http://www.herbmedpharmacol.com doi: 10.15171/jhp.2019.11

Journal of Herbmed Pharmacology

Protective effect of ivy leaf extract on paracetamol-induced oxidative stress and nephrotoxicity in mice

Pedram Moshaie-Nezhad[1], Seyed Mohammad Hosseini[2], Mohsen Yahyapour[3], Maryam Iman[1*], Ali Khamesipoure[4]

[1]Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Department of pathology, Babol branch, Islamic Azad University, Babol, Iran
[3]Faculty of Veterinary medicine, Babol branch, Islamic Azad University, Babol, Iran
[4]Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical. Sciences, Tehran, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 27 October 2018
Accepted: 1 December 2018

Keywords:
Ivy leaf extract
Oxidative stress
Medicinal plant
Protective effect
Kidney

ABSTRACT

Introduction: The aim of the study was to evaluate the protective effect of *Hedera helix* (ivy) leaf extract on paracetamol-induced oxidative stress and renal toxicity in mouse model.
Methods: Sixty male Swiss albino mice were randomly divided into six equal groups. Group A: as the control group, received NaCl 0.9%. Group B: received a single (i.p.) injection of paracetamol (600 mg/kg). Pretreatment groups: included T1 (50 mg/kg), T2 (100 mg/kg), T3 (200 mg/kg), and T4 (300 mg/kg) which were treated with ivy leaf extract in different doses. The mice were sacrificed under mild anesthesia and the blood samples were collected to titrate the levels of serum blood urea nitrogen (BUN), creatinine and, uric acid. Kidneys were removed for histopathological examination.
Results: Paracetamol administration significantly increased the BUN, creatinine and uric acid levels ($P < 0.05$). Treatment with Ivy leaf extract resulted in a significant reduction in serum creatinine, uric acid and BUN concentrations in comparison with the paracetamol group. Ivy leaf extract treatment similarly reduced histological alteration induced by paracetamol.
Conclusion: This study can be used as prove of reading that ivy leaf extract might be used to prevent renal damage induced by paracetamol.

Implication for health policy/practice/research/medical education:
Results demonstrate that ivy leaf extract might be used to prevent renal damage induced by paracetamol. This is a promising result for preparation of new drugs. Further studies are required to confirm the mechanisms responsible for nephron-protective activity.

Please cite this paper as: Moshaie-Nezhad P, Hosseini S, Yahyapour M, Iman M, Khamesipoure A. Protective effect of ivy leaf extract on paracetamol-induced oxidative stress and nephrotoxicity in mice. J Herbmed Pharmacol. 2018;9(1):64-68. doi: 10.15171/jhp.2019.11.

Introduction

Paracetamol (Acetaminophen or N-acetyl-p-aminophenol or APAP) is commonly used as an analgesic and antipyretic medicine, which causes liver and kidney necrosis in human and animals when used in high doses (1). Although nephrotoxicity is less prevalent than hepatotoxicity, but there is a possibility of acute renal failure development even in the absence of liver damage (2). N-acetylcysteine (NAC) is used for the treatment of liver toxicity and may cause increase liver glutathione level but it does not protect the kidneys against paracetamol (3). Since paracetamol might induce liver and renal damages, it is necessary to find a way to neutralize its effect.

Hedera helix, also known as Ivy is an evergreen plant which belongs to Araliaceous family with small, heart-shaped leaves. It is common in most parts of the world particularly in East Asia, India, and America (4,5). The ivy leaf extracts is used as anti-inflammatory, anti-cough, anti-neurologic pain, anti-rheumatism, antifungal, anti-worm, anti-spasm, and anti-asthma (4,6).

There are many studies on the effect of different plant extracts on paracetamol-induced renal toxicity; but none showed complete inhibition of the effect of paracetamol on kidneys (7-10). Despite of the common use of ivy, few studies have been conducted on the therapeutic and beneficial effects of this plant. Based on the given background, this study was carried out to investigate the protective effect of ivy leaf extract in the paracetamol-

***Corresponding author**: Maryam Iman, Email: iman1359@yahoo.com

induced nephrotoxicity and oxidative stress in mouse model.

Materials and Methods

Animals

Male Swiss albino mice (approximately 6–8 weeks old) weighing 22-25 g were purchased from Pasteur Institute of Iran and maintained in standard condition (temperature 25 ± 2°C) (53 ± 10% humidity) with free access to water and food under 12-hour light/dark cycles for the experiments. The mice were given time to adapt to the environment, at least one week before beginning the experiment.

