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Antidote activity and protective effects of *Lysiphyllum strychnifolium* (Craib) A. Schmitz extract against organophosphate pesticide in omethoate-treated rats

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1. Introduction

Pesticide poisoning is a global health problem,[1,2] with a serious epidemiological profile in Thailand.[3] Many regions of Thailand use organophosphate pesticides (OPs) to protect their crops against insect pests.[4,5] However, uncontrolled and inappropriate application of OPs lead to OP poisoning.[6] Health records at the Ramathibodi Poison Center attribute 24.3% of human exposures to OPs.[3] Moreover, OP residues in vegetables may pose serious risk to the wellbeing of consumers.[7,8] One toxic effect of OPs is the cholinergic syndrome, which is caused by acetylcholinesterase (AChE) inhibition.[9].

AChE (EC 3.1.1.7) hydrolyzes the neurotransmitter acetylcholine to acetate and choline. It is found mainly at neuromuscular junctions and cholinergic synapses in the central nervous system. Studies have demonstrated that OPs act directly on AChE activity by inhibiting plasma levels in animals and humans. This renders a biomarker potential for AChE in monitoring OP toxicity.[10–12] Therefore, natural compounds that lead to the successful recovery of AChE activity after OP poisoning may indicate their potential to serve as antidotes.

Lysiphyllum strychnifolium Craib or "Ya nang daeng" (*L. strychnifolium*) is classified as a family of *Leguminosae*.[13] Leaf decoction has been used to neutralize toxins in upper Northeastern Thailand.[14] Leaf, stem and root of *L. strychnifolium* are traditionally used to treat fever, alcohol intoxication and serve as an antidote to pesticide poisoning. Several bioactive ingredients from the *L. strychnifolium* extracts (LS) were reported such as trilobatin, quercetin (QR), 3,5,6,3',5pentahydroxyflavanonol3OαLrhamnopyranoside, 3,5,7trihydroxyc hromoone3OαLrhamnopyranoside, β-sitosterol, stigma sterol and gallic acid.[13,15] Preclinical studies have shown that LS (aqueous and ethanol) exhibited anti-HIV-1 integrase and anti-allergic activities with the most potent IC50 values of 11.2 and 6.4 μg/ml, respectively.[16,17] Moreover, different parts (stems/leaf) of LS (ethanol) have been shown to have medicinal properties. These include anti-tumor activity in cancer cell lines (stem)[15] and anti-malaria activity *Plasmodium berghei* in mice (leaf).[18]Aqueous extract LS leaf have been shown to have high levels of total phenolic compounds and strongly exhibit higher antioxidant activity than green tea.[19] More recently, the anti-inflammatory and anti-hyperuricemic effects of the leaf water LS have been reported.[13] A clinical study likewise reported that water LS leaf could detoxify alcohol intoxication.[20]

LS root or leaf or stem decoctions have been used to treat diarrhoea and neutralize food poisoning at Yasothon province, North-eastern part of Thailand with no reports of toxicity.[21] This study investigates the following actions of ethanol LS stem in omethoate (OM)-treated rats: (i) identify the active compounds using High Performance Liquid Chromatography coupled with

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ultraviolet-photodiode array detection (HPLC-DAD); (ii) antidote effect by determining the enzymatic activity of AChE; (iii) protective effect on the liver and kidney against OP poisoning.

2. Materials and methods

2.1. Crude extract preparation

Flowers, fruits, leaves and stems of *L. strychnifolium* were collected in July 2013 from Nakhon Pathom and Yasothon provinces, Thailand for taxonomic identification. Upon confirmation, a specimen was deposited at the Herbarium of the Department of Thai Traditional and Alternative Medicine, Thailand (a voucher specimen TTM no.0003601).

Stems have been identified as the plant part with the most potential for *L. strychnifolium* extraction (LS).[17] After collection, stems were washed in water, air dried under shade, ground to fine powder, and stored in amber glass bottles at room temperature (25 °C). The dried powders of *L. strychnifolium* stems (400 g) were extracted three times with 95% ethanol at room temperature. Ethanol from LS was removed by vacuum evaporation and was stored at −20 °C for analysis.

2.2. Chemicals

Purified myricetin, quercetin (QR) and kaempferol (Sigma-Aldrich, USA) were used as HPLC standards. Amplex®Acetylcholine/Acetlycholinesterase assay kit (Molecular probes, Invitrogen, USA) was used to assess enzymatic activity. OM at 50% w/v EC (domestic source) was used as positive control.

2.3. Standard and sample preparation

Stock solutions of standard myricetin, QR and kaempferol were prepared at a concentration of 0.5 μg/ml in acetonitrile. The ethanol LS stem was diluted in acetonitrile at a concentration of 2 mg/ml, and clarified through a 0.45 μm syringe filter before HPLC analysis.

2.4. Experimental procedures

2.4.1. HPLC analysis

ACE C18 reverse phase HPLC column (Agilent 1260 Co. LTD, USA), size 250 mm × 4.6 mm, 5 μm (Hichrom, UK) was used at a flow rate of 1 ml/min. Elution used a linear gradient of the mobile phase: 1% formic acid in water (solvent A): acetonitrile (solvent B). Gradient elution using solvent B was as follows: 0–5 min at 5%, 5–20 min at 15%, 20–55 min at 35% and 55–65 min at 5%. Detection was performed at UV 280 and 350 nm. Samples were analyzed in triplicate by injection of 10 μl volumes of the clarified filtrate.

