Synergistic protective effect of *Camellia sinensis* leaf buds and *Camellia sinensis* flowers against cisplatin-induced nephrotoxicity in rats and characterization of its bioactive compounds

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**ABSTRACT**

An effective antitumor drug like cisplatin has toxic effects leading to kidney injury. The authors investigated the nephroprotective action of *C. sinensis* leaf buds (CSB) and its flowers (CSF), both individually and in combined therapy against cisplatin-induced renal injury. Renal functional tests, oxidant, anti-oxidant and histopathology of kidney tissues are assessed. The major findings of the experiment is that the CSB and/or CSF ameliorate the successive conditions of (1) Renal dysfunction (Serum creatinine, urea, uric acid and glucose, protein level present in the urine) (2) The degree of lipid peroxidation (3) Antioxidant enzyme suppression and (4) The destructive renal architecture associated with drug-induced renal toxicity. Result suggests that the combined therapy of CSB and CSF was highly effective when compared to monotherapy in the complete regeneration of destructive cells by drug effect and could be an addition of a new source to the pharmaceutical industry as antioxidant nutrition supplement.

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1. Introduction

Cisplatin (CP) is a therapeutically effective anticancer medication used to treat a wide range of solid tumors. However, due to its severe side effects, notably nephrotoxicity, it has only limited usage in clinical practice (Abdel-Wahab et al. 2017). There is no effective medication to treat CP-induced nephrotoxicity in modern medicine but recently plants bioactive compounds and their analogs which possess strong antioxidant capabilities have made a significant contribution as drugs against kidney injury (Dachuri et al. 2020).

*Camellia sinensis* is the world’s oldest crop and has gained importance in multiple cultures. It has more than 700 chemicals that are useful to human health (Monda 2014). The study supports the opinion of Khan et al. (2009); Nasri et al. (2015) about the preventive action of *Camellia* leaf against nephrotoxicity but the investigation also stress the significance of the abundant resource like *C.sinensis* flowers which is gaining its attention in recent researches. It has similar metabolites as a tea plant and has excess compounds like myricetin, quercetin, kaempferol, mono-diglycosides, and saponins which elevate immune response in the human system (Li et al. 2011). Hence, the present study is aimed to investigate the efficacy of tea flowers along with the *Camellia* leaf buds and their synergic effect against CP-induced nephrotoxicity in rats and also focused to characterize its bioactive metabolites.

2. Results and discussions

Cisplatin (CP), being a lead chemotherapeutic drug, is also acknowledged for its nephrotoxicity side-effect. The findings of the investigation have demonstrated that the single dose of CP induced renal injury and it is manifested by abnormal shifts in renal functional markers which indicated the enzymes were being devoured by oxidative damage. This shows that depletion of the antioxidant system is a part of the mechanism of renal injury in CP-induced rats. Hence, the use of antioxidant therapy
could prevent the detrimental side effects of CP. In an attempt to ameliorate CP-induced renal injury, the crude extract of *C. sinensis* leaf buds (CSB) and *C. sinensis* flowers (CSF) were administrated individually and in combination. Outcomes illustrated a protective effect of both CSB and CSF when used as monotherapy. Furthermore, CSB and CSF combination at 100 mg/kg B.W was more effective, which pointed out the synergic effect when compared to the control group.

An alteration in body weight, organ index, and renal panel markers in CP administered groups were considered as the significant indicators in toxicological tests (Abdel-Wahab et al. 2017). The study outcome disclosed changes in body weight (12%\textsuperscript{V}R), Kidney ratio (185%\textsuperscript{V}R), and a steady rise in the level of urea (75.7%), creatinine (54.5%), uric acid (90.7%) in blood serum, and excretion of protein (724.5%), glucose (975.8%) in the urine of drug injected rats. In the histopathological signs (Figure S1), the authors established the CP intoxicated rats glomerular, proximal, distal tubules architecture is entirely damaged and inflammation is evidenced in the renal tissue. The complete necrosis by the drug cause renal vasculature which leads to renal vasoconstriction and thus suppresses the glomerular filtration rate. Hence the observed kidney index is due to the edema of nephritic parenchyma and body weight reduction might be related to impaired tubular reabsorption. Thus the dysfunction in the kidney retains the waste products in the blood and excretes excess glucose and protein. Previous reports by Abdel-Wahab et al. (2017) have demonstrated enhanced production of reactive oxygen species in the nephritic tissue following the CP administration that induced lipid peroxidation and elicit nephropathy. These extremely reactive free radicals were detoxified by endogenous antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), and mitigate the damage in the biological system (Kapoor et al. 2019). Consistent with the literature, the findings showed enhanced LPO level (81.6%) and suppressed antioxidant range in the renal tissue (GPx-66.5%, GR-57.04%, SOD-41.1%, and CAT-44%). The changes in the balance in the CP group are due to the oxidative stress triggered when one or more of these antioxidant components inside the system get declined due to drug administration.