Chemicals

Paracetamol powder was purchased from Sigma-Aldrich (Sigma-A5000). Ivy leaf extract was purchased from manufacturer Engelhard Arzneimittel GmbH & Co. KG (Germany).

Experimental design

According to our previous study, mice were divided randomly into six groups. Every group contains 10 mice (A, B, T1, T2, T3, and T4) as previously described in the study of Moshaie-Nezhad et al (5).

- Group A (Control): received NaCl 0.9% (200 mg/kg), orally for 7 days.
- Group B (Paracetamol): received a single intraperitoneal (i.p.) injection of paracetamol (600 mg/kg) on the day 8th. Paracetamol powder was dissolved in distilled water at 50°C and then cooled to 37°C. The paracetamol dose was determined as previously reported (11-13).
- Pretreatment groups included: T1, T2, T3, and T4, were orally treated with ivy leaf extract twice a day, every 12 hours for seven days with different doses (50, 100, 200 and 300 mg/kg). The ivy extract doses and the period of the treatment were selected based on previous reports (14-19).

On the day 9, the mice were killed under mild anesthesia 24 hours after the paracetamol administration, the blood samples were collected from different groups of mice to titrate the levels of serum blood urea nitrogen (BUN), creatinine and uric acid. The aforementioned subjects' kidneys were removed and kept in 10% buffer formalin solution for histopathological examination.

Biochemical analysis

Urea, creatinine and uric acid measurements

Urea, creatinine and uric acid levels were determined spectrophotometrically from serum samples using commercial test kits (Pars Azmon kits) and an auto analyzer machine (Alfa classic).

Histopathological assessment

The kidney tissues were put in a 10% buffer formalin solution. After 48 hours, paraffin blocks were prepared and subsequently, the kidneys were cut into sections using a rotary microtome at 5 µm stained with (H&E) and afterwards were examined under a light microscope. Changes in the experimental histopathologic parameters for kidney tissue were graded (20).

Statistical analysis

Statistical analyses were done using the statistical SPSS V22. The results were analyzed by one-way analyses of variance (ANOVAs), followed by Duncan's-test and expressed as mean ± SE. The $P \leq 0.05$ was considered as statistically significant.

Results

BUN, creatinine and uric acid levels

In the paracetamol group, chronic administration of paracetamol caused a significant increase in the levels of BUN, creatinine and uric acid when compared with the control group (Figure 1a, b, c). Administration of ivy leaf extract significantly reduced the BUN and uric acid activities in the pretreatment groups T2 (100 mg/kg)

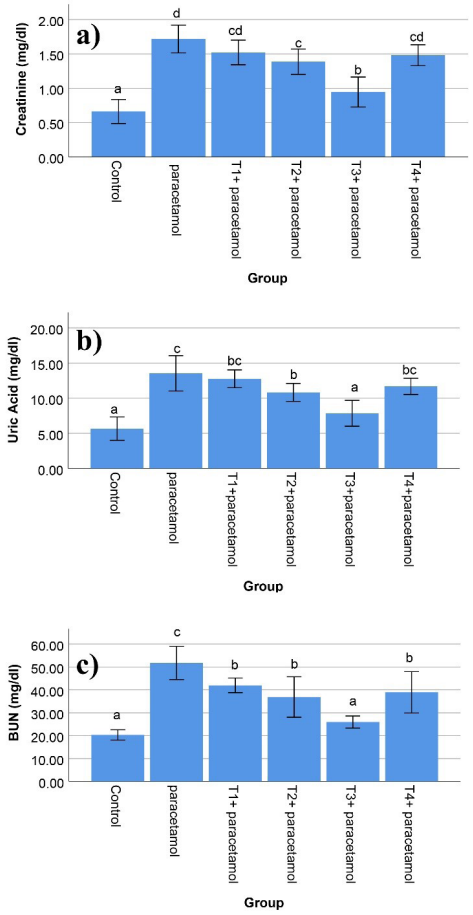

Figure 1. Serum of (a) creatinine, (b) uric acid and (c) bun levels in the mice at 24 h after paracetamol injection and ivy leaf extract treatment. Control group, paracetamol, t1, t2, t3 and t4. Abcd significant difference between the groups was shown after the Duncan post-test, each letter represents a group. The confidence level is 95.

