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Research Paper

Optimistic Influence of Commelina benghalensis L. and *Cissus quadrangularis* L. in Alleviating Protection Against Quinalphos Induced Nephrotic Damages

Environmental Toxicology

KEYWORDS : Oxidative stress, quinalphos, natural antioxidants, nephrotoxicity.

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ABSTRACT

The present study investigated the protective and the curative effect of Commelina benghalensis (CBE) and Cissus quadrangularis (CQE) against quinalphos (QP) induced oxidative stress (OS) in kidney tissue. Induction of nephrotoxicity by QP was confirmed from the rise in serum urea, uric acid and creatinine. Especially, the level of creatinine was increased significantly which is a marked indicator for kidney damage.Treatment with CBE and CQE restored the level of kidney markers thereby protecting kidney damages as substantiated by histomorphological analysis.Among the two plants, CQE showed better protection compared to CBE. The findings concluded thatCBE and CQE play a significant role in protecting cell damages from OS caused by QP.

INTRODUCTION

The frequent use of organophosphate insecticides (OPIs) has result their wide distribution in the environment and deleterious effect on the biological system [1]. OPIs induce OS leads to generation of free radicals and thereby altering the level of free radical scavenging activity of antioxidant enzymes. Kidney is a major detoxification organ, which is the site for the elimination of reactive metabolites of many xenobiotics and is frequently susceptible to their nephrotoxic effects [2]. QP and its metabolites generate reactive oxygen species (ROS) through OS, thereby increasing the level of lipid peroxidation (LPO) which leads to testicular tissue damage [3].

Green vegetables are rich in antioxidant activity than the dietary antioxidants such as milk, egg, etc. Several researchers have emphasized the nephroprotective potential of medicinal plants. Plants are a wealthy source of free radical scavenging molecules such as vitamins, phytosteroids (terpenoids), phenolic compounds, lignins, tannins, flavanoids, alkaloids, coumarins, and other metabolites, which are rich in antioxidant activity.

C. benghalensis Linn. (CBE) is a herb, predominantly located in tropical Asia and Africa. CBE is reported to have antimicrobial, antimalignant and analgesic activity [4]. *C. quadrangularis* Linn. (CQE) is another medicinal plant and used as a common food supplement in southern India. CQE possess antimicrobial, antiulcer, antioxidative, antiobesity, cholinergic activity [5]. Both CBE and CQE are reported to possess medicinally important phytochemicals such flavonoids, phenols and phytosteroids [6].

Recently, our study has confirmed that both plant extracts are having fertility enhancing properties and effectively restoring reproductive toxicity induced by QP [7]. However, there is no data to substantiate the role of CBE and CQE in recovering nephrotoxicity caused by QP. Hence, this study was aimed to study the toxic effect of QP in kidney and explore the beneficial effects of CBE and CQE in recovering QP induced OS via antioxidant activity.

MATERIALS AND METHODS

Preparation of plant extracts

Cold water extraction

Powdered plant material was dissolved in distilled water (5/100, w/v). Then it was kept in a mechanical shaker for 48 hours. The extract was filtered through filter paper using a Buchner funnel. The filtrate was quickly frozen at -20°C and dried for 48 hours using a vacuum freeze dryer (CHRIST ALPHA, Germany).

Animals

Healthy adult male Swiss albino mice of Wistar strain *Mus musculus* weighing 30-35 g (60-70 days old) were housed in a controlled environment at 23±1°C with alternating 12 hours light-dark cycles (Humidity - 50±5%). All experiments were conducted in accordance with the guidelines for animal care by the Institutional Animal Ethics Committee (IAEC) Bharathidasan University, India.

