Evaluation of protective effect of hydroalcoholic extract of saffron petals in prevention of acetaminophen-induced renal damages in rats

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Abstract

In recent years more attention has been given to herbal drugs in the treatment and prevention of drug toxicity because of the harmful effects of chemical drugs. In this study, directed for this purpose, research was conducted on the protective effect of hydro-ethanolic extract of saffron petals (SPE) against acetaminophen (APAP) induced acute nephrotoxicity. Twenty-four male Wistar rats were distributed into four groups of six each. Group I, as a control group, received normal saline (0.09%) orally (PO). Group II, as an intoxicated group was treated with APAP, PO (600 mg/kg). In the groups III and IV, SPE in a dose of 10 and 20 mg/kg along with APAP (600 mg/kg) was administered, respectively. At the end of the trial (8th day), blood was taken from the heart of rats for assessment of biochemical parameters and the right kidney was placed in 10% buffered formalin for histopathological evaluations. In the APAP treatment group, higher serum creatinine and uric acid were observed. SPE in a dose of 20 mg/kg significantly reduced serum creatinine and uric acid. In pathologic evaluation, a dose of 20 mg/kg of SPE prevented the kidney injuries induced by APAP. Tissues changes were in accordance with biochemical findings. It is likely that the SPE contributed to the prevention of acute nephrotoxicity induced by APAP.

Introduction

Acetaminophen (APAP or paracetamol), chemically named N-acetyl-p-aminophenol, is a widely used nonprescription analgesic and antipyretic medication. Use of APAP in therapeutic doses is safe. APAP is rapidly and almost completely absorbed from the gastrointestinal tract, but overdose of the drug leads to life-threatening or fatal hepatic necrosis and renal failure.[1] APAP is now the most common drug in self-poisoning, with a high rate of morbidity and mortality.[2] Many cases of poisoning with APAP in humans and animals have been reported.[3-7] Nephrotoxicity is less common than hepatotoxicity, but acute renal failure is possible, even in the absence of liver damage. Renal failure can occur even with therapeutic doses.[8] Renal effects of APAP have received less attention. Administration of N-acetyle cycstein (NAC) is used to treat APAP-induced hepatotoxicity but glutathione is more effective than NAC in preventing liver injury.[9] NAC leads to increased hepatic glutathione, but is not able to protect the kidney against APAP.[10] Moreover, it must be noted that conjugates of glutathione have been implicated in the formation of nephrotoxic compounds.[11] Uric acid is an end product of the metabolism of purine through the action of xanthine dehydrogenase or xanthine oxidase. Serum uric acid can be elevated due to reduced excretion by the kidney. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Low clearance values for creatinine indicate a diminished ability of the kidneys to filter waste products from the blood. As clearance levels of kidneys for excretion in the urine decrease, blood levels of uric acid and creatinine increase.[12,13] Several plants reported to contain antioxidant compounds act as hepatoprotective.[14-16] Saffron (*Crocus sativus* L.) is one of the most important and valuable herbs of Iran. Saffron is a perennial stemless herb with numerous medical properties used in traditional medicine.[17] Beneficial effects of saffron in the treatment or prevention of kidney disorders have been noted.[18,19] Various pharmacological studies have been done on saffron by researchers. Some effects such as anticonvulsants, antihypertensives,[20] antinociceptive and anti-inflammatory,[21] antidepressants,[22] antimicrobial,[23] anticancer and chemopreventive agent,[24,25] free radical scavenging,[18] and the prevention of cisplatin-induced nephrotoxicity using the stigma of saffron have been mentioned.[26]

Positive linear correlation was noted between the phenolic content and antioxidant capacity of the medicinal herbs.[27] Saffron petals are a rich source of flavonoids that can be potentially used as an antioxidant compound in pharmaceutical research.[28,29] Flavonoids are powerful antioxidants and prevent DNA damages.[30] Saffron petals are one of the easily accessible sources of natural antioxidants and several of them are obtained during the saffron processing. Several studies have shown that the saffron petals have a large variety of flavonoid compounds, glycosides and anthocyanins.[31] The concentration of 500 ppm of extract of saffron petals (SPE) is equal to a synthetic antioxidant (TBHQ) at the level of 100 ppm *in vitro*.[32] Antioxidants can neutralize free radicals in the environment and prevent the damaging effects of them. According to the available resources, effect of SPE in the prevention or treatment of nephrotoxicity induced by APAP has not been studied. SPE has been shown to be protective against APAP induced liver necrosis.[16] This observation prompted us to study whether SPE protects against APAP induced renal damage.

