial to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties.

In this context, *Capsicum annum* (*CA*), one of the commonly consumed spices, was chosen for the present study. Chinese and Native American traditional medicinal practice documents the use of this spice in healing various ailments. Accumulating evidence has shown that capsicum is enriched in antioxidant enzymes and exhibits a wide range of physiological and pharmacological properties (Govindarajan & Sathyanarayana, 1991) including cardio-protective, anti-neoplastic, and gastro-protective effect (Arora, Gill, Chauhan, & Rana, 2011; Basith, Cui, Hong, & Choi, 2016; Mózsik, Szolcsányi, & Rácz, 2005). Capsaicin, the active constituent of capsicum and a natural alkaloid, is also reported to protect the renal cells against cisplatin-induced nephrotoxicity, as well as diabetic nephropathy (Ríos-Silva et al., 2019; Shimeda et al., 2005). However, the renoprotective role of crude extract of *CA* against ethanol-induced nephrotoxicity has so far not been elucidated. So, the present study has been designed to evaluate the renoprotective efficacy of *CA* against ethanol-induced over-production of reactive oxygen species and tissue damage.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Ethanol was purchased from Merck, India. All other reagents used in the experiment were of analytical grade and were purchased either from Sigma-Aldrich, USA, or HiMedia Laboratories Pvt. Ltd., Mumbai, India. All Kits were purchased from Span Diagnostics Pvt. Ltd. TUNEL assay was performed using antibodies (Roche, Germany). Antibodies against Caspase-8, Caspase-9, and Caspase-3 were purchased from Cell Signaling Technology (Beverly, MA, USA). β-Actin antibodies and secondary antibodies (anti-mouse, anti-rabbit, and anti-goat) generated by Santacruz Biotechnology (Santacruz, CA, USA) were used.

2.2 | Plant material

Green *Capsicum annum L.* (*CA*) was selected for the study and purchased from the local vegetable market approved by Kolkata Municipal Corporation from November 2018 to February 2019. The identity of the vegetable was confirmed by the Botanical Survey of India (BSI), Government of India, Ministry of Environment and Forests, Howrah, West Bengal, India. A herbarium of the specimen was maintained in the institute library against voucher specimen no. RMC/PHY/MD/01/14.

2.3 | Aqueous extract preparation

After washing, samples were shadow dried, and then extracted in distilled water using a Soxhlet apparatus. The mixture was centrifuged and the supernatant was concentrated to dryness using a hot plate and stored in vials at −20°C for future use. The % yield was found to be 19% (w/w).

2.4 | Extract standardization and GC-MS analysis

Standardization of the extract with respect to the marker compound capsaicin was performed using High Performance Liquid Chromatography (UFLC, Shimadzu, Japan) as presented in our previous work (Das et al., 2018).

The phytoconstituents present in *AqCA* were analyzed using gas chromatography–mass spectrometry (GC-MS, Thermo scientific trace GC ultra) technique. First, 1 µl of the sample dissolved in methanol was injected and the mobile phase (carrier gas: He) was set to have a flow rate of 1.0 ml/min. The oven temperature program was initially set at 50°C–250°C with a hold time of about 2 min. Mass spectrum interpretation was carried out using the National Institute Standard and Technology (NIST)/National Bureau of Standard (NBS) and Wiley database.

2.5 | Animal selection

Male Wistar rats weighing 150–200 g were accommodated in autoclavable, polyvinyl cages, and maintained at ambient temperature (23°C–25°C) with 12:12 hr light: dark cycle. All rats had free access to standard diet and water, supplied *adlibitum*. The entire animal experiment was conducted with the prior approval of the Institutional Animal Ethics Committee (reg. no: 1795/PO/Ere/S14CPCSEA) formed by CPCSEA, Ministry of Environment, Government of India, New Delhi.

2.6 | Toxicity assessment and dose standardization

For inducing renal toxicity the dose of ethanol was determined to be 40% v/v ethanol, at 2 g per kg body weight (Basu, Das, & Datta, 2013). As described in the guidelines of OECD (TG 407, OECD, 2008) given by CPCSEA, groups of rats (n = 6) were administered the extract (i.p.) at doses ranging from 100 to 2,000 mg/kg body weight, and their mortality was detected. The calculated LD_{50} value, according to Lorke's method, (1983) was found to be 1,118.03 mg/kg. The *AqCA* was reflected as a non-toxic (Clarke & Clarke, 1967). For further study, we selected 1/8th and 1/4th of the maximum safe dose (1,000 mg/kg).

2.7 | Experimental design

The rats were randomly divided into six groups (n = 6), and treatment was carried out for a period of 30 days, as per the schedule mentioned below.

Group I: Control (Cont) rats; treated with normal mammalian saline (0.9 g %, i.p.).

Group II: Ethanol (EtOH)-treated rats; dose, 40% v/v ethanol (2 g per kg body weight, i.p.).

Group III: Co-treated rats; *AqCA* (125 mg/kg body weight, i.p.), followed by ethanol, (CA_{125} + EtOH).

Group IV: Co-treated rats; *AqCA* (250 mg/kg body weight, i.p.), followed by ethanol, (CA_{250} + EtOH).

Group V: Only extract treated rats (Only CA_{125}); *AqCA* (125 mg/kg body weight, i.p.).

Group VI: Only extract treated rats (Only CA_{250}); *AqCA* (250 mg/kg body weight, i.p.).

Intraperitoneal (i.p.) route for the administration of the drug has been chosen in the study to escape first-pass metabolism (Iwaniec & Turner, 2013) and also to minimize stress response initiated due to forced extract and alcohol administration. In addition, stress-induced enhanced release of glucocorticoids is also prevented, which is immunosuppressive (El-Guindy, de Villiers, & Doherty, 2007).

2.8 | Blood and tissue collection

At the end of the treatment period, the post-experimental body weight of all animals was recorded. The animals were then euthanized via intravenous ketamine injection, after being anesthetized via intraperitoneal injection of a combination of 100 mg/kg ketamine and 10 mg/kg xylazine (Mitra, Ray, Datta, Sengupta, & Sarkar, 2014). Blood was then cautiously collected by cardiac puncture for biochemical analysis according to our previous study (Das et al., 2018). The kidneys were excised immediately, washed in ice-cold saline and blotted dry. The weight of each kidney was recorded. A part of the tissue was dipped in fixative and set aside for histopathological studies and the residual part was used for biochemical studies.

2.9 | Determination of kidney weight to body weight ratio

Pre- and posttreatment body weight of all animals was noted. After sacrifice, each kidney was separated, weighed, and its relative weight to the body was calculated.

2.10 | Estimation of serum urea and creatinine levels

Serum urea and creatinine levels were estimated using kits to assess the kidney functioning. Blood Urea Nitrogen (BUN) level was also calculated. The results were expressed in mg/dl. Besides, BUN: creatinine ratio was also determined.

2.11 | Estimation of oxidative stress and antioxidant status in kidney

2.11.1 | Preparation of tissue homogenate

For oxidative stress measurement, a 10% w/v kidney tissue homogenate was prepared in ice-cold Phosphate buffer saline (PBS) as described earlier (Das et al., 2018).

2.11.2 | Preparation of mitochondrial fraction

For the preparation of mitochondrial fraction, the tissue homogenate was initially centrifuged at 5,000 rpm for 10 min and further processed as described previously (Das et al., 2018).

2.11.3 | Measurement of oxidative stress biomarkers: LPO and GSH level

Lipid peroxidation (LPO) was estimated as the concentration of malondialdehyde (MDA), according to Buege and Aust (1978). The absorbance was measured at 532 nm and the values were expressed as μmoles of MDA)/100 g tissue.

Reduced glutathione (GSH) content was estimated according to Sedlak and Lindsay (1968) using DTNB. GSH forms a yellow colored complex with DTNB with an absorbance maximum at 412 nm. Values were calculated and expressed as mg/100 g tissue.

2.11.4 | Measurement of antioxidant enzyme activities: SODs, catalase, GST, GPx, GR, and G6PD

Copper Zinc superoxide dismutase (Cu-Zn SOD) and Manganese-superoxide dismutase (Mn-SOD) activity were measured according to Marklund and Marklund (1974), based on the inhibition of pyrogallol auto-oxidation. The enzymatic activities were expressed in U/min/mg protein.

