Hepatoprotective and antioxidant effects of *Polygonum odoratum* L. extract against acetaminophen–induced hepatotoxicity in rats

Nuntiya Somparn*, Rungrat Jitvaropas**, Suphaket Saenthaweesuk***, Amornnat Thuppia***

Abstract

Introduction: *Polygonum odoratum* L. contains significant amounts of flavonoids which have been shown to possess strong antioxidant activity. We investigated the antioxidant and protective effect of *Polygonum odoratum* L. extract in rats which were induced to have liver injury by acetaminophen (APAP), a commonly used antipyretic/analgesic drug that can cause hepatotoxicity when used overdose.

Methods: Rats were administered with 500 or 1,000 mg/kg body weight (BW)/day of the plant extract for 7 days prior to exposure of APAP (3 g/kg BW). After 24 hours of APAP administration, blood samples were collected for determination of aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde (MDA) levels, and liver samples were examined for histopathology changes.

Results: Levels of AST, ALT and MDA were significantly higher in rats treated with APAP alone as compared to the control. Pre-treatment of the animal with the extract significantly reduced AST, ALT and MDA levels and liver injury.

Discussion: The increased levels of liver enzymes and MDA level in APAP treated rats were due to hepatocellular injuries. Pre-treatment with *Polygonum odoratum* L. extract could ameliorate oxidative stress and liver injury induced by APAP. The protective effect might be due to the antioxidant proprieties of this extract.

Key words: Antioxidant, Oxidative stress, Acetaminophen, *Polygonum odoratum* L.

Received: 1 July 2013 Accepted: 25 October 2013
Methods

Chemicals and drugs

Acetaminophen, 2, 2-diphenyl-1-picrylhydrazil (DPPH) and thiobarbituric acid (TBA) were purchased from Sigma chemical co. (St.Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant material

Fresh *P. odoratum* specimens were purchased from local market in Pathum Thani, Thailand during September 2010. The collected plant material (whole plant) was washed thoroughly in water, cut into small pieces and soaked in 95% ethanol at a ratio of 1:1 for one night at room temperature and then filtered. The filtrates were concentrated by rotary vacuum evaporation and then lyophilized with a freeze dryer. The extract was kept at -80 °C for analysis.

Introduction

Acetaminophen, also known as paracetamol or APAP (acetyl-para-aminophenol) is an over-the-counter drug, commonly used for its analgesic and antipyretic activities. At therapeutic doses, APAP is considered safe. However, it can cause severe hepatotoxicity and nephrotoxicity when used overdose. Once being absorbed into a body, APAP is activated and converted by cytochrome P450 in the liver to a toxic metabolite called NAPQI (N-acetyl-p-benzoquinoneimine). This substance causes oxidative stress and glutathione (GSH) depletion. The oxidative stress leading to lipid peroxidation contributes to the initiation of liver damaging process.

Generally, animals including human are protected from active oxygen species and oxidative stress by cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. In addition to this protective mechanism at the cellular level, it has been shown that food especially some plants and vegetable have health benefit against oxidative stress and other related diseases. Several studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects.

*Polygonum odoratum* L. (*P. odoratum*), member of Polygonaceae family, is a perennial herb which grows up to 30-35 cm high. It has 6-15 cm long-pointed leaves with a distinctive dark purple marking in the center of the leaves. *P. odoratum* is native plant of Southeastern Asia and is better known as Vietnamese mint or Vietnamese coriander.

*P. odoratum* has been shown to possess strong antioxidant activity and contains significant amounts of flavonoids in its leaves. Adding *P. odoratum* in daily meal may have beneficial health effects. Therefore, the present study was conducted to determine antioxidant potential of *P. odoratum* extract against APAP-induced hepatotoxicity in rats.

Phytochemical screening

The ethanolic extract was screened for the presence of some phytoconstituents according to the methods described by Harborne et al.

Ferric reducing/antioxidant power (FRAP) assay

The ability of *P. odoratum* extract to reduce ferric chloride was measured according to the method described previously. The FRAP reagent was freshly prepared from 300 mM acetate buffer pH 3.6, 10 mM of 2,4,6-tris (2-pyridyl) -1,3,5-triazine (TPTZ) solution in 40 mM HCL and 20 mM iron (III) chloride (FeCl$_3$·6H$_2$O) solution at a ratio 10:1:1 (v/v). The plant extract was diluted in 100% ethanol, added into FRAP reagent, and incubated for 5 minutes at room temperature and the absorbance at 593 nm was determined. The standard curve was constructed using iron (II) sulfate (FeSO$_4$) solution. The experiments were performed in triplicate.
**DPPH radical scavenging test**

The DPPH radical-scavenging activity test was applied following the method described previously. Briefly, 0.08 mM DPPH was prepared in 100% ethanol. DPPH solution, Tris buffer solution, and 80% ethanol were mixed in order to obtain a 1:1:1 ratio for 1.8 mL. Then, the plant extract (0.6 mL) was added and incubated for 30 minutes in the dark. The absorbance was read at 525 nm.