2.4.2. Animal treatment

Male and female Wistar rats weighting 200–250 g were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. All animals were housed in environmentally controlled conditions of 12 h light - 12 h dark cycle and a temperature of 25 ± 1 °C. Food and water were freely available. All rats were allowed 1 week to acclimate to the housing conditions before starting the experiment. All experimental protocols were approved by the Thammasat Animal Care and Use Committee, Thammasat University (No.009/2556).

2.4.3. Antidote study of LS

Thirty three Wistar rats were used in the experiment, a schematic overview of which is presented in Fig. 1. The negative control group (n = 3) received normal saline. Each of the two positive control groups (total n = 12) were injected with either OM (2.5 mg/kg/d, i.p.) or administered with 70 mg/kg of the LS. Six rats each comprised the three test groups (T1-T3).

T1 rats were given 70 mg/kg of LS by gavage for 7 days, after which OM was injected for 7 days. T2 rats were initially injected with OM for 7 days and then given the LS with the same dose as T1 for 7 days. T3 rats received the same treatment as T1 but the extract dose was increased to 350 mg/kg. Fourteen days after treatments, all rats were sacrificed with an overdose of CO_2. Immediately following the rats' demise, blood was collected by cardiac puncture. Brains, livers and kidneys were then excised. Liver and kidney tissues were preserved in 10% neutral buffered formaldehyde solution for histological analysis. Brain tissues were stored in cold PBS buffer for further analysis.

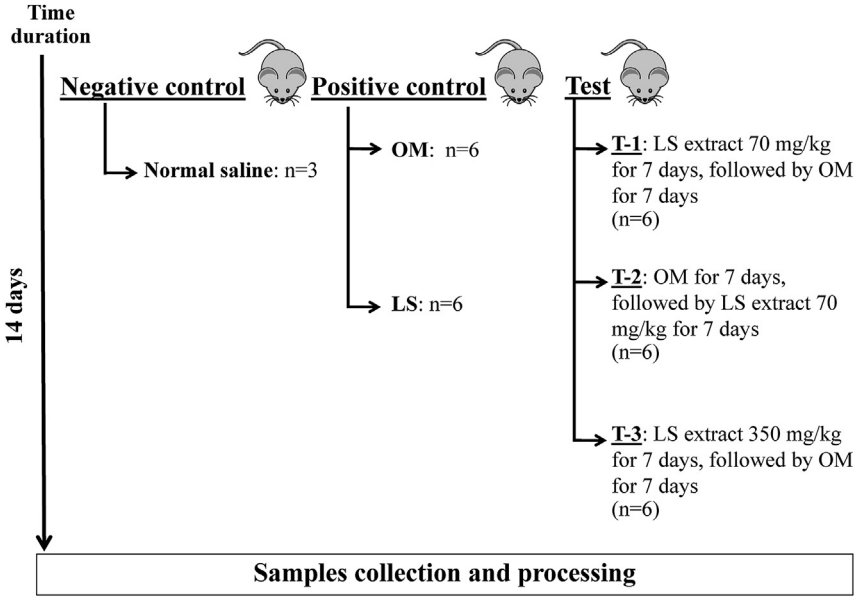

Fig. 1. Schematic overview of the animal experiment to study the antidote effects of the ethanol LS stem on organophosphate pesticide poisoning.