Catalase activity was determined by the decomposition of H_2O_2 at 240 nm, according to Aebi (1984). Change in the rate of absorbance was converted μmoles of H_2O_2/min/mg protein.

Glutathione Peroxidase (GPx) activity was measured according to Rotruc et al. (1973). Glutathione Peroxidase activity was expressed as μMoles of GSH consumed/min/mg protein.

Glutathione-S-Transferase (GST) was estimated according to Habig, Pabst, and Jakoby (1974). The absorbance was measured at 340 nm and values were calculated and expressed as μMoles of CDNB-GSH conjugate formed/min/mg protein.

Glutathione reductase (GR) was estimated according to Racker (1955) and the activity was expressed as μMoles of NADPH utilized/min/mg protein.

Moreover, Glucose-6-Phosphate Dehydrogenase (G-6-P-D) was estimated according to Balinsky and Bernstein (1963). The activity was expressed in terms of U/mg protein, in which one unit is equal to the amount of enzyme that brought about a change in OD of 0.01/min.

Protein content was estimated according to the methodology of Lowry, Rosebrough, Farr, and Randall (1951). All experiments were done in triplicate under the same experimental conditions. Absorbance at different wavelengths was obtained using a UV-VIS spectrophotometer (Systronics 118).

2.12 | Cytokine level

Commercially available ELISA kits were used to determine the level of tumor necrotic factor-alpha (TNF-α) and interleukin 6 (IL-6) and the values were expressed in pg/ml.

2.13 | Histopathological and histochemical studies

Biopsies of the kidney were immediately fixed in 10% formalin buffer for histopathological study. About 5 μm thick tissue sections were stained with Harris hematoxylin-eosin (H-E) stain (Banchroft, Stevens, & Turner, 1996). The microscopic investigation of these sections was carried out using a bright field microscope (Magnus, MLXi) and images obtained at 200X magnification. The area of the renal lesion was determined according to the formula of Singhal, Ganey, and Roth (2012).

Moreover, periodic acid Schiff (PAS) and feulgen staining were also performed on renal tissues to determine the changes in glycogen and DNA content, respectively. The sections were then observed and photographed using a digital camera (Olympus BX51) at (200X) and (400X) magnification. Images were analyzed using image analysis system (imageJ, NIH Software, Bethesda, MI), and the fractional area containing glycogen and DNA for each image were measured and expressed as % glycogen and % DNA content.

2.14 | Quantitation of fibrosis by confocal microscopy

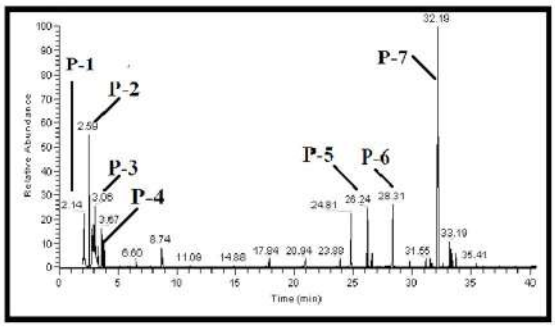
For the quantitation of fibrosis, the amount of collagen deposition was estimated by staining renal sections with Sirius Red (Roy et al., 2009). Laser scanning confocal microscope (Leica SP8, Germany) was used to view the tissue sections and pictures of stacked images were captured at 200X magnification. Further analysis of images was accomplished using image analysis software (imageJ, NIH Software, Bethesda, MI) and the segmental area of each image containing collagen deposition was determined and communicated as % collagen content.

2.15 | Apoptosis detection by TUNEL assay

TUNEL assay was executed on renal tissue sections for the detection of apoptosis using the TUNEL assay kit (Roche, Germany). DAPI was used for counterstaining the nuclei and the sections were then observed under a laser scanning confocal microscope (Leica SP8, Germany). The apoptotic index (AI) was calculated as per the equation below (Kitamura, Itoh, Noda, Matsuura, & Wakabayashi, 2004):

$$AI = (\text{Number of TUNEL positive cells/Total number of cells}) \times 100\%$$

(a)



(b)

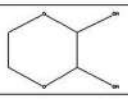
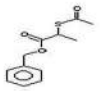
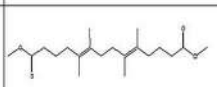
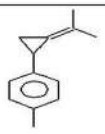
Peak No.	Molecular Weight	Chemical Structure	Compound Name
P-1	120		2,3-Dihydroxy 1,4-dioxane
P-2	170		2-[{(Methylsulfonyl) methyl} sulfanyl] ethanol
P-3	238		Benzyl 2-(acetyl sulfanyl) propionate
P-4	134		2-Hydroxy-2-methyl malonic acid
P-5	338		5,9-Tetradecadienedioic acid
P-6	194		1-(4-ethoxyphenyl)-1-hydroxy 2- propanone
P-7	172		p-(Isopropylidene cyclo-propyl)

FIGURE 1 Bio-active components present in aqueous extracts of *CA*. (a): Total ion chromatogram by GC-MS [Source: Das et al., 2018]. (b): Chemical structures of a few identified peaks along with their name and molecular weight.

2.16 | Western Blot analysis for Caspase 8, 9, and 3

Kidneys were homogenized in RIPA lysis buffer to prepare whole cell lysate, and the cytosolic fraction was prepared according to the standard protocol (Li, Xu, Dunbar, & Dhabuwala, 2003; Liang et al., 2013). Protease inhibitor supplemented buffers were used. About 50 μg of each sample was resolved on 10% SDS–PAGE and electrophoretically transferred to PVDF membranes. After blocking the membrane with 5% nonfat milk in TBST for 1 hr, the specific primary antibody was added and incubated at 4°C overnight. Consequently, secondary antibodies conjugated with HRP were added to the membrane. ECL detection was used to visualize the bands and the loading control was β-Actin. The relative density of the bands obtained were quantified using ImageJ software (imageJ, NIH Software, Bethesda, MI).

2.17 | Statistical analysis

The homogeneity of variances between the treated groups was confirmed and the level of significance of mean values between the treated sets was statistically analyzed using one way post hoc tests (Tukey's HSD test) of analysis of variances (ANOVA) using SPSS software version 20.0. Each experiment was repeated for a minimum of thrice. Values are presented as means ± *SE*. Level of significance ($p > .05$) was considered statistically non-significant.

3 | RESULTS

3.1 | GC-MS profiling of *AqCA*

Gas chromatography–mass spectrometry analysis of the extract showed the existence of several hydrocarbons together with other compounds such as ester products, fatty acyls, and ketone. The GC-MS chromatogram and the chemical structure of a few of these pharmacologically important components are presented in Figure 1.

3.2 | Kidney weight to body weight ratio

Administration of *AqCA* along with ethanol significantly decreased ($p \leq .001$) ethanol-induced increase in body weight (Table 1). Simultaneously, a significant decrease ($p \leq .001$) in the kidney weight of the only ethanol-treated group was also restored by both the doses of *AqCA*. Also, our records showed that the changes in both body and kidney weights were not dose-dependent in low-and high-dose *CA* supplemented groups.

3.3 | *CA* pre-treatment prevented ethanol-induced rise in serum markers

From Table 2, our results showed that the levels of serum urea, creatinine, and BUN are significantly elevated ($p \leq .001$) in the EtOH-treated group by 59%, 92%, and 59%, respectively, in comparison to the control group. Serum Urea and creatinine are sensitive and reliable biochemical indices of kidney function and its increase indicates renal dysfunction. In addition, BUN: Creatinine ratio was found to be significantly low ($p \leq .001$) in EtOH group compared to the control group. Concomitant administration of low and high doses of *CA* extract to the EtOH-treated group ameliorated the renal functions.

3.4 | *CA* extract enhanced the antioxidant activities and reduced ethanol-induced renal oxidative redox

Furthermore, Figure 2 depicts the changes in tissue antioxidant markers. Administration of ethanol caused significant ($p \leq .001$) elevation of lipid peroxidation, and Cu-Zn SOD activity by 66% and 57%, respectively, compared to the control group. Consecutively, ethanol also significantly depleted ($p \leq .001$) renal GSH level by 45%, Mn-SOD activity by 80%, and catalase activity by 55%. In the case of *AqCA* co-administered animals, the above changes were prevented and tissue antioxidant status was thus restored. Moreover, GSH, Mn-SOD, and catalase activities showed a dose-dependent response.

