RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2015.45.51

Antioxidant Effects of *Matricaria chamomilla* L. in Paraquat Induced Kidney Oxidative Damage in Rats

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ABSTRACT

Background and Objectives: Paraquat (PQ), one of the most widely used herbicides, is highly toxic to humans and animals. There is much information regarding its toxic effects on the lungs, but less is known about its toxicity in other organs. PQ is thought to play pivotal roles in the pathophysiology of acute renal failure and the progression of chronic kidney disease. The aim of this study was the effect of hydroalcoholic extract *Matricaria chamomilla* L. (*M. chamomilla*) against PQ-induced kidney injury in association with its antioxidant activity. **Methodology:** The male rats were treated with gavages' daily with PQ (5 mg/kg/day) and *M. chamomilla* (50 mg/kg/day) were administered alone or in combination for 7 days. After treatments, blood urea nitrogen and creatinine levels, Total Antioxidant Capacity (TAC), total thiol molecules (TTG) levels and catalase CAT activity in kidney tissue were measured. **Results:** Rats administered PQ showed increased blood urea nitrogen and creatinine levels and CAT activity, TAC and TTG was decreased compared with control rats. Co-administration of PQ with *M. chamomilla* extract increased TAC and TTG in kidney tissue as compared with PQ group. **Conclusion:** These results showed that PQ-induced nephrotoxicity may be caused by oxidative damage in rat kidneys and that *M. chamomilla* could protect kidneys from PQ-induced toxicity.

Key words: Paraquat, reactive oxygen species, *Matricaria chamomilla* L., kidney

Pharmacologia 6 (1): 45-51, 2015

INTRODUCTION

Paraquat (N,N-dimethyl-4,4-bipyridinium) (PQ), one of the most widely used herbicides, is highly toxic to humans and animals (Suntres, 2002). There is much information regarding its toxic effects on the lungs, but less is known about its toxicity in other organs (Suntres, 2002; Delaval and Gillespie, 1985; Dinis-Oliveira *et al*., 2008). Critical PQ poisoning is characterized by multiple-organ failure, involving mainly the lung (Smith, 1987; Papiris *et al*., 1995), kidney (Kim *et al*., 2009; Lock and Ishmael, 1979) and liver (Amirshahrokhi and Bohlooli, 2013; Hirai *et al*., 1992). The mechanisms involved in PQ-induced cell injury are not clearly understood. However, several studies demonstrated that Reactive Oxygen Species (ROS) may be important mediators in PQ-induced nephrotoxicity (Suntres, 2002; Webb, 1982). Unusual production of ROS directly damages some macromolecules and induces cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein oxidation and DNA damage (Sies, 1997; Halliwell, 2011). Thus, the administration of several compounds with antioxidant activity has been successfully used to prevent or ameliorate PQ-induced nephrotoxicity (Samai *et al*., 2007; Yoon *et al*., 2011). In the past few years, much interest has been laid on the

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role of naturally occurring dietary substances and herbal medicine for the control and management of various disease and poisoning (Stickel and Schuppan, 2007; Li *et al*., 2011; Cemek *et al*., 2010). *Matricaria chamomilla* L. (*M. chamomilla*) is an annual medicinal plant frequently used in herbal teas and for the extraction of bioactive constituents (i.e., sesquiterpenes, phenolics, coumarins, coumaroyl spermidine and polyacetylenes) that accumulate in the anthodia (Hojati *et al*., 2011; Namjooyan *et al*., 2011) Also, *M. chamomilla* has been used with inflammatory, antioxidant and antimicrobial effects (Abdoul-Latif *et al*., 2011; Owlia *et al*., 2007).

Increasing evidence demonstrates that applied *M. chamomilla* can neutralize oxidative damage induced by adverse conditions in animals (Cemek *et al*., 2008, 2010), though the mechanisms underlying these effects remain unclear. It has been reported that *M. chamomilla* comprise free radical-scavenging and antioxidative properties (Cemek *et al*., 2010; Namjooyan *et al*., 2011).