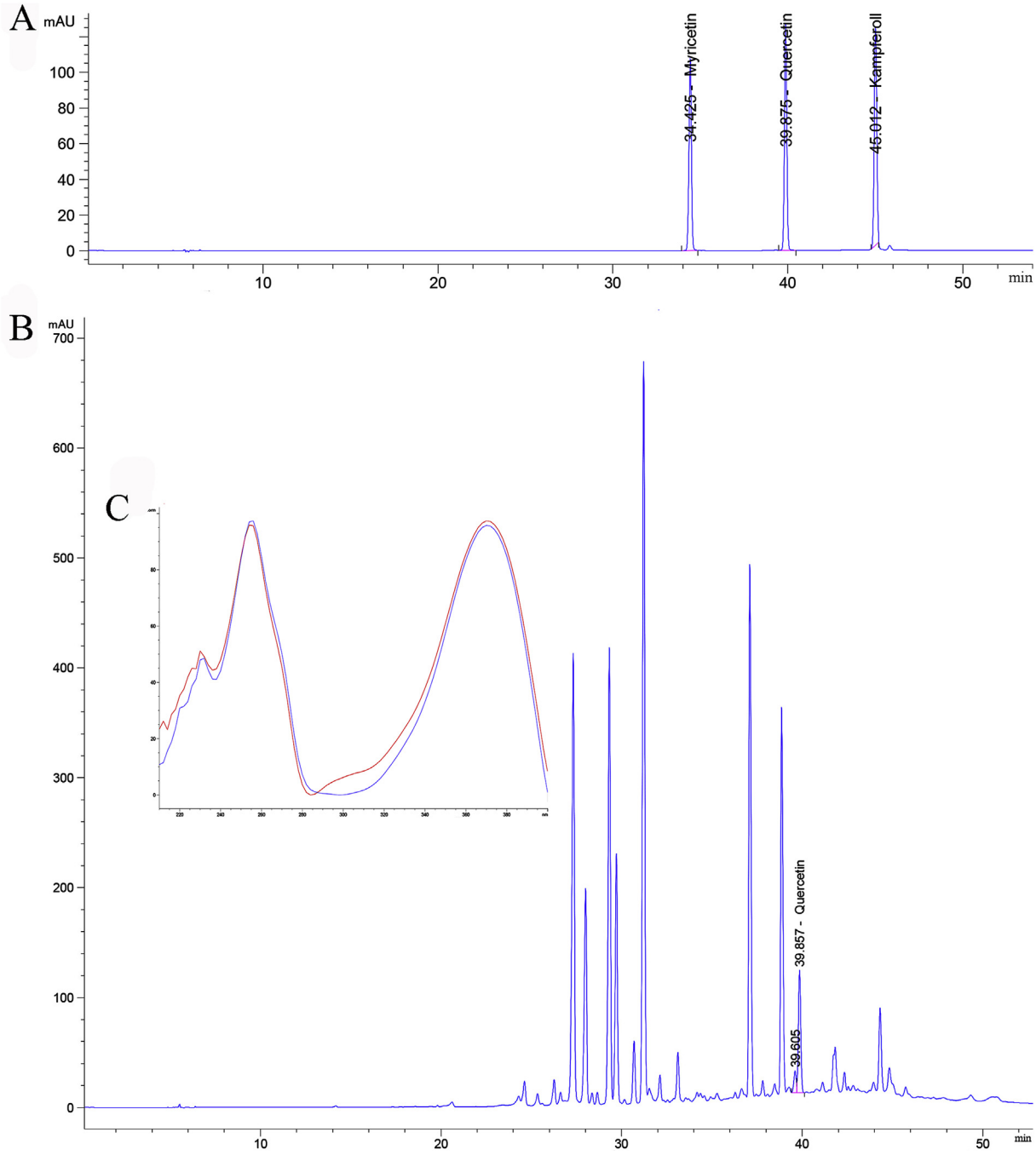

Fig. 2. Representative HPLC chromatograms of standard myricetin, QR and kaempferol (A). Chromatographic profile of the ethanol extract of LS stem was demonstrated at UV 350 nm (B). UV spectrum of target peak (blue line) was superimposed to a standard QR spectrum (red line) (C).

2.4.4. Brain tissue extraction

Brain tissues in ice cold lysis buffer (25 mM Tris-Cl, 1 mM EDTA, pH 7.4, 1% (v/v) Triton X-100 and 1 mM PMSF) were sonicated and incubated for 10 min at 4 °C. The lysate was centrifuged at 10,000×*g* for 10 min at 4 °C. The supernatant was collected for determining AChE activity. Protein concentration was quantified using the Bradford method[22] against a bovine serum albumin standard dilution series.

2.4.5. Determination of AChE activity in blood and brain homogenates

Amplex® Acetylcholine/Acetlycholinesterase assay kit (Molecular probes, Invitrogen, USA) was used to determine AChE activity in blood and brain homogenate samples. Samples were diluted in 1x reaction buffer supplied in the kit. Excitation and emission ranges were detected at 560 and 590 nm, respectively.

2.4.6. Histopathology

The livers and kidneys of all rats were dissected and fixed in 10% neutral-buffer formalin. The tissues were embedded in paraffin, sectioned to a 5 μm thickness, stained with hematoxylin-eosin, and examined under a light microscope (Leica DM3000 LED). Photomicrographs of the samples were taken. Assessment liver and kidney damage was modified from a previous study[23] using a grade