Renal tissue of only the ethanol-treated group also portrayed significantly elevated ($p \leq .001$) levels of GST, GPx, GR, and G6PD by 62%, 26%, 53%, and 54%, respectively, compared to control group. Co-treatment with *AqCA* (125 mg/kg body weight and 250 mg/kg body weight), ameliorated the above changes and restored them to near normal values. In addition, GPx and GR also showed a dose-dependent response.

3.5 | *CA* pre-treatment prevented ethanol-induced histo-morphological alterations

Our histological findings revealed that the administration of *CA* at both the doses prevented ethanol-induced degenerative changes. Normal renal tubular and glomerular architecture was observed in the control group, whereas ethanol-treated group showed dilation of the tubular lumen, widened capsular space, glomerular atrophy, cytoplasmic degeneration in cells of renal tubules, pyknotic nuclei, and renal tubules with neutrophilic infiltration and multiple foci of hemorrhage (Figure 3a). Co-administration of *AqCA* at both the doses, together with ethanol well-preserved the normal cellular morphology of both glomerulus and renal

TABLE 1 Effect of aqueous extracts of *CA* against ethanol-induced changes in kidney weight to body weight ratio

Groups	Initial body weight (g)	Final body weight (g)	Kidney weight (g)	Kidney weight: Body weight ratio (%)
Cont	176 ± 6.17	180 ± 4.02	1.0 ± 0.02	0.56 ± 0.03
EtOH	183 ± 5.32	196 ± 5.64*	0.62 ± 0.05*	0.32 ± 0.02*
CA_{125} + EtOH	182 ± 4.81	177 ± 4.21^{**ns}	0.83 ± 0.17^{**ns}	0.47 ± 0.012^{**ns}
CA_{250} + EtOH	180 ± 5.43	182 ± 6.22^{**ns}	0.91 ± 0.11^{**ns}	0.50 ± 0.03^{**ns}
Only CA_{125}	172 ± 4.28	176 ± 5.27^{**ns}	1.01 ± 0.04^{**ns}	0.57 ± 0.01^{**ns}
Only CA_{250}	178 ± 6.41	182 ± 6.61^{**ns}	0.98 ± 0.02^{**ns}	0.54 ± 0.022^{**ns}

Notes: The values are expressed as mean ± *SE* ($n = 6$). Data are analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Kramer post hoc analysis. $^{*}p \leq .001$ versus control, $^{**}p \leq .001$ versus EtOH, $^{ns}p > .05$ versus control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

TABLE 2 Effect of aqueous extracts of *CA* against ethanol-induced changes in serum marker enzymes

Groups	Urea (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	BUN: Creatinine ratio
Cont	25.05 ± 1.43	0.30 ± 0.12	11.71 ± 1.32	39.03 ± 4.02
EtOH	60.69 ± 1.89*	3.64 ± 0.06*	28.36 ± 2.05*	7.79 ± 2.16*
CA_{125} + EtOH	40.33 ± 2.23**#	0.50 ± 0.05**ns	18.85 ± 2.61**#	37.7 ± 2.91**ns
CA_{250} + EtOH	27.67 ± 1.76**ns	0.37 ± 0.02**ns	12.93 ± 1.58**ns	34.95 ± 3.17**ns
Only CA_{125}	24.37 ± 2.03**ns	0.36 ± 0.05**ns	11.39 ± 1.67**ns	31.64 ± 3.49**ns
Only CA_{250}	25.25 ± 1.61**ns	0.39 ± 0.07**ns	11.80 ± 2.06**ns	30.36 ± 2.11**ns

Notes: The values are expressed as mean ± *SE* (*n* = 6). Data are analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Kramer post hoc analysis. *$p \leq 0.001$ versus control, **$p \leq .001$ versus EtOH, #$p \leq .001$ versus control, ns$p > .05$ versus control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

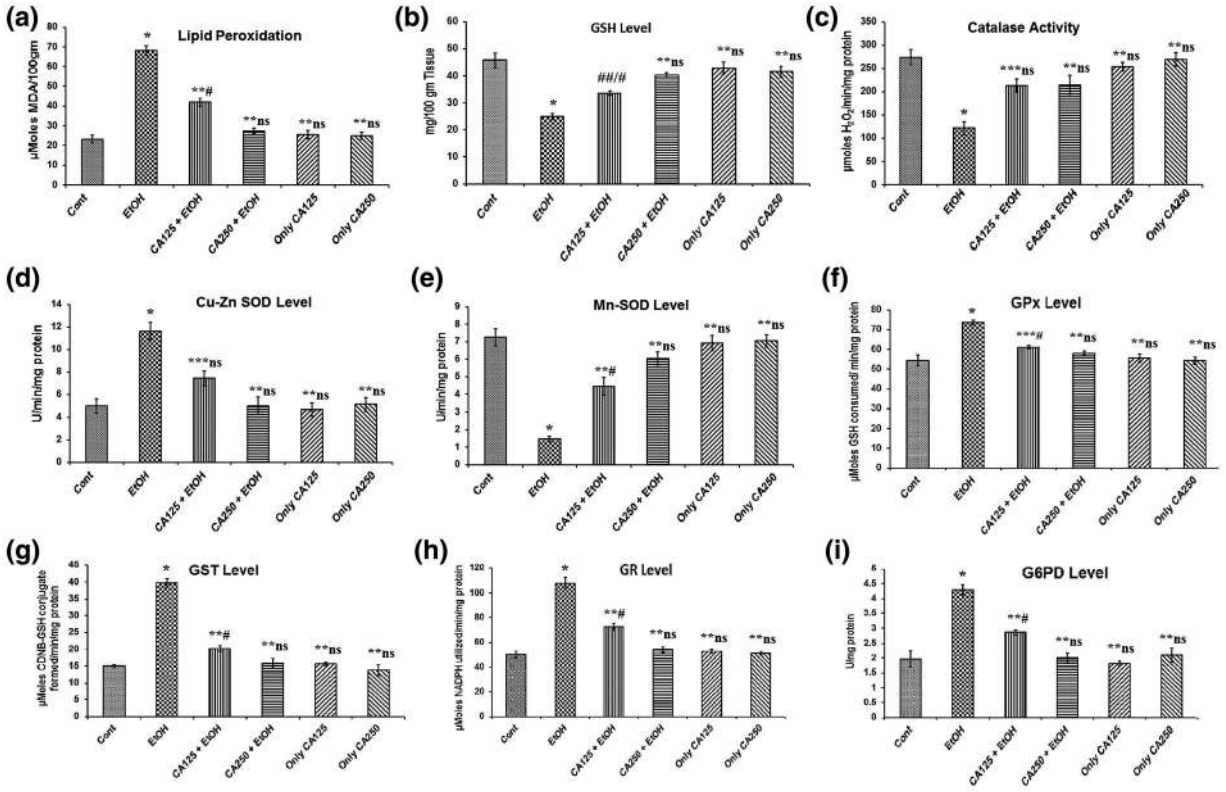


FIGURE 2 Effect of aqueous extracts of *CA* against ethanol-induced changes in tissue antioxidant status. The values are expressed as mean ± *SE*, (*n* = 6). Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. *$p \leq .001$ versus control, **$p \leq .001$ versus only ethanol, ***$p > .05$ versus only ethanol, #$p \leq .05$ versus control, ##$p \leq .01$ versus only ethanol, ns$p > .05$ versus. control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

tubules nearly equivalent to the control group, thus approving the preventive role of the *AqCA* against ethanol-induced cellular damage.

Periodic acid Schiff stained renal sections depicted the glycogen content of the tissue. Analysis of tissue sections of the ethanol-treated group revealed depletion of glycogen content compared to the control group (Figure 3c). However, groups co-treated with *AqCA* (125 and 250 mg/kg body weight) exhibited normal glycogen content, comparable to control group.

Moreover, Feulgen stained tissue sections revealed a decrease in DNA content, as indicated by modest magenta shade and indistinctly stained nuclei in the only ethanol-treated group. Groups receiving ethanol and extracts at different doses, depicted intensification of DNA content comparable to that of control and only extract treated groups. Thus, *AqCA* prohibited DNA damage induced by ethanol (Figure 3e).