and T3 (200 mg/kg) in comparison with the paracetamol group. The results showed that there was not a significant difference between the group T3 (200 mg/kg) and the control group. However, a significant difference was seen between the creatinine level in the group which received 200 mg/kg and the control group ($P < 0.05$). The most effective treatment was observed in the group which received 200 mg/kg of ivy leaf extract in comparison with the control group with respect to BUN and uric acid levels ($P > 0.05$). (Figure 1a, b, c)

Histologic examination

The histopathological findings showed a normal architecture of kidneys in the control group (Figure 2a), in contrary to the paracetamol treated group, hyaline casts, inflammatory cell infiltration, congestion, edema, degeneration, and necrosis were observed which were indications of renal damage (Figure 2b). In group treated with 50 mg/kg of the ivy extract, hyaline casts, congestion, edema, degeneration and necrosis were decreased. Furthermore, in the group which received 100 mg/kg, the hyaline cast was not observed (Figure 2c,d). In the group which were with 200 mg/kg a mild degeneration, congestion and necrosis with a minimal tissue damage were seen (Figure 2e). In the group treated with 300 mg/kg of the ivy extract, a mild necrosis, degeneration, edema with a moderate congestion were observed (Figure 2f). Histopathological parameters for kidney tissue are graded in Table 1.

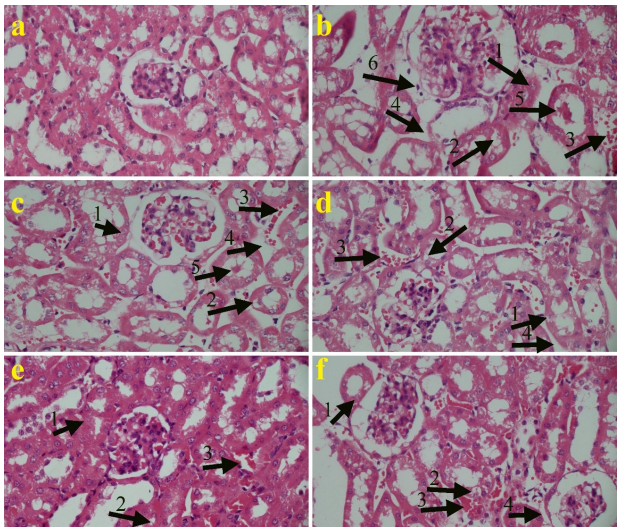

Figure 2. Histopathological observations; **a**. Control, **b**. Paracetamol (1: necrosis, 2: degeneration, 3: congestion, 4: edema, 5: hyaline casts, 6: inflammatory cell infiltration). **C**. T1 (1: necrosis, 2: degeneration, 3: congestion, 4: edema, 5: hyaline casts), **d**. T2 (1: necrosis, 2: degeneration, 3: congestion, 4: edema), **e.** T3 (1: necrosis, 2: degeneration, 3: congestion), **f**. T4 (1: necrosis, 2: degeneration, 3: congestion, 4: edema. (Kidney sections H&E, magnification x40).

Discussion

Acute toxicity of paracetamol is one of the most recognized causes of liver and kidney damages (3). The intensity of renal damage by paracetamol depends upon the system of cytochrome p450 and glutathione deposits. In the kidney, cytochrome p450 is mainly found in the proximal tubule and only a small amount in the glomerulus, distal tubes and collecting duct. On the other hand, due to the activities of absorption and secretion in the proximal tubule, the concentration of toxic metabolites is more in the tubules than the other parts (2,3).

The mechanism of acetaminophen-induced nephrotoxicity and hepatotoxicity has been reported by other investigators and showed that paracetamol causes oxidative stress and renal toxicity when used at high doses (21-26).

In our previous study, we have investigated the antioxidant properties of ivy leaf extract against acetaminophen induced oxidative damage and hepatotoxicity in mice. Administration of ivy extract at doses of 200 mg/kg was able to protect the liver against toxicity (5).