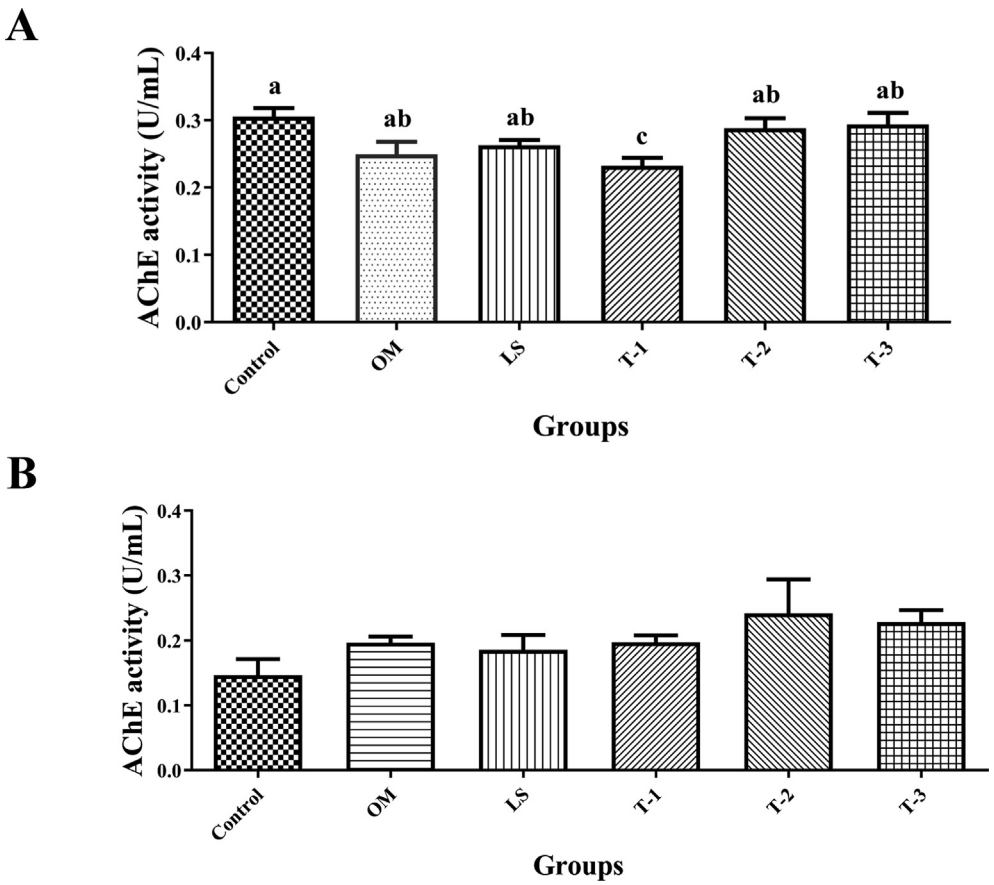

Fig. 3. The protective effect of the ethanol extract of LS against OM-induced inhibition of AChE activity in blood (A) and brain homogenates (B) of rats. Data (indicated as vertical bars in this graph) were expressed as mean ± SEM. Inter-group differences were analyzed with ANOVA, with post-hoc Dunnett's test. Significant differences ($p < 0.05$) are indicated by lower case letters above each bar. Absence of letters above each bar or letters in common between bars indicate non-significant differences ($p > 0.05$).

scale of 0–3 as follows: (i) grade 0: normal liver architecture, which includes the central vein (CV) in the lobule surrounded by hepatocytes characterized by a strongly eosinophilic granulated cytoplasm with distinct nuclei and intact hepatic sinusoids; (ii) grade 1: mild liver injury which includes hydropic changes, hepatocyte hypertrophy and swelling; (iii) grade 2: moderate liver injury including fatty changes, focal necrosis, ill-defined blood sinusoids between hepatocyte cords, mild to moderate hemorrhage or congestion and slight inflammatory cell infiltration; (iv) grade 3: massive necrosis which includes hemorrhage or congestion, moderate to severe inflammatory cell infiltration.

Kidney damage scores were as follows: (i) grade 0: intact renal corpuscle, renal tubules, proximal/distal convoluted tubules, glomerulus (GM), urinary space and Bowman's capsule, without indication of hemorrhage; (ii) grade 1: mild tubular injury but intact GM, hydropic changes and mild hemorrhage; (iii) grade 2: moderate tubular injury with glomerular degeneration and shrinkage, edema and widening of the tubular lining epithelium and lumen, respectively, focal tubular degeneration, moderate hemorrhage, and indication of inflammatory cell infiltration; (iv) grade 3: most severely affected samples that showed extensive renal injury which included glomerular degeneration, massive tubular necrosis/hemorrhage, and moderate to abundant inflammatory cell infiltration.

2.5. Statistical analysis

Normally distributed data were reported as mean ± standard error of mean (SEM). Significant differences were determined by using Student's t-test (for two groups) and/or one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test (for > two groups) using GraphPad Prism 8.1.2 (GraphPad software Inc., San Diego, California, U.S.A.). Two-tailed p value ≤ 0.05 was set as the significance threshold.

3. Results

3.1. Chromatographic profile of LS

We identified potential active compounds responsible for the antidote activity of ethanol LS stem on OP poisoning using chromatography of choice (HPLC). Retention times for the three internal standards (myricetin, QR and kaempferol) were 34.425, 39.875 and 45.012 min, respectively (Fig. 2A). In Fig. 2B, we identified a minor peak, whose retention time of 39.857 min was perfectly matched with the QR standard (39.875 min). UV spectrum analysis validated the perfect match where the lines of the minor peak (blue) superimposed those of the QR standard (red) (Fig. 2C). These results suggest a QR role for antidote activity against OP toxicity. The amount of QR in the extract was calculated by using linear regression (equation: y = 26.08999x − 3.08904 with R2 of 0.99998); at R2 of close 100%, yielded 19.6 mg/g extract.

3.2. Antidote effect of LS on AChE activity

To investigate the antidote effect of ethanol extract of the LS stem against OP poisoning, AChE activity in the blood and brain

homogenates of the treated-rats were assessed. Fig. 3A shows that AChE levels range from 0.25 to 0.30 U/mL across all comparisons. High AChE levels in the blood favored antidote activity and low levels did not. ANOVA results showed that T1 had reduced AChE activity (an unfavourable outcome) which was significantly different ($p < 0.05$) from T2-T3 and the three controls. Thus, T2-T3 treatment favored antidote activity, but was not significantly different from the controls ($p > 0.05$).