The extent of renal damage in H-E stained renal sections is also graphically depicted in Figure 3b as the percentage area of renal

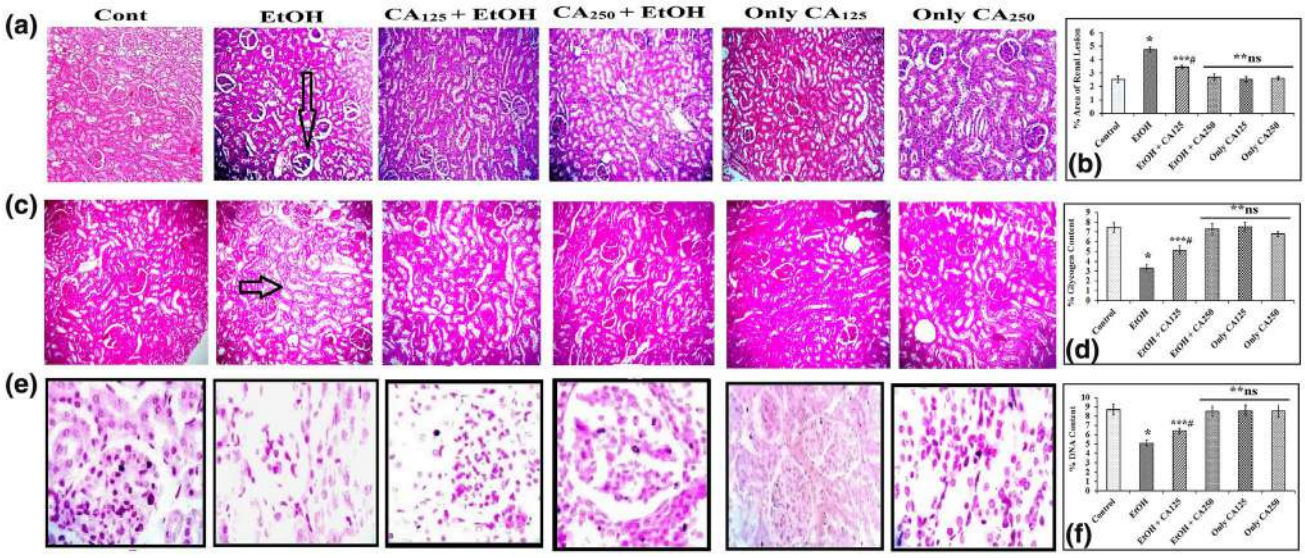


FIGURE 3 Effect of aqueous extracts of *CA* on renal architecture, glycogen content, and DNA content in control and experimental rats. (a): H&E stained paraffin-embedded renal sections (200X). The arrow indicates glomerular degeneration, (b): Graphical representation of percentage of renal lesion, (c): PAS stained renal tissue sections (200X), the arrow indicating glycogen depletion, (d): Graphical representation of percentage glycogen content, (e): Feulgen stained renal tissue sections (200X) depicting DNA content, (f): Graphical representation percentage DNA content. $^{*}p \leq .001$ versus control, $^{**}p \leq .001$ versus only ethanol, $^{***}p > .05$ versus only ethanol, $^{\#}p \leq .05$ versus control, $^{ns}p > .05$ versus control. All values are expressed as mean ± *SE*, ($n = 6$). Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

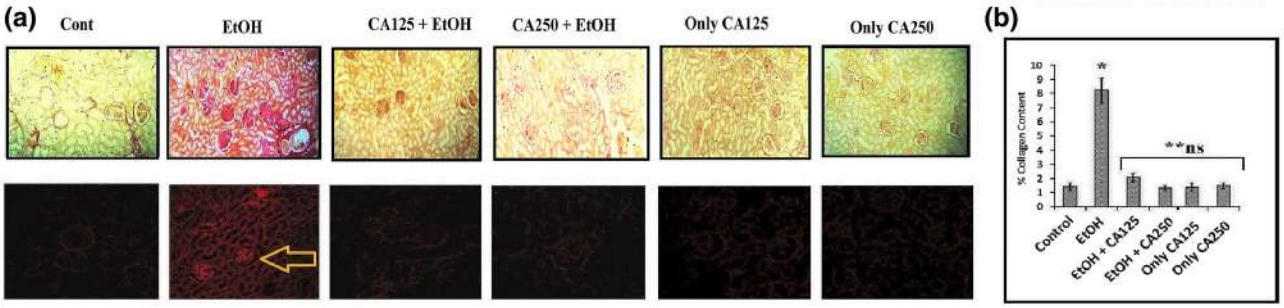


FIGURE 4 Effect of aqueous extracts of *CA* on Renal Collagen Content of Control and Experimental Rats. (a): Picro-Sirus Stained Renal Tissue Sections. Light microscope (200X magnification); Confocal microscope (400X magnification). (b): Graphical representation of percentage collagen content. $^{*}p \leq .001$ versus. control, $^{**}p \leq .001$ versus only ethanol, $^{ns}p > .05$ versus control. All values are expressed as mean ± *SE*, ($n = 6$). Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

lesions. It showed that the percentage area of renal damage in the only ethanol-treated group is significantly higher ($p < .001$) by 47% in comparison to the control group. Co-treatment with the *AqCA* at both the doses reduced the extent of renal damage, the higher dose being more effective. PAS and feulgen stained images were also analyzed to quantitate the percentage glycogen and percentage DNA content in renal tissues and the data are represented graphically in Figure 3d,f. It reveals that the percentage glycogen content and percentage DNA content in the renal tissue of rats receiving only ethanol is significantly depleted ($p < .001$) by 56% and 41%, respectively, as compared to the control group. However, the values were restored to near normal in groups co-supplemented with *AqCA*. The only extract-treated groups showed no change compared to the control group, thus revealing their non-toxic effect.

3.6 | *CA* pre-treatment prevented ethanol-induced fibrosis

Picro-sirus stained renal tissue in Figure 4 below depicts the amount of collagen deposition surrounding the glomerulus and renal tubules indicating fibrosis in rats receiving only ethanol. However, both the doses of *CA* extract decreased collagen

deposition, thus preventing renal fibrosis. The percentage of collagen content obtained by analyzing the images is represented graphically in Figure 4b. Analysis of values revealed that the collagen content in the renal tissue of rats receiving only ethanol was significantly elevated ($p < .001$) by 83% as compared to the control group. Co-supplementation with *CA* at both the doses restored the values comparable to control group.

3.7 | *CA* pre-treatment prevented ethanol-induced inflammatory response

Significantly higher ($p < .001$) level of TNF-α and IL-6 was observed in EtOH group in comparison to the control group. In the EtOH-treated group, the levels of TNF-α and IL-6 in serum augmented by 46% and 48% correspondingly. Co-supplementation with *AqCA* caused a noticeable decline in serum TNF-α and IL6 levels as revealed in Figure 5. In addition, a dose-dependent response was observed in our treatments, with the higher one being more potent in the alleviation of EtOH-induced inflammatory responses. No significant change was observed in only extract-treated groups when compared to control group.

3.8 | *CA* pre-treatment prevented ethanol-induced cellular apoptosis

To explicitly test whether *CA* extract protects the kidney from ethanol-induced apoptosis, we performed TUNEL assay. Our results showed that the number of apoptotic cells increased in only the ethanol-treated group, which, however, decreased to normal values upon *CA* co-treatment (Figure 6a). The histogram in Figure 6b depicts the apoptotic index of all experimental groups, revealing a significant decrease in the apoptotic index of co-treated groups as compared to the EtOH-treated group.

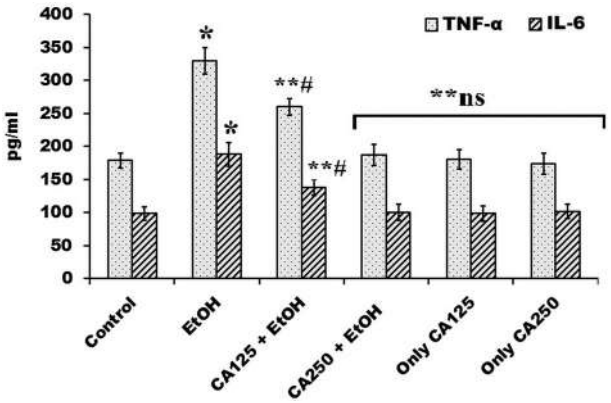


FIGURE 5 Effect of aqueous extracts of *CA* against ethanol-induced changes in cytokine levels. The values are expressed as mean ± *SE*, ($n = 6$). Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. $^{*}p \leq .001$ versus control, $^{**}p \leq .001$ versus only ethanol, $^{\#}p \leq .05$ versus control, $^{ns}p > .05$ versus control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

3.9 | *CA* pre-treatment prevented ethanol-induced caspase activation

Besides, to get an insight into the probable mechanism of action of *CA* in preventing apoptosis, western blot analysis was performed for caspase 8, caspase 9, and caspase 3. This analysis revealed an increase in activation of all the caspases in EtOH group in comparison to the control group. However, co-treatment with extracts of *CA* reverted the values to near normal (Figure 7a). Elevated expression of both caspase 8 and 9 represent the involvement of both the extrinsic and intrinsic pathways in ethanol-induced apoptosis. The corresponding histogram shows the cleaved Caspase 3/β-actin ratio in Kidney tissue (Figure 7b).