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Key words: Acetaminophen; rat; renal toxicity; saffron petals.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 21 January 2015.
Revision received: 4 March 2015.
Accepted for publication: 10 March 2015.

Veterinary Science Development 2015; 5:5821
doi:10.4081/vsd.2015.5821

Materials and Methods

Animals

Twenty-four Wistar rats weighing 220±20 g were individually housed in 25×22×20 cm stainless steel cages in the standard rat house (22-25ºC on a 12 h light-dark cycle) and were fed on a pellet diet (Javaneh Khorasan Co, Mashhad, Iran). The rats had free access to food and distilled water.

Animal ethics

All the rats received humane care in accordance with the approval of Institutional Ethics Committee rules of the Agriculture Faculty of Birjand University. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes were followed.

Preparing the petal extract

Saffron was collected from Hajiabad Village (Kashmar) in Khorasan-Razavi province, northeast Iran, in December 2012 (Figure 1) and the samples were identified by the Agricultural Faculty of Birjand University in Iran. The voucher number of specimen (No. 2669) was deposited in the herbarium of Birjand University as well. To prepare the SPE, the samples were dried in shade and then pulverized with a grinder (Hamilton Beach Brand, Canada). Hydroalcoholic extract of SPE was prepared using 50 g of dried powder in 1000 mL of 80% v/v ethanol and shaking for 24 h. The mixture was filtered through a No. 1 Whatman filter paper and then oven-dried at 40°C for 24 hours. The yield (w/w) was 30% after the final powdered extract was weighed and calculated.

Treatment schedule

One week after the adaptation period, rats were divided into 4 groups of 6 animals. Experimental groups include: Group I: received 5 ml normal saline (0.9% NaCl) daily by gavage method for 7 days. Group II: received 5 ml normal saline (0.9% NaCl) daily for 6 days. On the 7th day rats in this group received APAP (600 mg/kg) by gavage method. Group III: received low dose of SPE (10 mg/kg) daily for 6 days. On the 7th day the rats received APAP (600 mg/kg) by gavage method. Group IV: received high dose of SPE (20 mg/kg) daily for 6 days. On the 7th day they received APAP (600 mg/kg) by gavage method. The SPE doses were selected based on the previous reports and pilot studies.[29] SPE was dissolved in normal saline and the pure powder of APAP was dissolved in 20% alcohol, while all the solutions were administered by oral gavage. After 24 hours of APAP intoxication, rats were euthanized by ether and then sacrificed.

Biochemical and histopathological analysis

The blood sample was collected by cardiac puncture and the serums separated by centrifugation of the samples at 750 *g* for 15 min at room temperature, they were then stored at –21°C for further analysis. The concentration of creatinine was determined by the Jaffé method.[33] Colorimetry and uricase method were used to measure uric acid level (Uric acid TOOS kit, Pars Azmoon Co, Iran). After necropsy, the right kidney was removed and fixed in 10% formalin buffer at room temperature. After fixing the tissue, it was thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared afterwards and embedded in soft paraffin. Tissue sections of about 5 μm were obtained, and stained by hematoxylin and eosin (H&E), then observed under a light microscope.[34]

Statistical analysis

All the tested parameters were subjected to statistical analysis which was done by One-way Analysis of Variance (ANOVA) and means were compared by Dunnett's comparison. The level of significance was set at $P<0.05$.

Results

Biochemical results obtained from measurements of creatinine and uric acid are shown in Figure 2. In rats that received the toxic dose of APAP, creatinine and uric acid levels were significantly increased compared with the control group. The results showed that both doses of 10 and 20 mg/kg of SPE significantly decreased cre-

Figure 1. Purple colored petal of saffron (*Crocus sativus*).