The aim of the present study was therefore to investigate whether *M. chamomilla* treatment prevents PQ-induced nephrotoxicity. For this purpose, we have examined oxidative effects of PQ and possible protective effect of *M. chamomilla* on tissue damage of rat kidney. We have also examined tissue Total Antioxidant Capacity (TAC), total thiol molecules (TTG) levels for protein oxidation and catalase CAT activity in kidney tissue and Blood Urea Nitrogen (BUN) and creatinine (Cr) levels in order to evaluate kidney function.

MATERIALS AND METHODS

Chemicals: Ethylenediamine Tetra Acetic Acid (EDTA), dithiobis-2-nitrobenzoic acid (DTNB), tris base and 2, 4, 6-tripyridyl-S-triazine (TPTZ), hydrogen peroxide (H_2O_2) and paraquat were used in this study. All other chemicals were obtained from the Sigma.

Animal treatment: Male Wistar rats (180-250 g) were obtained from the animal colony of the Pastor Institute, Iran. Animals were maintained under standard conditions of temperature (22±1°C), humidity (45-55%) and light (12/12-h light/dark cycle). The rats in control group (n = 5) were treated with the saline solution. The rats in PQ-treated group (n = 5) were orally given solution of PQ (5 mg/kg/day) by gastric gavage. The rats in hydroalcoholic extract *M. chamomilla* were orally given by gastric gavage (50 mg/kg/day) (n = 5). The rats in PQ (5 mg kg^{-1})+*M. chamomilla* flower (50 mg kg^{-1}) group (n = 5) were orally given aqueous solution by gastric gavage was administered for 7 consecutive days. The experiments were conducted according to the ethical rules approved by Institutional Review Board (IRB).

Sample collection: The whole kidney dissects were homogenized in phosphate saline (100 mM) containing EDTA (1 mM, pH 7.4, 1:10 w/v) and centrifuged. The supernatant was separated and used for biochemical analysis (Nabavi *et al*., 2012).

Plant materials and extraction procedure

Plant material: The powder of *M. chamomilla* (Asteraceae) were collected from the region of Hamadan, Iran and air-dried at 40°C. The plant was deposited at the herbarium of the Faculty of Pharmacy in Hamadan University.

Preparation of the aqueous extract: Dried and finely powdered aerial parts (1000 g) were extracted with ethanol 50% (3×5 L) at room temperature for 4 weeks. After removal of the solvent in vacuuo at 50°C, the residue (300 g, 30%, w/w) was stored at 4°C in sealed vials until usage (Macchioni *et al*., 2004).

Biochemical analysis

Estimation of kidney markers: BUN and Cr level in were estimated according to the standard procedure of kits (Pars Azemon kit, Iran).

Measurement of biomarkers of oxidative stress

Catalase (CAT) activity assay: Catalase (CAT) activity was assayed in the samples by measuring the absorbance decrease at 240 nm in a reaction medium containing H_2O_2 (10 mM), sodium phosphate buffer (50 mM, pH ¼ 7.0). One unit of the enzyme is defined as 1 mol H_2O_2 as substrate consumed/min and the specific activity is reported as units/mg protein (Johansson and Borg, 1988).

Assay of Total Antioxidant Capacity (TAC): It was measured by Ferric Reducing Ability of Plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (Benzie and Strain, 1999).

Assay of total thiol molecules (TTG): To evaluate the plasma total thiol molecules, DTNB was used as a reagent. DTNB reacts with thiol molecules and create a yellow complex which has good absorbance at 412 nm in spectrophotometer (Hu and Dillard, 1994).

Total protein: Protein concentrations in the samples were measured by the Bradford method using concentrated Coomassie blue reagent. Bovine serum albumin was used as the standard (Bradford, 1976).

Statistical analysis: Mean and standard error values were determined for all the parameters and the results were expressed as Mean±SEM. All data were analyzed with SPSS Version 11.5 employing one-way ANOVA followed by Turkey *post hoc* test. Differences between groups were considered significant when $p<0.05$.