4 | DISCUSSION

Scientific and investigational studies have demonstrated that the routine consumption of a large volume of ethanol has deleterious effects on the kidney (De Marchi et al., 1993). Several studies had also confirmed the association between nephrotoxicity and oxidative stress in many experimental models (Bashan, Bashan, Secilmis, & Singirik, 2014; Sahu, Kuncha, Sindura, & Sistla, 2013). Increased levels of serum creatinine and urea have been considered as an important index for assessing nephrotoxicity (Ali, Ismail, & Bashir, 2001; Anwar, Khan, Amin, & Ahmad, 1999). The elevated serum levels of serum urea, creatinine, BUN, and reduced BUN: creatinine ratio in the ethanol-treated experimental group in the present study is an indicator of the severity of kidney damage. Increased level of creatinine signifies ethanol-induced alteration in glomerulus filtration rate that might lead to a reduced rate of renal creatinine clearance as is observed by Ojeda, Barrero, Nogales, Murillo, and Carreras (2012). According to Nechifor and Dinu, (2011), the deleterious impacts of ethanol on kidney are due to the increased activities of alcohol dehydrogenase, lactate dehydrogenase, and malate dehydrogenase. Recently, Shanmugam, Mallikarjuna, and Reddy (2011) have reported that ethanol-induced oxidative stress causes a significant decrease in the levels of antioxidant enzyme viz, catalase, and superoxide-dismutase activities along with an increase in MDA in the kidney of the rats, which is in accordance to our observation.

Long-term exposure to alcohol (ethanol) increases enzyme activities related to the recycling and consumption of kidney glutathione (Dinu, Nechifor, & Movileanu, 2006). In the present study, a low level of GSH along with elevated levels of GST, GPx, GR, and G6PD were observed in the only ethanol-treated group. The decreased level of GSH in kidney of rats treated with alcohol is also reported in earlier studies (Kode, Rajagopalan, Penumathsa, & Menon, 2004; Rodrigo et al., 2002). Amplified GST activity may be correlated with the observed decrease in the GSH level of the kidney. Decreased GSH level

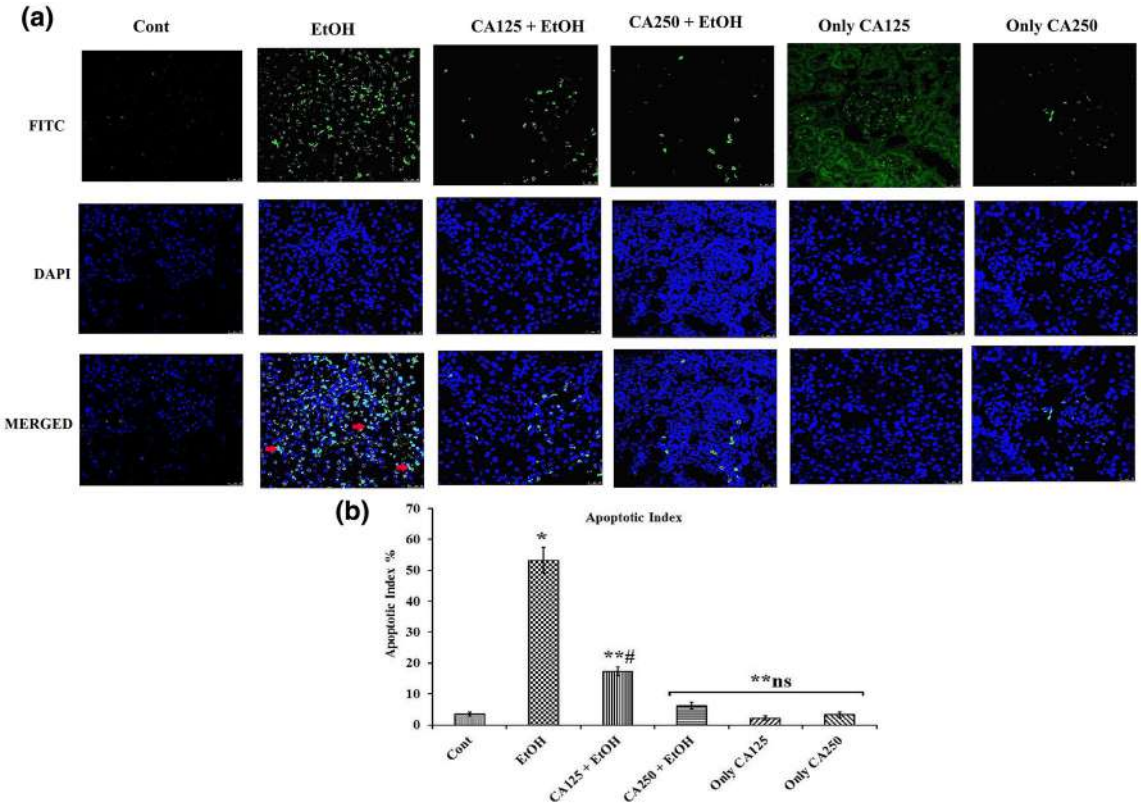


FIGURE 6 Effect of aqueous extracts of *CA* against ethanol-induced commencement of apoptosis. (a) TUNEL (green) stained renal tissue sections of different treated groups and nucleus counterstained with DAPI (blue), viewed at 200X magnification. Co- localization of both the blue and green stain in the merged images represents the TUNEL-positive apoptotic cells. Bar = 25μm. (b) Apoptotic index (%) was quantified from the TUNEL positive cells. The corresponding histogram represents values expressed as mean ± *SE*, (*n* = 6). Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. $^{*}p \leq .001$ versus control, $^{**}p \leq .001$ versus only ethanol, $^{\#}p \leq .05$ versus control, $^{ns}p > .05$ versus control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

may be due to an enhanced GSH utilization in conjugation reaction mediated by increased GST activity (Dinu et al., 2006). Increased GST activity also implies its activation owing to oxidative stress (Aniya & Daido, 1994).

Both SOD and catalase are antioxidants enzymes that play a crucial role against cellular oxidative damage, and the level of both these enzymes is reported to be diminished in diabetic nephropathy (Mitra, Tripathy, & Ghosh, 2020). A decrease in the activity of catalase in this study indicates H_2O_2 accumulation within renal tissue (Sullivan-Gunn & Lewandowski, 2013). Accumulated H_2O_2 and lowered antioxidant enzymes might lead to DNA damage and cell injury (Mitra et al., 2020), suggesting the role of ethanol-induced ROS in cellular damage. Enhanced Cu-Zn-SOD activity in the present study may indicate the production of superoxide anion in excess amount. This superoxide anion is ultimately converted to H_2O_2. Exhaustion of Mn-SOD might be due to time-dependent leakage of mitochondrial SOD into the cytosol, thus representing a loss of mitochondrial membrane integrity.

Our histological and histochemical inspections also ascertain the preventive role of the *AqCA* against oxidative damage. Increased neutrophil infiltration as observed in ethanol-treated group might contribute to oxidative stress in kidney (Latchoumycandane et al., 2014). The decrease in glycogen content as depicted in Figure 3c,d was directly linked to the alternation in carbohydrate metabolism resulting in inactivation of the enzyme glycogen synthase kinase 3β in renal cells of the ethanol-treated group (Mariappan et al., 2014). The development of the magenta color in feulgen test is not only specific for the localization of DNA in chromosomes but at the same time, the intensity of the reaction may also be considered to be an index of the amount of DNA present in the cell (Sharma & Sharma, 2014). Hence, the moderate magenta color in feulgen stained renal tissue sections of rats treated with ethanol found in our study (Figure 3e,f) indicates the reduction in DNA content and the recovery from this state after co-treatment with *CA* extract. The reduction in DNA content may be attributed to an increase in DNA fragmentation or loss of DNA repair mechanism. Several studies have reported a strong correlation between loss of DNA content or DNA fragmentation and cellular apoptosis induced by several stressful conditions (Aldrovani, Mello, Guaraldo, & de Campos Vidal, 2006; Maria, Vidal, & Mello, 2000), and probably including exposure to ethanol as found in this study.