**Experimental animal and design**

Forty two adult male Sprague-Dawley rats weight 200-250 g were used. The animals were housed in specific standard laboratory condition for one week. The conditions were kept in a temperature-controlled environment (25 ± 1°C), 55 ± 5% relative humidity and with regular 12 hours light/ 12 hours dark cycles. All animals were fed with standard rat chow diet and water ad libitum.

The animals were divided into 7 groups, each of which consisted of 6 rats. The animals in group I reserved as normal control were administered only 5% Tween 80 throughout the duration of the experiment. Those in group II (APAP treated) received 5% Tween 80 for 7 days followed by APAP (3 g/kg BW/day, po) on day 8. The animals in group III and IV were administered 500 and 1,000 mg/kg BW/day of the plant extract, respectively, for 7 days. Group V and VI were administered 500 and 1,000 mg/kg BW/day of the plant extract, respectively for 7 days followed by APAP (3 g/kg BW/day, po) on day 8. Those in group VII (positive control) received 5% Tween 80 for 7 days followed by APAP (3 g/kg BW/day, po) and N-acetylcysteine (NAC) 400 mg/kg BW (ip injection) at 3 hours after APAP administration. Animals were anaesthetized and euthanized 24 hours after the administration of APAP. The study protocol was approved by the Ethics Review Committee of Faculty of Medicine, Thammasat University.

**Measurement of liver function markers**

The blood was centrifuged at 2,000 rpm 4°C for 10 minutes to separate the serum. The levels of liver function markers including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the laboratory unit at Thammasat University Hospital.

**Assay of oxidative status**

Assay of malondialdehyde (MDA) as a marker of lipid peroxidation was performed in serum as thiobarbituric acid reactive products (TBAR), by a previously described by Draper et al. (1993). In brief, 150 µl of serum was reacted with 125 µl of 10% TCA, 125 µl of 5 mM EDTA, 125 µl of 8% SDS, and 10 µl of 0.5 µg/ mL of butylatedhydroxytoluene (BHT). The mixture was left for 10 minutes, then 0.6% thiobarbituric acid (TBA) was added in an equal volume and the mixture was heated for 30 minutes in a boiling water bath. After cooling to room temperature the mixture was centrifuged at 25 °C 10,000 xg for 10 minutes. The absorbance of the supernatant was measured at 532 nm. A standard curve was generated by using appropriated concentrations of standard 1,1,3,3-tetraethoxypropane (TEP) (0.3-10 µmol/ L).

**Histopathological examination of rat liver**

The rat liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The serial sections were cut 5 µm thick and stained with hematoxylin-eosin (H&E) and examined under a photomicroscope.

**Statistical analysis**

Data were expressed as mean ± S.E.M. All statistical analyses were performed by one way analysis of variance (ANOVA). P < 0.05 was considered significant.

**Results**

**Phytochemical analysis and antioxidant activity**

The phytochemical analysis revealed that the ethanolic extracts of P. odoratum contained flavonoids, alkaloids, phenolic compounds and tannins but lacked saponins. The extract was screened for its possible antioxidant activity through two methods: DPPH and FRAPS assays. The scavenging activity (DPPH value) of the extract was 50.25 ± 0.61 mg/mL whereas the DPPH value...
of the potent antioxidants including vitamin E and butylatedhydroxytoluene (BHT) were 14.79 ± 0.78 and 19.71 ± 0.79 mg/mL, respectively. For reducing property, FRAP value of the extract was 28.37 ± 0.18 μM/μg whereas the FRAP value of vitamin E was 192.68 ± 12.18 μM/μg. These results indicated that the *P. odoratum* extract exhibited moderate antioxidant activity toward scavenging free radicals but not as strong as the potent antioxidants.

**Protective effect of *P. odoratum* against APAP induced hepatotoxicity**

Treatment of rats with APAP alone significantly increased the levels of serum ALT and AST levels, compared with the normal control rats. This reflected the failure of liver function due to APAP-induced hepatotoxicity. Pre-treatment with 500 or 1,000 mg/kg BW/day of the *P. odoratum* extract or treatment with NAC significantly reduced the elevation of serum AST and ALT levels (*P < 0.05*) (Figure 1). The obtained results revealed a protection of the *P. odoratum* extract against the hepatotoxic effect by APAP.

Since oxidative stress contributes to the development of APAP-induced hepatotoxicity, the serum MDA level was measured as a marker of lipid peroxidation. The serum MDA was significantly elevated in animals treated with APAP compared to control animals (*P < 0.05*) (Figure 2). However lipid peroxidation level was restored to normal by pre-treatment with the *P. odoratum* extract which demonstrated preventive effect on accumulation of lipid peroxidation.