RESULTS

Effect *M. chamomilla* extract on the kidney markers: Figure 1 and 2 shows the Mean±SE of variables related to creatinine (Cr) level and Blood Urea Nitrogen (BUN) in animals test. PQ caused a significant increase in Cr and BUN when compared to control ($p<0.01$). *Matricaria chamomilla* caused a significant decrease in Cr and BUN when compared to PQ group ($p<0.01$). Coadministration of *M. chamomilla* with PQ significantly reduced PQ induced Cr and BUN ($p<0.01$) (Fig. 1a-b).

Effect *M. chamomilla* extract on the oxidative stress parameters

Catalase (CAT) activity: PQ caused a significant increase in CAT activity when compared to control ($p<0.05$). *Matricaria chamomilla* caused a significant decrease in CAT activity when compared to PQ group ($p<0.05$). Coadministration of *M. chamomilla* with PQ significantly reduced PQ induced CAT activity ($p<0.05$) (Fig. 2a).

Total thiol molecules (TTG): No significant difference was observed TTG in PQ group when compared to control. *Matricaria chamomilla* caused a significant increase in TTG when compared to PQ group ($p<0.05$). Coadministration of *M. chamomilla* with PQ significantly induced PQ induced TTG ($p<0.05$) (Fig. 2b).

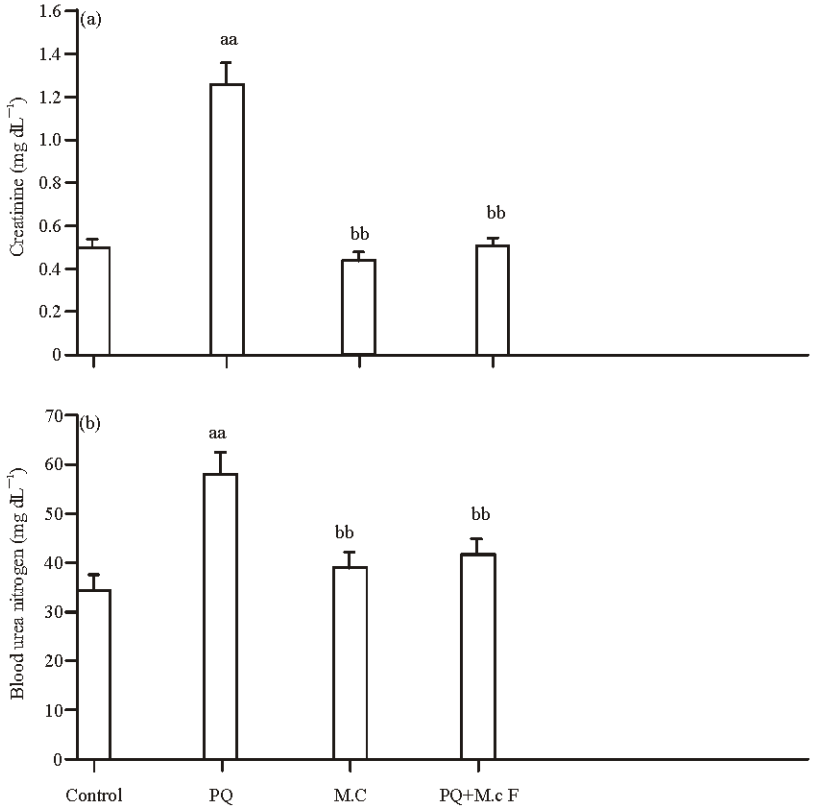

Fig. 1(a-b): (a) Creatinine (mg dL^{-1}) and (b) Blood urea nitrogen (mg dL^{-1}) level of blood rats. ^{aa}: Significantly different from control group at $p<0.05$, ^{bb}: Significantly different from PQ group at $p<0.05$, PQ: Paraquat, M.c: *M. chamomilla*, PQ+M.c F: Paraquat+flower of *M. chamomilla*