The progress of chronic kidney disease (CKD) also is characterized by renal fibrosis, including the loss of epithelial cell membrane structure and function, and acquisition of more fibroblastic cells leading to the production of extracellular matrix elements, such as collagen

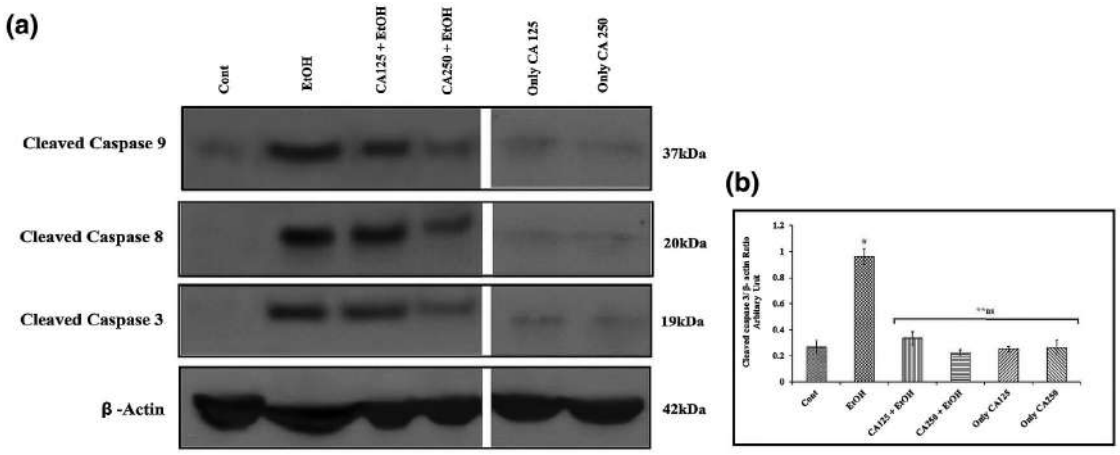


FIGURE 7 Effect of aqueous extracts of *CA* against ethanol-induced activation of the apoptotic pathway. (a) Relative western blot analysis of cleaved caspase 8, cleaved caspase 9, and cleaved caspase 3. A white demarcating line indicates band taken from different gels. (b) Histogram depicting cleaved caspase 3/ β-actin ratio, values expressed as mean ± *SE* (n = 6) of relative arbitrary units of the bands of immunoblots, conducted with separate experiments in each group. Data were analyzed by ANOVA followed by Tukey's Kramer post hoc analysis. $^{*}p \leq .001$ versus control, $^{**}p \leq .001$ versus only ethanol, $^{ns}p > .05$ versus control. Cont-control, EtOH-ethanol treated, CA_{125}—*Capsicum annum* at 125 mg/kg body weight dose, CA_{250}—*Capsicum annum* at 250 mg/kg body weight dose.

and fibronectin (Kim, Seok, Jung, & Park, 2009). In the present study, picro-sirus stained kidney sections (Figure 4), light and confocal microscopy depict an increase in collagen deposition surrounding the glomerulus and renal tubules when compared to the control group. Co-administration of *AqCA* restored the above changes.

Activation of inflammatory cells induced by ethanol, promote the production of TNF-α and IL-6. These cytokines are known to participate in inflammation and acute-phase protein synthesis (Tilg & Diehl, 2000). This literature coincides with our result, which reveals a rise in the levels of TNF-α and IL-6 owing to ethanol treatment (Figure 5).

Moreover, it is well known that reactive oxygen species injure multiple bio-molecules including DNA, protein, and lipids. They also activate certain signaling pathways leading to necrosis and apoptosis (Soghra Parhizgar et al., 2016). TUNEL assay in the present study confirms an increase in apoptotic cells in kidney sections of EtOH group. Excess apoptosis may lead to atrophy and renal dysfunction, as indicated in the present study by changes in serum parameters. This apoptosis might be the result of multiple mechanisms, including ethanol-mediated toxicity, induction of oxidative stress, inhibition of survival genes (*c-Met*), and induction of pro-apoptotic signaling molecules (TNF-α and Fas ligand). (McVicker, Tuma, & Casey, 2007).

Recent studies revealed that both the intrinsic and extrinsic pathways of apoptosis via death receptor are activated during renal obstruction (Campbell et al., 2008). Caspase 3 is the executioner caspase that is activated both via the intrinsic as well as the extrinsic pathway. In the present study, increased expression of primary apoptotic proteins like activated caspase 8 and caspase 9 in EtOH group also confirms activation of both intrinsic and extrinsic apoptotic pathways, thus leading to activation of caspase 3 (Figure 7). This result is in accordance with the study conducted by Banerjee et al. (2016). These changes are reverted in *CA* extract co-treated group.

This nephroprotective efficacy of *CA* against ethanol-induced oxidative damage may be attributed to its free radical scavenging activity and a huge resource of antioxidant vitamins which has already been reported in our previous study (Das, Basu, Sen, & Datta, 2016). Besides, the absence of heavy metals also makes the vegetable safe for consumption (Das et al., 2016). As stated earlier, polyphenols and other secondary plant metabolites act as natural antioxidants and prevent oxidative tissue damage. Besides, the active component capsaicin, a transient receptor potential vanilloid 1 (TRPV1) receptor agonist is also reported to prevent renal damage by regulating blood pressure, inflammation, and oxidative stress (Ueda, Tsuji, Hirata, Takaoka, & Matsumura, 2008; Yu, Ma, & Wang, 2018). Moreover, GC-MS analysis has established that the extract possesses several phytoconstituents like glycosides (2,3-Dihydroxy 1,4-dioxane), ester derivative (2-[{(Methylsulfonyl) methyl} sulfanyl] ethanol), short-chain fatty acids and carboxylic acids (Benzyl 2-(acetyl sulfanyl) propionate & 2-Hydroxy-2-methyl malonic acid), long-chain fatty acids ((5,9-Tetradecadienedioic acid), ketone (1-(4-ethoxyphenyl)-1-hydroxy 2- propanone)), and aromatic derivatives. These compounds along with other polyphenols, flavonol, and terpenoids present in *AqCA* are known to possess antioxidant, anti-inflammatory, alcohol dehydrogenase inhibitory, immuno-modulatory, and ethanolytic activity (Das et al., 2018; Wang et al., 2019), and might contribute to enhanced antioxidant status and renal cell protection against ethanol-induced oxidative stress and apoptosis.

5 | CONCLUSION

It can, thus, be concluded from the present study that the administration of *AqCA* could efficiently prevent ethanol-induced production of excess free radicals and alteration in antioxidant status. Moreover, it can also prevent ethanol-induced loss of both structural

and functional integrity of renal cells. The various phytoconstituents present may act synergistically to provide protection against ethanol-induced oxidative stress. Based on these findings, future pharmacognostic approaches on the isolation of different bioactive components will lead to further exploration of its therapeutic use as an anti-oxidant and renoprotective agent. Being easily available almost all over the world, *CA* can also be established as a promising candidate as part of the functional food.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors regarding the publication of this article.

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REFERENCES

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121–126.

Aldrovani, M., Mello, M. L. S., Guaraldo, A. M. A., & de Campos Vidal, B. (2006). Nuclear phenotypes and DNA fragmentation in tendon fibroblasts of NOD mice. *Caryologia*, *59*(2), 116–124. https://doi.org/10.1080/00087114.2006.10797906

Ali, B. H., Ismail, T. B., & Bashir, A. A. (2001). Sex difference in the susceptibility of rats to gentamicin nephrotoxicity: Influence of gonadectomy and hormonal replacement therapy. *Indian Journal of Pharmacology*, *33*, 369–373.

Aniya, Y., & Daido, A. (1994). Activation of microsomal glutathione S-transferase in tent-butyl hydroperoxide-induced oxidative stress of isolated rat liver. *Japanese Journal of Pharmacology*, *66*, 123–130.