The current investigation utilized plant phytochemicals as prototypes for counteracting organ toxicity without compromising the drug activity. The analysis of the literature suggests green tea improved antioxidant defense, renal Pi transport, and energy metabolism, to protect against gentamicin-induced nephrotoxicity (Khan et al. 2007). Observation by Xu et al. (2012) stated the tea flowers are natural polymer with antioxidant and antitumor activities. The authors confirmed the findings that in the treated group of CSB or/and CSF along with the CP, the kidney index was decreased by 44.5%, and 35.8%, respectively compared to the CP group. The individual dose of CSB or CSF produced significant reduction of 17.0%, 39.1%, 44.8% and 14.9%, 37.9%, 34.7% respectively in terms of urea, creatinine, uric acid and decreased the urinary excretions by 63.3%, 62.5%, and 60.5%, 57.6% respectively, compared with the drug intoxicated group. It significantly (P < 0.05) reduced the elevation of LPO level by 49.7% and 46.0%, and the activity of GPx, GR, CAT, and SOD were increased by 180.9%, 136.1%, 125.5%, 112.4% and 159.9%, 103.2%, 104.5%, 99.8% respectively, compared with the CP group. The rats treated with 100 mg/kg of CSB extract (Group III)
showed less impact of cisplatin in the histopathology of the glomeruli and tubule structure of the kidney. Less expansion of lumen and vacuolation was also visible. In the treated group with 100 mg/kg of CSF extract (Group IV), there was no significant difference in the pathology of glomeruli but the tubules showed focal mild eosinophilic material within the lumen. Inflammatory infiltrates in the interstitium was less scattered and both the extracts showed less blood vessel congestion in the tissue.

Yapar et al. (2009) and Mowafy et al. (2011) demonstrated the supplementation of royal jelly with green tea, and red grape resveratrol with green tea as synergic therapy respectively to decrease CP-induced toxicity by reducing oxidative stress and systemic inflammation. Research by Jain et al. (2011) claimed the antioxidant activity of herbs was elevated by the combined therapy of various plants chemical constituents. The authors also support the hypothesis and suggest that Camellia sinensis flowers could be a potential adjuvant along with the leaf buds to treat nephritic tissues. The plant comprises distinct compounds especially floral metabolites including those found in pollen and nectar, which have a wide range of beneficial functions. This is a first investigation to report the interaction between the phytochemicals of floral and leaf buds from the shoot system of the same plant Camellia sinensis in treating nephrotoxicity. Treatment with CSB and CSF at 100 mg/kg. B.W significantly (P < 0.05) reduced serum urea, creatinine, uric acid by 32.1%, 58.9%, 68.8% respectively, and urine glucose, protein by 78.2% and 74.9%. The combined therapy more effectively reduced the renal LPO level (58.7%) and provided maximum protection by increasing anti-oxidant enzymes by 230.2%, 163.9%, 182.2%, and 118.7% respectively compared to the CP induced group (Tables S1–S4). The combined therapy reassured the intoxicated rats gained normalcy against cisplatin administrated renal injury (Figure S1). This established the synergistic protective effect of Camellia extract. In the present study, the responsible volatile compounds were identified using GC-MS techniques (Figures S2 and S3) and the identified compounds where subjected to their pharmaceutical applications based on the available literature and are summarized in Tables S5 and S6. The occurrence of these compounds in the C. sinensis plant could be an addition of new sources to the pharmaceutical industry as an antioxidant nutrition supplement.

3. Conclusion

The synergic effect of C. sinensis leaf buds and its flowers reversed the chemotherapy-induced nephropathy. The combined chemical constituents’ activity has proven to be an essential feature in the antioxidant treatment due to several important considerations, such as increasing activity through the use of multiple active components, minimizing required doses, reducing both cost and adverse side effects. As a result, the authors propose Camellia flowers as second resource along with the Camellia leaves for patients with drug-induced kidney damage.

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