There are few studies in which the chemical constituents of ivy extract have been analysed. An HPLC with diode array detector (HPLC-DAD) method was developed and validated to assess the content of α-hederin and hederacoside C in various ivy extracts (27). In another report, α-hederin, hederacoside C, hederagenin and oleandrine in the methanol extract of ivy leaf and some biological fluids were analysed using LC–MS-MS (28) in both studies focus was on the analysis of saponins in ivy leaf. Yu et al quantified six main phenolics in ivy leaf extract using HPLC and capillary electrophoresis (29). The properties of ivy extract components especially

Table 1. Histopathologic changes induced by paracetamol and different doses of treatment groups of ivy leaf extract in kidney tissues

Group	Hyaline cast	Inflammatory cell infiltration	Congestion	Edema	Degeneration	Necrosis
Control	-	-	-	-	-	-
Paracetamol (600 mg/kg)	+	+	++	++	+++	++
T1 (50 mg/kg)	+	-	+	+	++	++
T2 (100 mg/kg)	-	-	+	+	++	+
T3 (200 mg/kg)	-	-	+	-	+	+
T4 (300 mg/kg)	-	-	++	+	+	+

Histopathologic assessments of the experimental parameters were graded as follows: (-) showing no changes and (+), (++) and (+++) indicating: mild, moderate and severe changes respectively.

rutin, quercetin, saponins, and flavonoids motivated to investigate nephroprotective effect of ivy leaf extract in paracetamol-induced renal toxicity.

It was concluded that paracetamol administration resulted in a significant increase in the BUN, creatinine, and uric acid levels, indicating a significant damage to the kidneys accompanied by degeneration of tubular epithelium and inflammatory cell infiltration in the kidneys (30,31). The results of histological studies are also consistent with biochemical findings. Moreover, histopathology of kidneys' findings confirms the protective activity of ivy leaf extract against the paracetamol-induced renal damage as it is shown by the reduction of kidney lesions such as hyaline casts, inflammatory cell infiltration, congestion, oedema, degeneration and necrosis especially at the dose of 200 mg/kg of ivy leaf extract. Nonetheless, due to the effect of ivy leaf extract at the dose of 300 mg/kg, mild necrosis, degeneration, oedema with moderate congestion were observed. This shows the negative effects of high doses of ivy leaf extract on the kidney tissues. This might be due to pro-oxidant effect of the extract which has been shown to occur in some conditions such as high doses (32).

Conclusion

The findings of this study demonstrated that Ivy leaf extract is effective against paracetamol-induced nephrotoxicity in mouse model. The protective effect of ivy extract was confirmed by histopathological observation. Furthermore, the protective effect of the extract could be related to its biologically active compounds. This study provides evidence that ivy leaf extract might be beneficial for prevention of renal damage induced by paracetamol.

Authors' contributions

PMN performed the experiments. PMN, SMH and MY contributed to the design of the study. MI supervised the research and AK revised the manuscript. All authors read the final version and confirmed the manuscript publication.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

The experimental procedures were approved by the Animal Care and Use Committee of Baqiyatallah University of Medical Sciences (97000151); in accordance with the Helsinki Declaration of Animal Rights.

Funding/Support

None.

References

1. Ozkaya O, Genc G, Bek K, Sullu Y. A case of acetaminophen (paracetamol) causing renal failure without liver damage in a child and review of literature. Ren Fail. 2010; 32:1125-7. doi: 10.3109/0886022X.2010.509830.
2. McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicol Appl Pharmacol. 2012;264:387-94. doi: 10.1016/j.taap.2012.08.015.
3. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. J Med Toxicol. 2008;4:2-6.
4. Trute A, Gross J, Mutschler E, Nahrstedt A. In vitro antispasmodic compounds of the dry extract obtained from *Hedera helix*. Planta Med. 1997;63:125-9.
5. Moshaie-Nezhad P, Faed Maleki F, Hosseini SM, Yahyapour M, Iman M, Khamesipour A. Hepatoprotective and antioxidant activities of hedera helix extract on acetaminophen induced oxidative stress and hepatotoxicity in mice. Biotech Histochem. 2019. doi: 10.1080/10520295.2019.1566569.
6. Huntley A, Ernst E. Herbal medicines for asthma: a systematic review. Thorax. 2000;55:925-929.
7. Hamid ZA, Budin SB, Jie NW, Hamid A, Husain K, Mohamed J. Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. J Zhejiang Univ Sci. 2012; 13: 176-85.
8. Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol. 2008;118:318-323. doi: 10.1016/j.jep.2008.04.025.
9. Khoursandi LS, Ourazizadeh M. Protective effect of *Curcuma longa* extract on acetaminophen induced nephrotoxicity in mice. Daru. 2008;16:155-9.
10. Gulnaz H, Tahir M, Munir B, Sami W. Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. Biomedica. 2010;26:9-15.
11. Janbaz KH, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol-and CCl4-induced hepatotoxicity in rodents. Fitoterapia. 2002;73:557-563.
12. Li C, Liu J, Saavedra JE, Keefer LK, Waalkes MP. The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. Toxicology. 2003;189:173-80.
13. Slitt AM, Dominick PK, Roberts JC, Cohen SD. Effect of ribose cysteine pretreatment on hepatic and renal acetaminophen metabolite formation and glutathione depletion. Basic Clin Pharmacol Toxicol. 2005;96:487-94.
14. Dworacka M, Krawczyk A, Brytska V. Anti-inflammatory, antimicrobial activity and influence on the lungs and bronchus of *Hedera helix* leaves extract. Acta Pol Pharm. 2017;74:1159-66
15. Lutsenko YU, Bylka WI, Matlawska I, Darmohray RO. *Hedera helix* as a medicinal plant. Herba Polonica.2010 ; 56: 83-96.
16. Gepdiremen A, Mshvildadze V, Süleyman H, Elias R. Acute and chronic antiinflammatory effects of *Hedera colchica* in