Anwar, S., Khan, N. A., Amin, K. M. Y., & Ahmad, G. (1999). Effects of Banadequl Buzoor in some renal disorders. *Hamdard Medicine*, *42*, 33–36.

Arora, R., Gill, N. S., Chauhan, G., & Rana, A. C. (2011). An overview about versatile molecule capsaicin. *International Journal of Pharmaceutical Sciences and Drug Research*, *3*, 280–286.

Bagnardi, V., Blangiardo, M., La Vecchia, C., & Corrao, G. (2001). Alcohol consumption and the risk of cancer: A meta-analysis. *Alcohol Research & Health: The Journal of the National Institute on Alcohol Abuse and Alcoholism*, *25*(4), 263–270.

Balinsky, D., & Bernstein, R. E. (1963). The purification and properties of glucose-6-phosphate dehydrogenase from human erythrocytes. *Biochimica et Biophysica Acta*, *67*, 313–315. https://doi.org/10.1016/0926-6569(63)90239-6

Banchroft, J. D., Stevens, A., & Turner, D. R. (1996). *Theory and practice of histological techniques* (4th ed.). New York, NY: Churchil Livingstone.

Banerjee, B., Nandi, P., Chakraborty, S., Raha, S., Sen, P. C., & Jana, K. (2016). Resveratrol ameliorates benzo (a) pyrene-induced testicular dysfunction and apoptosis: Involvement of p38 MAPK/ATF2/iNOS signaling. *The Journal of Nutritional Biochemistry*, *34*, 17–29. https://doi.org/10.1016/j.jnutbio.2016.04.003

Bashan, I., Bashan, P., Secilmis, M. A., & Singirik, E. (2014). Protective effect of L-arginine on gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmacology*, *46*, 608–612. https://doi.org/10.4103/0253-7613.144915

Basith, S., Cui, M., Hong, S., & Choi, S. (2016). Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules*, *21*, 966–994. https://doi.org/10.3390/molecules21080966

Basu, S., Das, M., & Datta, G. (2013). Protective activity of ethanolic extract of amorphophallus campanulatus against ethanol induced hepatotoxicity in rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(Suppl 2), 412–417.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, *52*, 302–310.

Campbell, M. T., Dagher, P., Hile, K. L., Zhang, H., Meldrum, D. R., Rink, R. C., & Meldrum, K. K. (2008). Tumor necrosis factor-alpha induces intrinsic apoptotic signaling during renal obstruction through truncated bid activation. *Journal of Urology*, *180*, 2694–2700.

Clarke, E. G. C., & Clarke, M. L. (1967). *Garner's Veterinary toxicology*. London, UK: Bailliere, Tindall & Cassell.

Das, M., Basu, S., Banerjee, B., Sen, A., Jana, K., & Datta, G. (2018). Hepatoprotective effects of green Capsicum annum against ethanol induced oxidative stress, inflammation and apoptosis in rats. *Journal of Ethnopharmacology*, *227*, 69–81. https://doi.org/10.1016/j.jep.2018.08.019

Das, M., Basu, S., Sen, A., & Datta, G. (2016). Nutritional profile, mineral content and in vitro antioxidant potency of *Capsicum annum* L. Cultivated in 24 parganas [south], West Bengal, India. *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*, 182–188.

De Marchi, S., Cecchin, E., Basile, A., Bertotti, A., Nardini, R., & Bartoli, E. (1993). Renal tubular dysfunction in chronic alcohol abuse–effects of abstinence. *The New England Journal of Medicine*, *329*, 1927–1934. https://doi.org/10.1056/NEJM199312233292605

Desai, S. N., Patel, D. K., Devkar, R. V., Patel, P. V., & Ramachandran, A. V. (2012). Hepatoprotective potential of polyphenol rich extract of *Murraya koenigii* L.: An in vivo study. *Food and Chemical Toxicology*, *50*, 310–314. https://doi.org/10.1016/j.fct.2011.10.063

Dinu, D., Nechifor, M. T., & Movileanu, L. (2006). Ethanol-induced alterations of the antioxidant defense system in rat kidney. *Journal of Biochemical and Molecular Toxicology*, *19*, 386–395. https://doi.org/10.1002/jbt.20101

El-Guindy, N. B. S., de Villiers, W. J., & Doherty, D. E. (2007). Acute alcohol intake impairs lung inflammation by changing pro-and anti-inflammatory mediator balance. *Alcohol*, *41*, 335–345. https://doi.org/10.1016/j.alcohol.2007.07.002

Epstein, F. H., & Lieber, C. S. (1988). Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. *New England Journal of Medicine*, *319*(25), 1639–1650. https://doi.org/10.1056/NEJM198812223192505

Govindarajan, V. S., & Sathyanarayana, M. N. (1991). Capsicum–production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. *Critical Review of Food Science Nutrition*, *29*, 435–474.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*, 7130–7139.

Iwaniec, U. T., & Turner, R. T. (2013). Intraperitoneal injection of ethanol results in drastic changes in bone metabolism not observed when ethanol is administered by oral gavage. *Alcoholism: Clinical and Experimental Research*, *37*, 1271–1277. https://doi.org/10.1111/acer.12105

Kim, J., Seok, Y. M., Jung, K. J., & Park, K. M. (2009). Reactive oxygen species/oxidative stress contributes to progression of kidney fibrosis following transient ischemic injury in mice. *American Journal of Physiology- Renal Physiology*, *297*, F461–F470. https://doi.org/10.1152/ajprenal.90735.2008

Kitamura, T., Itoh, M., Noda, T., Matsuura, M., & Wakabayashi, K. (2004). Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on intestinal tumorigenesis in adenomatous polyposis coli gene knockout mice. *International Journal of Cancer*, *109*, 576–580. https://doi.org/10.1002/ijc.20012

Klein, H., & Harmjanz, D. (1975). Effect of ethanol infusion on the ultrastructure of human myocardium. *Postgraduate Medical Journal*, *51*, 325–329. https://doi.org/10.1136/pgmj.51.595.325

Kode, A., Rajagopalan, R., Penumathsa, S. V., & Menon, V. P. (2004). Influence of a thiazole derivative on ethanol and thermally oxidized sunflower oil-induced oxidative stress. *Fundamental and Clinical Pharmacology*, *18*, 565–571. https://doi.org/10.1111/j.1472-8206.2004.00274.x

Latchoumycandane, C., Nagy, L. E., & McIntyre, T. M. (2014). Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation. *Free Radical Biology and Medicine*, *69*, 403–416. https://doi.org/10.1016/j.freeradbiomed.2014.01.001

Leal, S., Jorge, D. O. R., Joana, B., Maria, S. S., & Isabel, S. S. (2017). Heavy alcohol consumption effects on blood pressure and on kidney structure persist after long-term withdrawal. *Kidney and Blood Pressure Research*, *42*(4), 664–675. https://doi.org/10.1159/000482022

Li, H., Xu, L., Dunbar, J. C., & Dhabuwala, C. B. (2003). Role of mitochondrial cytochrome c in cocaine-induced apoptosis in rat testes. *Urology*, *61*, 646–650. https://doi.org/10.1016/S0090-4295(02)02263-X

Liang, J., Yu, Y., Wang, B., Lu, B., Zhang, J., Zhang, H., & Ge, P. (2013). Ginsenoside Rb1 attenuates oxygen-glucose deprivation-induced apoptosis in SH-SY5Y cells via protection of mitochondria and inhibition of AIF and cytochrome c release. *Molecules*, *18*, 12777–12792. https://doi.org/10.3390/molecules181012777

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., … Ezzati, M. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: A systematic analysis for the global burden of disease study 2010. *Lancet*, *380*, 2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, *54*, 275–287. https://doi.org/10.1007/BF01234480

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Manzo-Avalos, S., & Saavedra-Molina, A. (2010). Cellular and mitochondrial effects of alcohol consumption. *International Journal of Environmental Research and Public Health*, *7*, 4281–4304. https://doi.org/10.3390/ijerph7124281

Maria, S. S., Vidal, B. D. C., & Mello, M. L. S. (2000). Image analysis of DNA fragmentation and loss in V79 cells under apoptosis. *Genetics and Molecular Biology*, *23*(1), 109–112. https://doi.org/10.1590/S1415-47572000000100020

Mariappan, M. M., Prasad, S., D'Silva, K., Cedillo, E., Sataranatarajan, K., Barnes, J. L., … Kasinath, B. S. (2014). Activation of glycogen synthase kinase 3β ameliorates diabetes-induced kidney injury. *Journal of Biological Chemistry*, *289*, 35363–35375. https://doi.org/10.1074/jbc.M114.587840

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, *47*, 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

McVicker, B. L., Tuma, D. J., & Casey, C. A. (2007). Effect of ethanol on pro-apoptotic mechanisms in polarized hepatic cells. *World Journal of Gastroenterology: WJG*, *13*(37), 4960. https://doi.org/10.3748/wjg.v13.i37.4960

Mitra, A., Ray, A., Datta, R., Sengupta, S., & Sarkar, S. (2014). Cardioprotective role of P38 MAPK during myocardial infarction via parallel activation of alpha-crystallin B and Nrf2. *Journal of Cell Physiology*, *229*, 1272–1282.