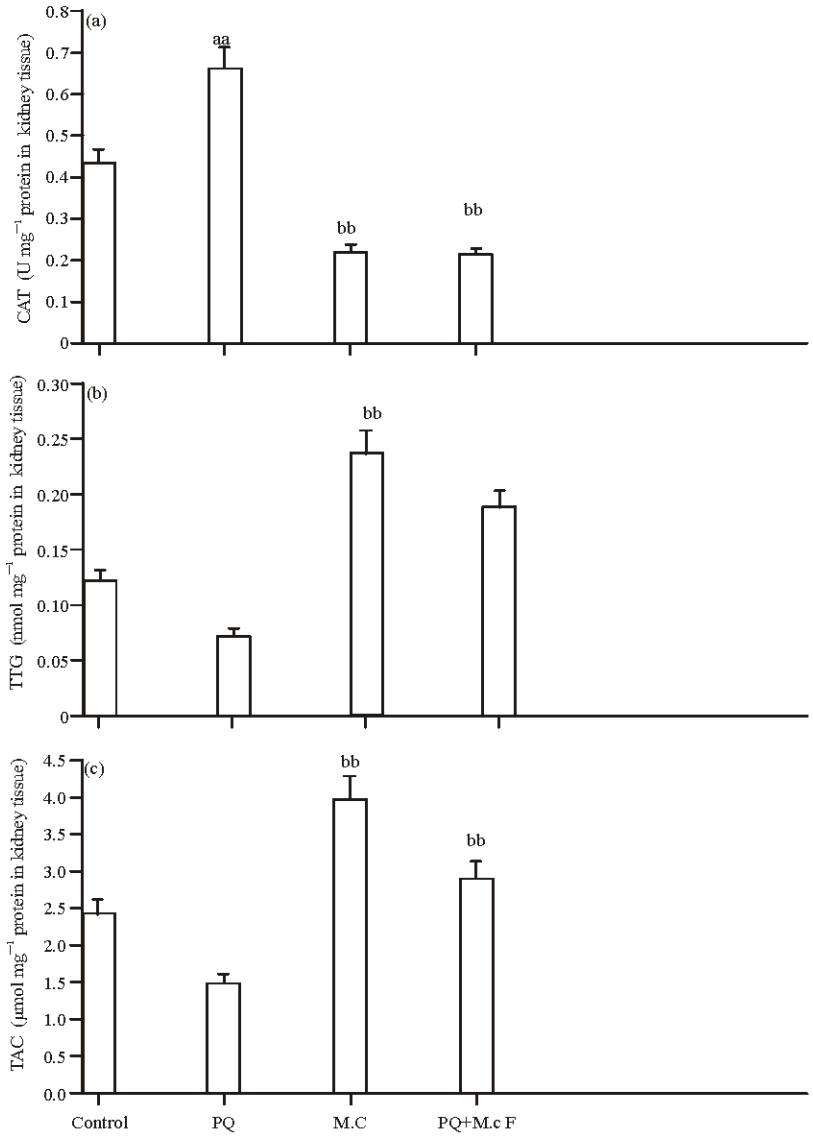

Fig. 2(a-c): (a) Catalase (CAT) activity (b) Total thiol molecules (TTM) and (c) Total Antioxidant Capacity (TAC) in kidney tissue of rats. [aa]: Significantly different from control group at $p<0.05$, [bb]: Significantly different from PQ group at $p<0.05$, PQ: Paraquat, M.c: *M. chamomilla*, PQ+M.c F: Paraquat+flower of *M. chamomilla*

Total Antioxidant Capacity (TAC): No significant difference was observed TAC in PQ group when compared to control group. Treatment with *M. chamomilla* increased TAC as compared to PQ group ($p<0.05$). Coadministration of PQ with *M. chamomilla* significantly increased PQ induced TAC level ($p<0.05$) (Fig. 2c).

DISCUSSION

This study evaluated the antioxidant capacity of hydroalcoholic extract of *M. chamomilla* against the renal toxicity induced by PQ in rat. PQ induced oxidative stress in renal and Cr and BUN levels in serum. Additionally, the level of TAC and TTG were decreased by PQ treatment. *Matricaria chamomilla* extract reduced the alterations in these parameters. It can be inferred that PQ causes kidney damage or nephrotoxicity via the propagation of free radicals while *M. chamomilla* prevented the kidney damage by scavenging of free radicals and stabilizing the oxidative status. Significant increase in the Cr and BUN levels in serum indicating severe damage to kidney samples (Singh *et al*., 2008).