Brain sample results showed that AChE levels ranged from 0.15 to 0.23 U/mL across all comparisons (Fig. 3B), considerably less than those in the blood. Although the trend of AChE activity between the treated and controls was similar to those in the blood, the differences between the comparisons were non-significant ($p > 0.05$).

3.3. Protective effect of LS on the vital organs

Histology was used to explore the protective effects of the ethanol LS in the liver and kidney. Fig. 4A-F delineate the histological differences between treated and controls in the liver. T2 and the negative control were graded 0 indicating absence of liver damage (Fig. 4A and E). The pesticide positive control (OM), graded as 3, showed the most impairment (Fig. 4B), considerably more than the damage observed in T1 and T3, both graded as 2 (Fig. 4D and F) and LS positive control graded as 1 (Fig. 4C).

Kidney histopathology differences between treated and controls are shown in Fig. 5A-F. T2 and the negative control were graded 0 indicating absence of kidney damage (Fig. 5A and E). The pesticide

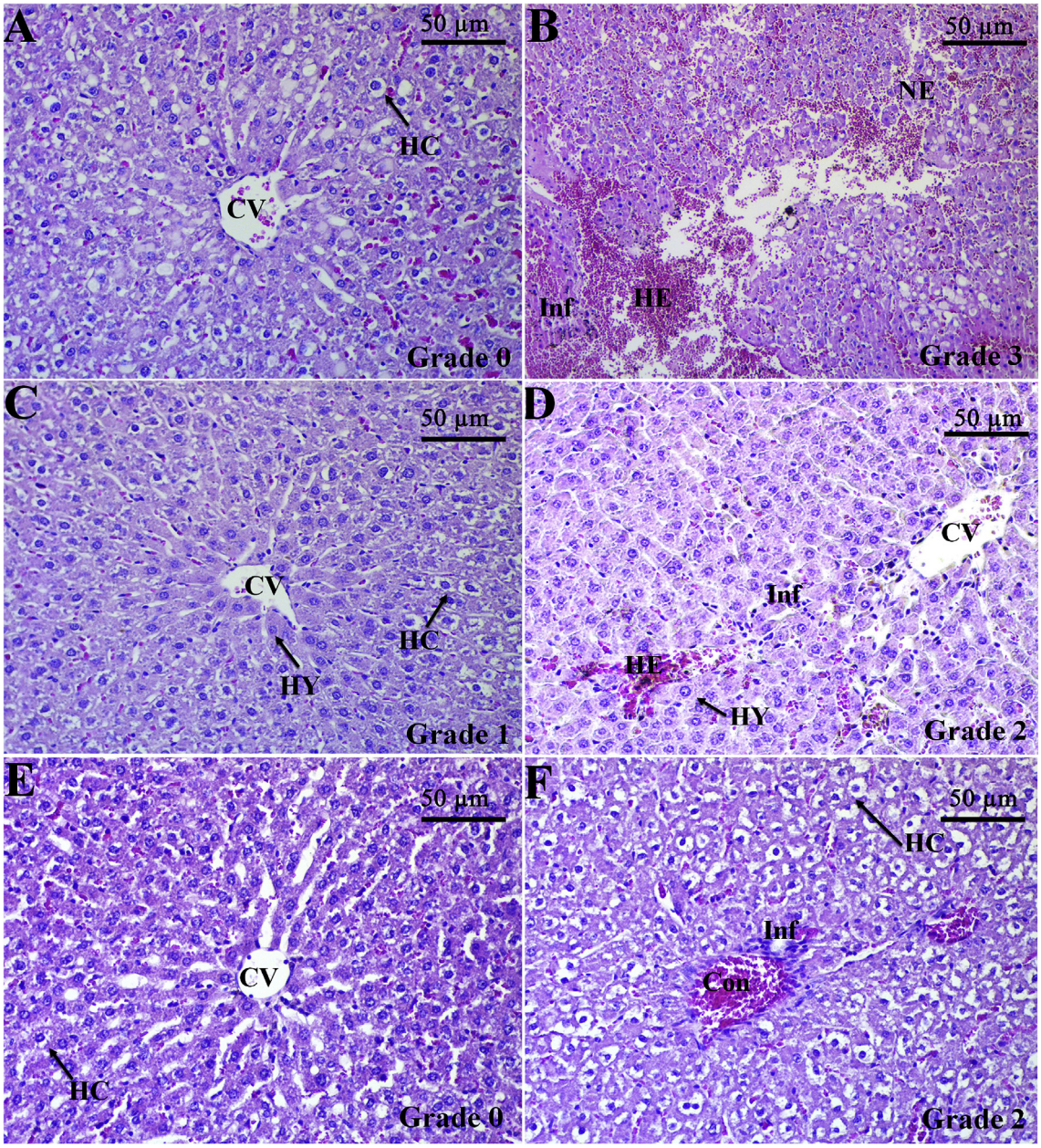

Fig. 4. Histopathological analysis of the liver in rats given the LS before and after OM treatment. Paraffin-embedded samples were stained with hematoxylin-eosin, and observed with light microscopy. A: negative control (saline); B: positive control (OM); C: positive control (LS); D–F: treated (T1-T3). CV, central vein; HC, hydropic changes; Inf, inflammatory cells infiltration; HE, hemorrhage; NE, massive necrosis; HY, hepatocyte hypertrophy; Con, congestion. Magnification was ×200.