Experimental design

The animals were divided into 9 groups randomly and each group contained 6 animals (n=6). Mode of administration is oral and the period of treatment is 7 days. Group 1 was served as control (Water), group 2 mice were orally treated with QP alone (7.5 mg/kg BW for 7 days), groups 3 and 4 mice received plant extract alone (CBE 400 mg/kg BW, CQE 350 mg/kg BW respectively). Groups from 5 to 8 mice were treated with QP along with plant extract. Groups 5 and 6 were served as protective (QP and simultaneous treatment of CBE) and curative groups of CBE (7 days QP treatment followed by next 7 days CBE treatment). Likewise, groups 7 and 8 mice were used for CQE protection (QP and simultaneous treatment of CQE) and curative treatment (7 days QP treatment followed by next 7 days CQE treatment). Group 9 was used as a withdrawal. The dose of CBE, CQE and QP was determined based on the previous studies [8, 9 and 4]

Blood and organ collection

Blood was collected by cardiac puncture and allowed to clot at room temperature. Serum was collected from the clotted blood by centrifugation (Cooling centrifuge - REMI) at 1500×g for 15 minutes at 4°C and stored at -80°C until analyses. Serum was used for biochemical analysis. Organs were excised immediately, washed with ice-cold physiologic saline solution (0.9%, w/v), blotted and weighed. Small representative pieces were fixed at 10% buffered-neutral formalin for routine histopathology.

Serum and Histomorphological analysis

The sera were assayed for kidney markers (Urea, uric acid and creatinine) by using Biosystem kits, Spain. Histology of kidney was studied by the method of Humason [10].

Statistical analysis

All data were expressed as mean ± SD. The statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS (version 17.0, Cary, NC, USA) and the individual com-

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parisons were obtained by Duncan's Multiple Range Test. A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS AND DISCUSSION

Observations of the present study reveal that the exposure to QP rigorously reduced the weights of kidney to several folds in QP administered mice (Group 2). However, oral supplementation of CBE and CQE showed significant improvement in kidney weight. Both protective and curative treatment of CBE and CQE exhibited almost similar effect (Table 1).

Free radicals have been implicated in several biological processes potentially important in glomerular diseases and also their role in neutrophil mediated glomerular diseases [11]. Rise in the levels of urea, uric acid and creatinine in serum are indicative of renal injury, which confirms functional damage to the kidney. The level of urea can be increased by many other factors such as dehydration, antidiuretic drugs and diet. On the other hand, creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level [12]. Oral administration of QP (Group 2) significantly ($p<0.05$) increased the level of serum kidney markers. Treatment of plant extracts brought back the level to normal. In CBE treatment, simultaneous treatment showed better recovery than later treatment, whereas, in the CQE treatment both concurrent and subsequent treatment considerably maintained. CQE treatment offered maximum nephroprotection compared to CBE treatment. There were no changes in plant extracts alone treated groups (Groups 3 and 4) compared with control (Group 1) (Table 2). Histoarchitecture of kidney also confirmed the effect of OS triggered by QP. Mice intoxicated with QP exhibited severe glomerular and tubulo-interstitial necrosis which was characterized by hydropic degeneration of the glomerular and tubular cells with complete destruction of the tubular lumen (from hydropic degeneration and tubular casts) (Fig. 1(2)) when compared to the normal mouse kidney (Fig. 1(1)). However, concurrent treatments with CBE and CQE ameliorated (Fig. 1 (5) and (7)) normal glomeruli (GL) encapsulated by normal Bowman's capsule (BM). Similarly, post supplementation of plant extract tremendously restored when compared to QP intoxicated group (Group 2) (Fig. 1 (6) and (8)). Histology of kidney clearly exhibited the efficacy of CBE and CQE as nephro-protectors. Some studies have claimed that flavonoids and terpenoids are antioxidant agents which interfere with free radical formation [13] thereby inhibiting the free radical mediated damage [14]. Therefore, we believed that the increased antioxidant activity of CBE and CQE might be due to the presence of high level of phytosterols, phenolics and flavonoids.