Mitra, D., Tripathy, A., & Ghosh, D. (2020). Antidiabetic and antioxidative efficacy of the constituents present in the fraction solvents of *Curcuma amada* rhizomes: An experimental study on the diabetic rat model. *Journal of Food Biochemistry*, *44*(3), e13154.

Molina-Jijón, E., Tapia, E., Zazueta, C., El Hafidi, M., Zatarain-Barrón, Z. L., Hernández-Pando, R., … Pedraza-Chaverri, J. (2011). Curcumin prevents Cr (VI)-induced renal oxidant damage by a mitochondrial pathway. *Free Radical Biology and Medicine*, *51*(8), 1543–1557. https://doi.org/10.1016/j.freeradbiomed.2011.07.018

Mózsik, G., Szolcsányi, J., & Rácz, I. (2005). Gastroprotection induced by capsaicin in healthy human subjects. *World Journal of Gastroenterology*, *11*, 5180–5184.

Nechifor, M. T., & Dinu, D. (2011). Ethanol-induced redox imbalance in rat kidneys. *Journal of Biochemical and Molecular Toxicology*, *25*(4), 224–230. https://doi.org/10.1002/jbt.20379

OECD. (2008). *OECD guidelines for the testing of chemicals*. Retrieved from https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg407-2008

Ojeda, M. L., Barrero, M. J., Nogales, F., Murillo, M. L., & Carreras, O. (2012). Oxidative effects of chronic ethanol consumption on the functions of heart and kidney: Folic acid supplementation. *Alcohol and Alcoholism*, *47*(4), 404–412. https://doi.org/10.1093/alcalc/ags056

Pachinger, O., Mao, J., Fauvel, J. M., & Bing, R. J. (1975). Mitochondrial function and excitation-contraction coupling in the development of alcoholic cardiomyopathy. *Recent Advances in Studies on Cardiac Structure and Metabolism*, *5*, 423–429.

Palani, S., Raja, S., Kumar, R. P., Soumya, J., & Kumar, B. S. (2009). Therapeutic efficacy of *Pimpinella tirupatiensis* (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. *International Journal of PharmTech Research*, *1*, 925–934.

Piano, M. R. (2017). Alcohol's effects on the cardiovascular system. *Alcohol Research*, *38*, 219–241.

Racker, E. (1955). *Methods in enzymology* (2nd vol.). New York, NY: Academic Press.

Ratna, A., & Mandrekar, P. (2017). Alcohol and cancer: Mechanisms and therapies. *Biomolecules*, *7*(3), 61. https://doi.org/10.3390/biom7030061

Regan, T. J. (1990). Alcohol and the cardiovascular system. *JAMA*, *264*, 377–381. https://doi.org/10.1001/jama.1990.03450030101041

Ríos-Silva, M., Santos-Álvarez, R., Trujillo, X., Cárdenas-María, R. Y., López-Zamudio, M., Bricio-Barrios, J. A., … Huerta, M. (2019). Effects of chronic administration of capsaicin on biomarkers of kidney injury in male Wistar rats with experimental diabetes. *Molecules*, *24*(1), 36.

Rodrigo, R., Trujillo, S., Bosco, C., Orellana, M., Thielemann, L., & Araya, J. (2002). Changes in (Na+ K)-adenosine triphosphatase activity and ultrastructure of lung and kidney associated with oxidative stress induced by acute ethanol intoxication. *Chest*, *121*, 589–596. https://doi.org/10.1378/chest.121.2.589

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, *179*, 588–590. https://doi.org/10.1126/science.179.4073.588

Roy, S. G., De, P., Mukherjee, D., Chander, V., Konar, A., Bandyopadhyay, D., & Bandyopadhyay, A. (2009). Excess of glucocorticoid induces cardiac dysfunction via activating angiotensin II pathway. *Cell Physiology and Biochemistry*, *24*, 1–10. https://doi.org/10.1159/000227803

Sabiu, S., O'Neill, F. H., & Ashafa, A. O. T. (2016). The purview of phytotherapy in the management of kidney disorders: A systematic review on Nigeria and South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(5), 38–47.

Sahu, B. D., Kuncha, M., Sindura, G. J., & Sistla, R. (2013). Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. *Phytomedicine*, *20*, 453–460. https://doi.org/10.1016/j.phymed.2012.12.001

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, *25*, 192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Shanmugam, K. R., Mallikarjuna, K., & Reddy, K. S. (2011). Effect of alcohol on blood glucose and antioxidant enzymes in the liver and kidney of diabetic rats. *Indian Journal of Pharmacology*, *43*, 330–335. https://doi.org/10.4103/0253-7613.81504

Sharma, A. K., & Sharma, A. (2014). *Chromosome techniques: Theory and practice*. London: Butterworth-Heinemann. ISBN: 978-0-408-70942-2.

Shimeda, Y., Hirotani, Y., Akimoto, Y., Shindou, K., Ijiri, Y., Nishihori, T., & Tanaka, K. (2005). Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. *Biological and Pharmaceutical Bulletin*, *28*(9), 1635–1638. https://doi.org/10.1248/bpb.28.1635

Singhal, R., Ganey, P. E., & Roth, R. A. (2012). Complement activation in acetaminophen-induced liver injury in mice. *Journal of Pharmacology and Experimental Therapeutics*, *341*, 377–385. https://doi.org/10.1124/jpet.111.189837

Soghra Parhizgar, S. H., Mousa-Al-Reza, H., Mohammad, S., Alireza, E., Reza, M., Zohreh, N. E. Y., & Abolfazl, K. R. (2016). Renoprotective effect of plantago major against nephrotoxicity and oxidative stress induced by cisplatin. *Iranian Journal of Kidney Diseases*, *10*, 182–188.

Sullivan-Gunn, M. J., & Lewandowski, P. A. (2013). Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with aging sarcopenia. *BMC Geriatrics*, *13*(1), 104. https://doi.org/10.1186/1471-2318-13-104

Tilg, H., & Diehl, A. M. (2000). Cytokines in alcoholic and non-alcoholic steatohepatitis. *New England Journal of Medicine*, *343*, 1467–1476. https://doi.org/10.1056/NEJM200011163432007

Ueda, K., Tsuji, F., Hirata, T., Takaoka, M., & Matsumura, Y. (2008). Preventive effect of TRPV1 agonists capsaicin and resiniferatoxin on ischemia/reperfusion-induced renal injury in rats. *Journal of Cardiovascular Pharmacology*, *51*(5), 513–520. https://doi.org/10.1097/FJC.0b013e31816f6884

Wang, J., Xu, J., Gong, X., Yang, M., Zhang, C., & Li, M. (2019). Biosynthesis, chemistry, and pharmacology of polyphenols from Chinese *Salvia* Species: A review. *Molecules*, *24*(1), 155.

World Health Organization. (2011). *Global status report on alcohol and health*. Geneva, Switzerland. https://www.who.int/substance_abuse/publications/alcohol_2014/en/

World Health Organization. (2018). *Global status report on alcohol and health 2018*. Geneva, Switzerland.. https://www.who.int/substance_abuse/publications/global_alcohol_report/en/

Yu, S. Q., Ma, S., & Wang, D. H. (2018). Activation of TRPV1 prevents salt-induced kidney damage and hypertension after renal ischemia-reperfusion injury in rats. *Kidney and Blood Pressure Research*, *43*(4), 1285–1296. https://doi.org/10.1159/000492412

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