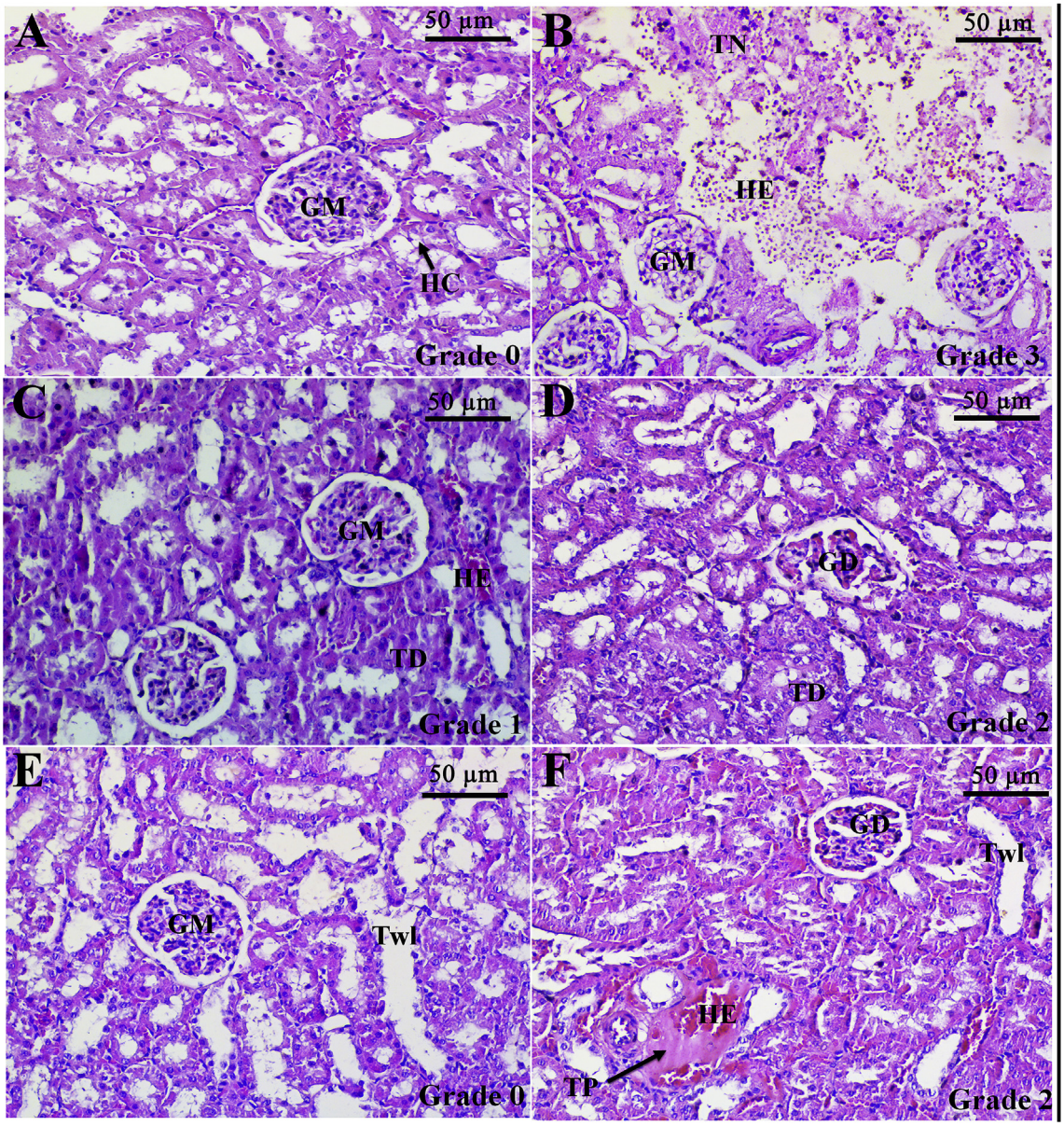

Fig. 5. Histopathological analysis of the kidney in rats after treatment with OM and ethanol LS. Paraffin-embedded samples were stained with hematoxylin-eosin, and observed with light microscopy. A: negative control (saline); B: positive control (OM); C: positive control (LS); D–F: treated (T1-T3). GM, glomerulus; HC, hydropic changes; HE, hemorrhage; GD, glomerular degeneration; TN, focal tubular necrosis; TP, tubular eosinophilic protein; TD, focal tubular degeneration; Twl, tubular widened lumen. Magnification was ×200.

positive control, graded as 3 showed the most impairment (Fig. 5B) more than those seen in T1 and T3, both graded as 2 (Fig. 5D and F) and the LS positive control graded as 1 (Fig. 5C).