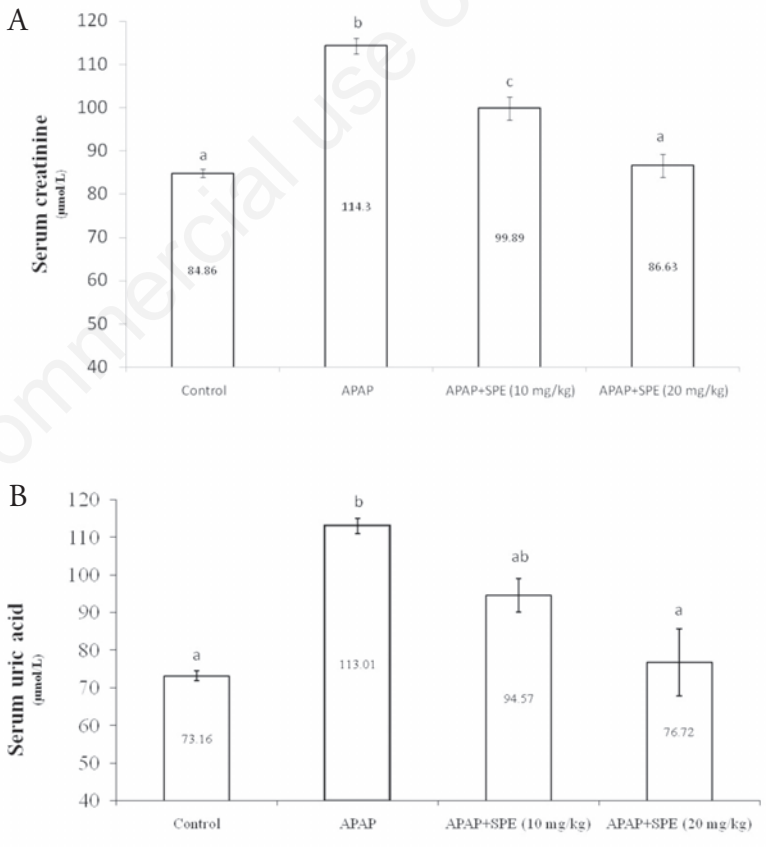

Figure 2. Effect of saffron petal extract (SPE) on acetaminophen (APAP)-induced renal damage in rats. Kidney damage was assessed 24 h after administration of APAP. Data are Mean ± SEM of 24 rats for serum creatinine (A) and serum uric acid (B). Control: received normal saline for 7 days orally; 2, intoxicated with APAP: received normal saline orally in first six days and on the seventh day received a dose of 600 mg APAP per kg body weight orally; 3, Groups received low dose of SPE (10 mg/kg body weight) in the first six days and on the seventh day, an hour after gavage of extract, received a dose of 600 mg APAP per kg orally; 4- Groups received low dose of SPE (20 mg/kg body weight) in the first six days and on the seventh day, an hour after gavage of extract, received a dose of 600 mg APAP per kg orally; [a,b,c]Values not sharing a common superscript letter in each column differ significantly at $P<0.05$.

atinine levels compared with APAP, but the reduction by dose of 20 mg/kg was more. High dose of SPE (20 mg/kg) significantly decreased elevated levels of serum uric acid caused by APAP ($P<0.05$). Low dose of SPE (10 mg/kg) reduced elevated serum levels of uric acid numerically. Rats that received APAP had severe kidney damage such as glomerular and tubular necrosis, interstitial tubular nephrosis and inflammation in the kidney. However, when the SPE was combined with APAP, kidney damages were less than in the case of APAP alone. In rats that received normal saline, normal kidney tissue appearance was observed (Figure 3A). In rats that received APAP alone, evidence of necrosis and impaired renal glomeruli, tubules and interstitial tissue were seen (Figure 3B). In rats that received a toxic dose of APAP along with low doses of SPE (10 mg/kg), kidney damage was less severe than the intoxicated rats. Hyaline casts and mild interstitial nephritis were observed in this group (Figure 3C). In rats treated with a high dose of SPE (20 mg/kg), histologic appearance of the kidney was almost similar to the normal kidney. Tubules necrosis and impaired renal glomeruli were considered insignificant (Figure 3D).