Matricaria chamomilla prevented the kidney injuries with a subsequent restoration of these parameters due to the presence of flavonoids, phenols, saponins, tannins and alkaloids (Babenko and Shakhova, 2006). In this study, our results showed that PQ induced Cr and BUN levels in blood.

Although the mechanism involved in PQ nephrotoxicity has been extensively studied, they are not yet fully elucidated. Generation of ROS plays a major part in the development of PQ-induced toxicity and especially nephrotoxicity (Yoon *et al*., 2011; Vaziri *et al*., 1979; Lock and Ishmael, 1979).

It has been shown that PQ generates ROS and stimulates lipid peroxidation in the kidney (Lock and Ishmael, 1979; Sood *et al*., 2011). PQ also inhibits the antioxidant capacity and increase protein oxidation and lipid peroxidation in chronic exposure (Ranjbar *et al*., 2002). As a result the balance normally present in cells between free radical formation and protection against them is disturbed causing oxidative damage to cell components, e.g., proteins, lipids and nucleic acids especially in certain organelles such as plasma membranes, mitochondria, microsomes and lysosomes in the kidney and other tissues (Forbes *et al*., 2008; Patra *et al*., 2001; Araujo and Wilcox, 2014). In agreement with previous observations PQ significantly increased the activities of CAT and reduced TAC and TTG in renal tissues indicating PQ induced oxidative damage (Araujo and Wilcox, 2014; Chang and Yoon, 2012; Yoon *et al*., 2011). Current research has therefore focused on the therapeutic potential of antioxidants against paraquat-induced toxicity, especially in recent years, the concept of chemoprevention by naturally occurring dietary substances has been strengthened (Suntres, 2002; Okolonkwo *et al*., 2013; Kim *et al*., 2006). *Matricaria chamomilla* provides a dietary source of biologically active compounds that help prevent a wide variety of diseases. *Matricaria chamomilla* and herbal medicine extract contain polypohenols, chiefly catechins and their derivatives which possess antioxidant, antimutagenic and anticarcinogenic effects that reduce the risk of various forms of cancer, cardiovascular and renal disorders against certain environmental agents (Cemek *et al*., 2008, 2010; Kovacik *et al*., 2009; Singh *et al*., 2011; Abt *et al*., 1995). *Matricaria chamomilla* has been shown to lower cisplatin-induced nephrotoxicity and oxidative stress (Salama *et al*., 2011; Salama, 2012). The present results show that *M. chamomilla* significantly enhanced antioxidant defense mechanism albeit differentially in kidney tissues. The activity of CAT significantly increased in renal in PQ group but *M. chamomilla* modulate its activity. However, *M. chamomilla* induced TAC and TTG against PQ nephrotoxicity.

Thus, *M. chamomilla* consumption offered a significant protection from PQ-induced oxidative damage as evident by significant increase of either CAT in kidney tissue in combination PQ and *M. chamomilla* treated compared to the rats treated with PQ alone. TAC and TTG significantly increased in the kidney tissue were improved compared to values obtained in PQ treated rats.

CONCLUSION

The results of present study showed that PQ induces generation of free radicals that causes oxidative damage to kidney tissue. In contrast *M. chamomilla* reduces oxidative stress by virtue of its antioxidant properties in the kidney. Taken together, these beneficial effects of *M. chamomilla* were able to ameliorate PQ-induced nephrotoxicity and oxidative damage.

Based on our present observations and already known numerous health benefits, we propose that *M. chamomilla* may provide a cushion for prolonged therapeutic option against pesticides nephrotoxicity without harmful side effects.

ACKNOWLEDGMENT

This study was supported by a grant from Vice Chancellor of Research of Hamadan University of Medical Sciences.

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