4. Discussion

The principal findings of this study include the following: (i) HPLC identified QR as the phytochemical compound in LS; (ii) OM integrated into LS (T1-T3) influenced AChE activity; (iii) statistical significance ($p < 0.05$) of AChE levels between treated (T) and controls were found in the blood but not in the brain. AChE levels in the blood were significantly elevated in T2 and T3 over that for T1, but not when compared with the controls. (iv) Post-treatment histopathology profiles of the liver and kidney were similar where T2 and negative controls were graded 0; these indicate safe use of LS.

A previous study has demonstrated that biological activity of QR from ethanol extract derived from stems of plants the same as LS (*L. strychnifolia* = *Bauhinia strychnifolia*).[15] Not only have QR-derived extracts been demonstrated to exhibit antioxidant and neuroprotective properties,[24,25] they have also been found with hepatoprotective activity.[24,26]

Our OM incorporated treated (T) groups had a marked effect on AChE activity. Significantly higher AChE levels induced by T2 and T3 over that of T1 demonstrated the potent action of LS. Previous studies reported that phytoconstituents from the medicinal plants showed anti-AChE activity.[27,28] Retention of AChE activity attributed to T2 and T3 demonstrated the protective effect of LS. The T3 feature of 5 times LS suggests a dose-dependent mechanism of LS. It may be that the timing and arrangement of OM delivery into the treated rats may have influenced the outcomes. Action of LS in our

results on AChE activity was similar in the blood and in the brain. That difference, however, is that statistical significance ($p < 0.05$) was found in blood AChE activity but not in the brain. Non-significant outcome ($p > 0.05$) from our brain analysis may have been due to small sample sizes ($n \leq 6$). Synaptosomal AChE levels in the cerebellum and hippocampus of treated-rats have been shown to be markedly affected in a lead-neurotoxicity study.[29] In a study of another Thai herb, *Thunbergia laurifolia* (TL) was reported as a neuroprotective agent which preserved AChE activity in mice exposed to lead.[30,31] Moreover, inhibition of brain cholinesterase (ChE) activity was found to be treatment-related.[32] In this study, we have not only presented evidence of LS action against OP poisoning, but demonstrated the safety of its use. Moreover, murine toxicity has been found with uses of ethanol extract of the leaf of LS.[18]

The lack of toxicity observed in the histopathology samples from the gavaged subjects might due to the protective effect of phenolic contents with potent antioxidant activity.[19] Natural compounds with hepatoprotective and nephroprotective activities from *Bauhinia hookeri* have been reported in a polyphenol-rich fraction (BHPF) against CCl4-induced acute hepatorenal toxicity in mice.[33]

5. Conclusion

This study is the first report the antidote effect and protective role of *L. strychnifolium* against OP poisoning. The evidence we have presented regarding the action of LS and the safety of its use renders the utility of *L. strychnifolium* in counteracting the effects of OP poisoning. These results suggest that the LS plant species could be used as a novel antidote agent to treat OP overexposure. We believe that the rigor in which we undertook this study is proportional to the level of evidence presented here. However, similar *in vivo* studies bearing larger sample sizes would validate or modify our findings. On a functional note, our next project would involve the mechanism of LS action against OP poisoning wherein we focus on the acetylcholine receptors in the brain of treated-rats.

Author's contributions

SS conceived the idea and designed of the study, performed HPLC analysis, determined AChE activities, collected and interpreted the data, wrote and final revised manuscript. RD analyzed the histological evaluation and wrote manuscript. TY, WY and SP performed animal experiments. AK prepared the sample extract. NK participated in the conceptualization of the design. JP advised for statistical analysis. SD conducted and advised for using the dose of the extract.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.03.001.