Discussion

Biochemical and pathologic findings represent kidney injury with toxic dose of APAP. These results are consistent with the findings of Zhao *et al.* and Kelkar *et al.*[35,36] Orally APAP (600 mg/kg) in rats produces nephrotoxicity indicated by significantly elevated serum levels of uric acid and creatinine. In general, harmful effects of APAP have been attributed to the production of its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). The metabolite is normally detoxified by reaction with glutathione and becomes soluble and is excreted through the kidneys. When large amounts of APAP are taken, overproduction of toxic metabolites causes depletion of hepatic glutathione reserves, therefore compromising the antioxidant status of cells. NAPQI covalently binds to cellular proteins, oxidizes protein sulfhydryls, and produces oxidative stress in association with liver necrosis.[37] In the liver, once glutathione is depleted, NAPQI covalently binds to cellular proteins and lipids initiate lipid peroxidation and result in damage to the kidneys.[10] Some constituents are hypothesized to be involved in APAP-induced renal toxicity such as cytochrome P-450 enzymes, glutathione S-transferase, prostaglandin endoperoxidase synthase (PGES), N-deacetylase, tumor necrosis factor (TNF)-α and oxidative stress.[38,39] NAC protects against APAP hepatotoxicity, but is unable to protect against APAP nephrotoxicity.[40] The results show that a dose of 20 mg/kg of SPE had a better protective effect than dose of 10 mg/kg. In the present study, histopathological findings also indicate renal interstitial necrosis and severe damage to the glomeruli and tubules by APAP. Slight glomerular and tubular damages were seen in prescription of SPE along with APAP and there was no evidence of necrosis. The findings of the present study indicate a protective effect of SPE against nephrotoxicity induced by APAP. Different pharmacological effects have been seen from saffron and its active constituents. Inhibition of oxidative stress induced by free radical oxygen species and increased glutathione synthesis could be mentioned in the reduction and prevention of acute toxicity and renal tubular damages caused by cisplatin.[41] It has been shown that the stigma of saffron has protective effect against gentamicin-induced nephrotoxicity in rats.[42] Also, it has been shown in mice that the extract of saffron stigma has a protective effect on the liver tissue against the effects of rifampin.[43] Aqueous saffron extract and crocin, its active constituent, can protect rats from oxidative damages due to renal ischemia-reperfusion.[18] Crocin is effective in prevention of acute liver damages induced by aflatoxin B1.[44] It is likely that the anti-oxidant properties of SPE are a protective mechanism that prevents free radicals from being produced by cytochrome p-450 system. Antioxidants can neutralize free radicals in the environment and prevent the harmful effects of them. However, it is likely that the SPE increases supply of glutathione in the liver.[45] SPE might be a potential candidate agent against APAP-induced nephrotoxicity, but further studies are required to identify the side effects of the saffron petals as a rich source of antioxidants.

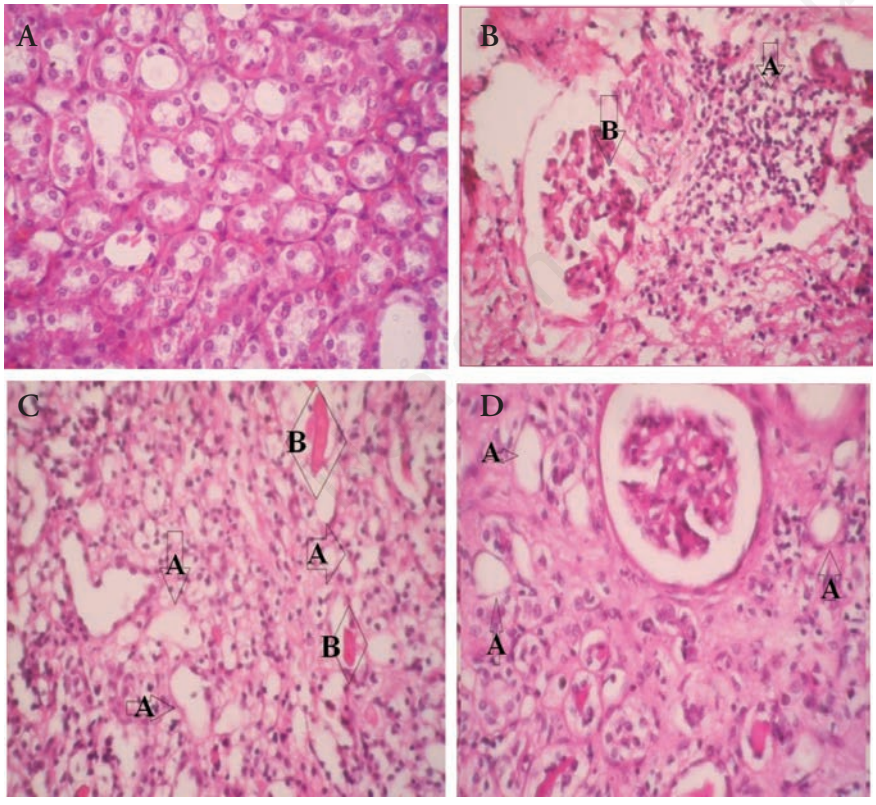

Figure 3. A) Light microscopic appearance of the rat kidney of the healthy rat; normal renal proximal and distal tubules are seen; B) light microscopic appearance of the rat kidney of poisoned rat; interstitial nephritis (arrow A) and glomerulonephritis (arrow B) are seen; C) light microscopic appearance of the kidney of a poisoned rat treated with low dose of saffron petal extract (SPE), (10 mg/kg); mild interstitial nephritis (arrow A) and hyaline casts (arrow B) are seen; D) light microscopic appearances of the kidney of a poisoned rat treated with high dose of saffron petal extract (SPE), (20 mg/kg); mild necrosis in renal tubules (arrow A) are seen (H&E stain, 40×).

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