rats. Ethnopharmacol. 2004;94:191-5.
17. Gepdiremen A, Mshvildadze V, Süleyman H, Elias R. Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Phytomedicine. 2005;12:440-4. doi: 10.1016/j.phymed.2004.04.005
18. Girish C, Koner BC, Jayanthi S, Ramachandra Rao K, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. Fundam Clin Pharmacol. 2009;23:735-45. doi: 10.1111/j.1472-8206.2009.00722.x.
19. Kaur G, Jabbar Z, Athar M, Alam MS. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chem Toxicol. 2006;44:984-993.
20. Aydin G, Gokçimen A, Oncu M, Cicek E, Karahan N, Gokalp O. Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. Turk J Vet Anim Sci. 2003;27(5):1131-40.
21. Moshaei Nezhad P, Iman M, Maleki FF, Khamesipour A. Hepatoprotective effect of *Descurainia sophia* seed extract against paracetamol-induced oxidative stress and hepatic damage in mice. J Herbmed Pharmacol. 2018;7:267-72. doi: 10.15171/jhp.2018.40
22. Naguib YM, Azmy RM, Samaka RM, Salem MF. *Pleurotus ostreatus* opposes mitochondrial dysfunction and oxidative stress in acetaminophen-induced hepato-renal injury. BMC Complement Altern Med. 2014;14:494. doi: 10.1186/1472-6882-14-494.
23. Olaleye MT, Rocha BJ. Acetaminophen-induced liver damage in mice: effects of some medicinal plants on the oxidative defense system. Exp Toxicol Pathol. 2008; 59:319-27. doi: 10.1016/j.etp.2007.10.003
24. Honmore V, Kandhare A, Zanwar AA, Rojatkar S, Bodhankar S, Natu A. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharm Boil. 2015; 53: 571-581.
25. Ameer B, Greenblatt DJ. Acetaminophen. Ann Intern Med. 1977;87:202-209.
26. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs. N Engl J Med. 1994;331:1675-9 .
27. Demirci B, Goppel M, Demirci F, Franz G. HPLC profiling and quantification of active principles in leaves of *Hedera helix*. Int J Pharm Sci. 2004;59:770-4.
28. Gaillard Y, Blaise P, Darré A, Barbier T, Pépin G. An unusual case of death: suffocation caused by leaves of common Ivy (*Hedera helix*). Detection of hederacoside C, α-hederin, and hederagenin by LC—EI/MS-MS. J Anal Toxicol. 2003;27:257-262.
29. Yu M, Shin YJ, Kim N, Yoo G, Park S, Kim SH. Determination of saponins and flavonoids in ivy leaf extracts using HPLC-DAD. J Chromatogr Sci. 2014; 53:478-83. doi: 10.1093/chromsci/bmu068.
30. Cekmen M, Ilbey YO, Ozbek E, Simsek A, Somay A, Ersoz C. Curcumin prevents oxidative renal damage induced by acetaminophen in rats. Food Chem Toxicol. 2009;47:1480-4. doi: 10.1016/j.fct.2009.03.034.
31. Das J, Ghosh J, Manna P, Sil PC. Taurine protects acetaminophen-induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation. Toxicology. 2010;269:24-34.
32. Rafieian-Kopaei M, Baradaran A, Rafieian M. Oxidative stress and the paradoxical effects of antioxidants. J Res Med Sci. 2013;18(7):628.