References

1. Good AM, Kelly CA, Bateman DN. Differences in treatment advice for common poisons by poisons centres–an international comparison. *Clin Toxicol*. 2007;45:234–239.
2. Litchfield MH. Estimates of acute pesticide poisoning in agricultural workers in less developed countries. *Toxicol Rev*. 2005;24:271–278.
3. Wananukul W, Sriapha C, Tongpoo A, Sadabthammarak U, Wongvisawakorn S, Kaojarern S. Human poisoning in Thailand: the Ramathibodi Poison center's experience (2001–2004). *Clin Toxicol*. 2007;45:582–588.
4. Thapinta A, Hudak PF. Pesticide use and residual occurrence in Thailand. *Environ Monit Assess*. 2000;60:103–114.
5. Tawatsin A, Thavara U, Siriyasatien P. Pesticides used in Thailand and toxic effects to human health. *Med Res Arch*. 2015;3:1–10.
6. Plianbangchang P, Jetiyanon K, Wittaya-areekul S. Pesticide use patterns among small-scale farmers: a case study from Phitsanulok, Thailand. *Southeast Asian J Trop Med Publ Health*. 2009;40(2):401–410.
7. Harnpicharnchai K, Chaiear N, Charerntanyarak L. Residues of organophosphate pesticides used in vegetable cultivation in ambient air, surface water and soil in Bueng Niam subdistrict, Khon Kaen, Thailand. *Southeast Asian J Trop Med Publ Health*. 2013;44:1088–1097.
8. Sapbamrer R, Hongsibsong S. Organophosphorus pesticide residues in vegetables from farms, markets, and a supermarket around Kwan Phayao lake of northern Thailand. *Arch Environ Contam Toxicol*. 2014;67:60–67.
9. Marrs TC. Organophosphate poisoning. *Pharmacol Therapeut*. 1993;58(1):51–66.
10. Hobbiger F. The inhibition of acetylcholinesterase by organophosphorus compounds and its reversal. *Proc Roy Soc Med*. 1961;54:403–405.
11. Cabello G, Valenzuela M, Vilaxa A, et al. A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. *Environ Health Perspect*. 2001;109:471–479.
12. Theirmann H, Kehe K, Steinritz D, et al. Red blood cell acetylcholinesterase and plasma butyrylcholinesterase status: important indicators for the treatment of patients poisoned by organophosphorus compounds. *Arh Hig Rada Toksikol*. 2007;58:359–366.
13. Sato VH, Chewchinda S, Nuamnaichati N, et al. Pharmacological mechanisms of the water leaves extract of *Lysiphyllum strychnifolium* for its anti-inflammatory and anti-hyperuricemic actions for gout treatment. *Phcog Mag*. 2019;15:98–106.
14. Chamratpan S, Homchuen SA. Ethnobotany in upper northeastern Thailand. *Acta Hortic*. 2005;675:6774.
15. Yuenyongsawad S, Bunluepuech K, Wattanapiromsakul C, Tewtrakul S. Anticancer activity of compounds from *Bauhinia strychnifolia* stem. *J Ethnopharmacol*. 2013;150:765–769.
16. Bunluepuech K, Tewtrakul S. Anti-HIV-1 integrase activity of Thai medicinal plants in longevity preparations. *Songklanakarin J Sci Technol*. 2011;33(6):693–697.
17. Bunluepuech K, Chatchai W, Madaka F, Tewtrakul S. Anti-HIV-1 integrase and anti-allergic activities of *Bauhinia strychnifolia*. *Songklanakarin J Sci Technol*. 2013;35(6):659–664.
18. Somsak V, Noilod J, Chaciyo S, Kraithep S. Antimalarial activity of ethanolic leaf extract of *Bauhinia strychnifolia* in mice infected with *Plasmodium berghei*. *Malar Contr Elimin*. 2015;4:1–7.
19. Itharat A, Sayompark S, Hansakul P, Dechayont B. In vitro antioxidant activities of extracts of *Bauhinia strychnifolia* stems and leaves: comparison with activities in green tea extracts. *Med Aromatic Plants*. 2016;5:1–7.
20. Luengthong N, Lin C, Muangman V, Wongyai S. Preliminary study of the effect of *Bauhinia strychnifolia* Craib on blood alcohol levels in healthy volunteers. *Afr J Tradit, Complementary Altern Med*. 2016;12(2):177–188.
21. Chuakul W, Saralamp P, Boonpleng A. Medicinal plants used in the Kutchum district, Yasothon province, Thailand. *Thai J Phytopharm*. 2002;9(1):22–49.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–254.
23. Alarami AMJ. Histopathological changes in the liver and kidney of Albino mice on exposure to insecticide, dimothoate. *Int J Curr Microbiol Appl Sci*. 2015;4(7):287–300.
24. Dajas F. Life or death: neuroprotective and anticancer effects of quercetin. *J Ethanopharmacol*. 2012;143:383–396.
25. Maalik A, Khan FA, Mumtaz A, et al. Pharmacological applications of quercetin and its derivatives: a short review. *Trop J Pharmaceut Res*. 2014;13:1561–1566.
26. Weng C-J, Chen M-J, Yeh C-T, Yen G-C. Hepatoprotection of quercetin against oxidative stress by induction of metallothionein expression through activating MAPK and PI3K pathways and enhancing Nrf2 DNA-binding activity. *N Biotechnol*. 2011;28:767–777.
27. Adewusi EA, Steenkamp V. *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa. *Asian Pac J*

Trop Med. 2011;4(10):829–835.
28. Adewusi EA, Moodley N, Steenkamp V. Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. *South Afr J Bot*. 2011;77:638–644.
29. Basha DC, Rani MU, Devi CB, Kumar MR, Reddy GR. Perinatal lead exposure alters postnatal cholinergic and aminergic system in rats brain: reversal effect of calcium coadministration. *Int J Dev Neurosci*. 2012;30(4):343–350.
30. Phyu MP, Tangpong J. Protective effect of *Thunbergia laurifolia* (Linn.) on lead induced acetylcholinesterase dysfunction and cognitive impairment in mice. *BioMed Res Int*. 2013:1–6.
31. Tangpong J, Satarug S. Alleviation of lead poisoning in the brain with aqueous leaf extract of the *Thunbergia laurifolia* (Linn.). *Toxicol Lett*. 2010;198:83–88.
32. Herr DW, Mwanza J-C, Lyke DF, Graff JE, Moser VC, Padilla S. Relationship between brain and plasma carbaryl levels and cholinesterase inhibition. *Toxicology*. 2010;276(3):172–183.
33. Ai-Sayed E, Abdel-Daim MM, Kilany OE, Karonen M, Sinkkonen M. Protective role of polyphenols from *Bauhinia hookeri* against carbon tetrachloride-induced hepato- and nephrotoxicity in mice. *Ren Fail*. 2015;37:1198–1207.