In conclusion, the present study showed for the first time that the aqueous extract of CBE and CQE significantly restored the QP induced toxicity in kidney However, simultaneous treatment of CQE showed beneficial effects on QP induced toxicity and it is believed that treatment of CBE and CQE could have healed the kidney through the antioxidant mechanisms.

Table 1. Effect of C. *benghalensis* and C. *quadrangularis* on kidney weight of QP exposed male mice

Groups	Kidney (g)	
	Right	Left
Control	0.334 ± 0.01^{ab}	0.285 ± 0.01^{c}
QP	0.226 ± 0.02^{c}	0.172 ± 0.02^{fg}
CBE alone	0.321 ± 0.02^{b}	0.3198 ± 0.02^{a}
CQE alone	0.356 ± 0.02^{ab}	0.3024 ± 0.001^{b}
QP+CBE (Preventive)	0.25 ± 0.03^{c}	0.258 ± 0.001^{ef}
QP+CBE (Curative)	0.360 ± 0.04^{a}	0.2614 ± 0.004^{e}
QP+CQE (Preventive)	0.246 ± 0.04^{c}	0.2744 ± 0.003^{d}
QP+CQE (Curative)	0.32 ± 0.01^{b}	0.26 ± 0.007^{e}
Withdrawal	0.22 ± 0.04	0.144 ± 0.023^{g}

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	52.31 ± 2.13^{g}	2.29 ± 0.49^{f}	1.30 ± 0.38^{f}
QP	176.42 ± 4.83^{b}	8.28 ± 0.22^{b}	18.54 ± 2.20^{b}
CBE alone	50.80 ± 0.94^{gh}	2.28 ± 0.22^{f}	1.398 ± 0.18^{f}
CQE alone	45.96 ± 4.52^{h}	2.34 ± 0.33^{f}	1.38 ± 0.09^{f}
QP+CBE (Preventive)	121.65 ± 2.28^{c}	5.27 ± 0.26^{d}	8.91 ± 0.21^{c}
QP+CBE (Curative)	105.79 ± 5.32^{d}	7.35 ± 0.27^{c}	10.72 ± 0.62^{c}
QP+CQE (Preventive)	62.44 ± 3.5^{f}	3.68 ± 0.31^{e}	5.99 ± 0.49^{d}
QP+CQE (Curative)	93.61 ± 2.75^{e}	4.14 ± 0.43^{e}	3.60 ± 0.48^{e}
Withdrawal	241.30 ± 6.38^{a}	9.82 ± 0.91^{a}	28.92 ± 3.50^{b}

Table 2. Effect of *C. benghalensis* and *C. quadrangularis* on kidney markers of QP administered male mice

All values are mean ±SD. Values with different superscripts are significantly different among the groups by ANOVA with Duncan's multiple range test at $p<0.05$.

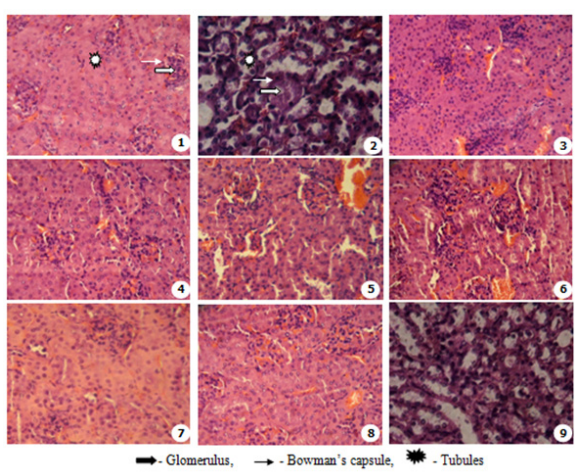

figure 1. i.e Glomerulus, Bowman's capsule and tubules is not clearly visible

(1) Control (2) QP treated (3) CBE alone (4) CQE alone (5) QP+CBE (preventive) (6) QP+CBE (curative) (7) QP+CQE (preventive) (8) QP+CQE (curative) (9) Withdrawal.

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