Cellular changes including infiltration and necrosis suggested high levels of lipid peroxidation which were found more prominent in APAP treated when compared to normal control. Interestingly, pre-treatment with the *P. odoratum* extract presented healing of the liver and less degree of cellular damages (Figure 3). Thus, amelioration of the histopathological changes that occurred in *P. odoratum* treated group indicated the hepatoprotective effects of *P. odoratum* against APAP-induced hepatotoxicity.

![Figure 1](image1.jpg)  
**Figure 1** Effect of *P. odoratum* extract on APAP-induced rise in serum transaminase levels

Serum aspartate transaminase (AST) is shown in figure 1A. Serum alanine transaminase (ALT) is shown in figure 1B. Values are expressed as means ± S.E.M. (n = 6), # Significant difference at *P < 0.05* when compared with control, * Significant difference at *P < 0.05* when compared with APAP alone, (NAC = N-acetylcysteine)
Figure 2  Effect of *P. odoratum* extract on APAP-induced lipid peroxidation

Values are expressed as means ± S.E.M. (n = 6), # Significant difference at P < 0.05 when compared with control.

* Significant difference at P < 0.05 when compared with APAP alone. (NAC = N-acetylcysteine)

Figure 3  Effect of *P. odoratum* extract on APAP-induced histopathological changes in the liver

Representative slides from corresponding groups. (NAC = N-acetylcysteine)
Discussion and Conclusion

The induction of hepatocellular damage or necrosis by APAP higher dose has been demonstrated in several studies of experimental animals and humans\(^\text{10}\). The hepatotoxicity of APAP has been attributed to the formation of toxic metabolites. When a part of APAP is activated by hepatic cytochrome p450, it transforms into a highly reactive metabolite, N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of proteins. GSH depletion, subsequently, leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid, and this ultimately leads to liver damage due to higher doses of APAP\(^\text{10, 11}\). Reactive metabolites can exert initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH) or binding to enzymes, lipids, nucleic acids and other cell structures\(^\text{12}\).

Malondialdehyde (MDA) is one of the end products in the lipid peroxidation process. The increase in TBARS level in liver induced by APAP suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism\(^\text{13}\). Therefore, reducing lipid peroxidation in liver tissue of \(P. \) odoratum treated groups indicated that the \(P. \) odoratum extract possessed antioxidative properties.

Serum AST and ALT level are parameters for detecting liver injury and are commonly used as a biochemical marker to evaluate liver injury. Administration of APAP caused a significant elevation of AST and ALT enzymes level which damaging the structural integrity of liver because they were located in cytoplasm and released into circulation after cellular damages indicating development of hepatotoxicity\(^\text{14-16}\). The pre-treatment of \(P. \) odoratum could reduce the increased serum marker enzymes AST and ALT level. The ability of the extract to protect APAP induced liver damage might be due to its antioxidant properties.

Previous studies have demonstrated that plants extracts contain antioxidant and hepatoprotective activities through regulatory action on cellular permeability, stability and suppressing oxidative stress. A number of scientific reports documented that certain flavonoids have a protective effect on the liver due to their antioxidant properties\(^\text{17-19}\).

The study of Gali H. et al. (1996) found out that tannins had potent antioxidants of anti-inflammatory activities\(^\text{20}\). Okuda T. et al. (1983) also reported that tannins might prevent the destructive effects of lipid peroxide in liver cells by lowering the levels of lipid peroxide in liver cells\(^\text{21}\). Moreover, flavonoids have been reported to possess antioxidant and anti-inflammatory activities\(^\text{22}\).

The antioxidant effects of natural product have been reported to be mostly due to phenolic compounds\(^\text{23-26}\). In our preliminary phytochemical analysis, the ethanolic extract of \(P. \) odoratum contained flavonoids, alkaloids, phenolic compounds and tannins but lacked saponins. The protective effects of the extract could be due to the presence of one or the other of the active principles in the plant. Therefore, these active pharmacological agents may be responsible for the hepatoprotective effects observed in this study. This hypothesis is yet to be validated.

Furthermore, the protective effect of \(P. \) odoratum extract against APAP intoxication has been studied in ICR mice by Jaruchotikamol (2000), the study used 50% ethanolic extract of dried \(P. \) odoratum and administrated at the dose of 1,000 and 2,000 mg/kg BW/day for 3 days prior to 200 mg/kg BW APAP injection. The protective effect of the extract was evidenced and no toxicity was found\(^\text{27}\).

However, dietary supplements are usually consumed longer than 3 days and the low dose of administration should be considered. They may have species differences in drug response as well. Therefore, this present study was designed to evaluate the 1 week effect of \(P. \) odoratum ethanolic extract at lower administration doses (500 and 1,000 mg/kg BW/day) in rats. Interestingly, although the extraction method, dosage, route of APAP administration and specie of animal are different, \(P. \) odoratum extract can process antioxidant and hepatoprotective
effects against APAP induced hepatotoxicity. Nevertheless, the presence of active compounds and mechanism of action may be different due to different extraction method, source of plant and animal species.

From our results, it could be concluded that increased serum marker enzymes and lipid peroxidation level in acetaminophen treated rats was due to hepatocellular damage. Pre-treatment with *P. odoratum* extract could prevent liver damages induced by acetaminophen.

**Acknowledgement**

This work was supported by Faculty of Medicine, Thammasat University, Thailand. The authors thank Dr. Pholawat Tingpej and Dr. Wanwarang Hiriote from Preclinical Science, Faculty of Medicine as well as Ms. Debra Kim Liwiski from The Language Institute, Thammasat University for language editing of the manuscript and valuable suggestions.

**References**


บทคัดย่อ
ฤทธิ์ป้องกันเซลล์ตับและฤทธิ์ต้านอนุมูลอิสระสลับกันของสารสกัดผักแพวต่อการเหนี่ยวนำาภาวะตับเป็นพิษด้วยอะเซตามิโนเฟน

นันทิยา สมภาร*, รุ่งรัตน์ จิตวโรภาส**, ศุภเกต แสนทวีสุข***, อมรณัฏฐ์ ทับเปีย***

* สาขาเภสัชวิทยา สถานวิทยาศาสตร์พรีคลินิก คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต ตําบลคลองหนึ่ง อำเภอคลองหลวง จังหวัดปทุมธานี ๑๒๑๒๐
** สาขาชีวเคมี สถานวิทยาศาสตร์พรีคลินิก คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต ตําบลคลองหนึ่ง อำเภอคลองหลวง จังหวัดปทุมธานี ๑๒๑๒๐
*** สาขากายวิภาคศาสตร์ สถานวิทยาศาสตร์พรีคลินิก คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต ตําบลคลองหนึ่ง อำเภอคลองหลวง จังหวัดปทุมธานี ๑๒๑๒๐

ผู้ให้ติดต่อ: นันทิยา สมภาร สาขาเภสัชวิทยา สถานวิทยาศาสตร์พรีคลินิก คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต ตําบลคลองหนึ่ง อำเภอคลองหลวง จังหวัดปทุมธานี ๑๒๑๒๐ โทรศัพท์: ๖๖๒-๙๒๖๙๖๒๙ โทรสาร: ๖๖๒-๙๒๖๙๗๑๑ อีเมล: nunthy_tom@hotmail.com

บทนำ: ผักแพวประกอบด้วยสารกลุ่มอิมัยซึ่งมีฤทธิ์ต้านอนุมูลอิสระ ส่งผลในการศึกษาในสัตว์ทดลองได้ประเมินฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ป้องกันพิษจากอะเซตามิโนเฟน ซึ่งเป็นยาบรรเทาปวด ลดไข้ที่ใช้โดยทั่วไป หากได้รับเกินขนาดอาจเกิดพิษต่อตับได้.

วิธีการศึกษา: หนูแรทได้รับสารสกัดผักแพวขนาด ๕๐๐ และ ๑,๐๐๐ มิลลิกรัมต่อกิโลกรัมน้ำาหนักตัวต่อวัน เป็นเวลา ๗ วัน ก่อนถูกเหนี่ยวนำาภาวะตับเป็นพิษด้วยอะเซตามิโนเฟนขนาด ๓ กรัมต่อกิโลกรัมน้ำาหนักตัว หลังจากนั้น ๒๔ ชั่วโมง ทำาการเก็บตัวอย่างเลือดเพื่อประเมินระดับเอนไซม์ตับ เช่น aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde (MDA) และเก็บเนื้อเยื่อตับเพื่อประเมินลักษณะทางจุลกายวิภาค.

ผลการศึกษา: ระดับเอนไซม์ AST, ALT และ MDA ในสัตว์ทดลองกลุ่มที่ได้รับอะเซตามิโนเฟนมีระดับสูงขึ้นเมื่อเทียบกับกลุ่มควบคุม. การได้รับสารสกัดผักแพวสามารถลดระดับ AST, ALT, MDA และลดการบาดเจ็บของเซลล์ตับได้

วิจารณ์และสรุปผลการศึกษา: การได้รับสารสกัดผักแพวสามารถลดการบาดเจ็บของเซลล์ตับจากการตกตามในเพนได้ผลดังกล่าวไม่เกิดจากคุณสมบัติในการด้านอนุมูลอิสระของสารสกัด

คำาสำาคัญ: สารต้านอนุมูลอิสระ, ภาวะเครียดออกซิเดชัน, อะเซตามิโนเฟน, ผักแพว