Original Article

Effect of *Ocimum americanum* Water Leaf Extract on Antioxidant System in Rat

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Abstract

Introduction: *O.americanum* has been reported to possess various medicinal properties. No study has been conducted in the antioxidant properties of *O.americanum* from the Thailand region.

Objectives: The objective of this study was to examine the antioxidant and cytoprotective aspects of the herb *O.americanum.*

Methods: Aqueous leaf extract of *O.americanum* was screened for phytochemical contents. Antioxidant properties were determined by the DPPH method. 24 male Sprague-Dawley rats were fed aqueous leaf extract for 28 days. Blood was drawn and collected to estimate the levels of BUN, creatinine, AST, ALT, and ALP and then the rats were sacrificed. Their liver tissues were collected and processed for histological study. Western blot analysis was performed to determine the expression of γ-GCL.

Results: Phytochemical contents of *O.americanum* are phenolic compounds, alkaloids, cardiac glycosides, tannin, and steroids. The phenolic content was 41.27 ± 1.86 mg/gram of gallic acid equivalents. DPPH assay of the free radical scavenging activity determined that IC_{50} was 36.91 ± 0.66 µg/ml (compared to BHT = 12.34 ± 1.14 µg/ml). Throughout the 28 days of the experiment, the rats did not seem to be adversely affected by the extract and were found to have normal ranges of serum AST, ALT, ALP, BUN, and creatinine. The histology of their liver tissue was normal. The protein expression of γ-GCL, which is a key enzyme in GSH biosynthesis, was found to have increased significantly compared with normal controls.

Conclusions: The study found that *Ocimum americanum* extract does have antioxidative properties and can induce cytoprotective properties *in vivo*.

Keywords: Antioxidative properties, DPPH, γ-GCL, Liver histology, *Ocimum americanum*

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Introduction

Different forms of free radicals are continuously generated at low levels in cells to regulate several physiological functions and are eliminated by an antioxidative system in the body. An imbalance between free radical production and elimination results in oxidative stress[1]. Enhanced antioxidant activity can reduce the oxidative damage from free radicals, and thus can decrease the risk of developing many free radical-induced illnesses,[2] such as malignancy, autoimmune, cardiovascular, and neurodegenerative diseases.[3] Several plants have been reported to produce antioxidant activity from their phytochemical contents: such as flavonoid, tannin, benzyl-isothiocyanate, and other phenolic compounds.[4,5]

There are many methods to assess the antioxidative capacity of a compound. Among them, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is one of the widely used methods. It is based on a free radical scavenging property of an antioxidative substance in which the DPPH radical, with an absorption of 515-525 nm, is converted into a colorless compound after reduction by the antioxidant.[6,7]

Gamma glutamylcysteinylglycine (GSH) is the crucial endogenous antioxidant compound involved in cellular redox homeostasis[8] and the glutathione redox system is the body's first-line defense against oxidative stress.[9] GSH is synthesized in the cytosol. The rate-limiting step of GSH biosynthesis requires the cytosolic enzyme gamma glutamate-cysteine ligase (γ-GCL) for glutamylcysteine formation[10] and then glutathione synthetase to link glycine to glutamylcysteine to form GSH.[11]

The genus *Ocimum* which belongs to the family Lamiaceae, has been widely used as a dietary plant. In Thailand, there are 5 species in this genus which are *O.americanum* L., *O.basilicum* L., *O.gratissimum*, *O.tenuiflorum* L. and *O.kilimandscharicum* Baker ex Gurke.[12] There is scientific research to support the health benefits of some of these species. Among them is *Ocimum americanum.*

O.americanum, commonly known as hoary basil has been reported to possess various medicinal properties. Its seed has been used to treat headaches, nausea, vomiting, constipation[13] and fungal infection.[14] Its seed extract could inhibit bacterial growth such as *Salmonella* spp, *Escherichia coli* O157:H, *Campylobacter jejuni* and *Clostridium perfringens*.[15] Water extract from Indian *O.americanum* has been reported to possess hypoglycemic and antioxidant effects.[16] Moreover, previous phytochemical studies suggested that *O.americanum* contains flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids.[17, 18] The objective of this study was to examine the antioxidant and cytoprotective aspects of the herb *O.americanum.*

Methods

2.1 Plant material identification

The plant was collected from Pathum Thani province, Thailand in January 2016. The botany identification was performed by the Department of Pharmacognosy and pharmaceutical sciences, Prince of Songkla University (Herbarium Specimen SKP 095 15 01 01).

2.2 Preparation of the extract

2,500 g of air-dried leaves of *O. americanum* were chopped finely, added 1 ratio of hot water to the chopped leaves, boiled in a stainless boiler for 2 hours (100 ºC), and then filtered with cheesecloth. The clear filtrate was collected and freeze-dried (-80 ºC) and evaporate with FTS system evaporator model Dura Dry (Science Engineer International CO., LTD., USA). The weight of the water extract was recorded. The percentage yield was 10.40%.[19]

2.3 Preliminary phytochemical testing

The extract was screened for its phytochemical contents by the standard method according to Thai Herbal Pharmacopoeia Vol. I, 1998.

2.4 Estimation of total phenolic content

Total phenolics were determined by a spectrophotometric method. The absorbance of the solution was recorded at 725 nm. A standard curve of absorbance versus concentration of gallic acid was plotted. The percentage of total phenolic content was calculated and expressed as % gallic acid.[20]

2.5 DPPH assay

Free radical scavenging capacity of the water extract of *Ocimum americanum* (OA) was tested by a modified DPPH assay in three in vitro models using butylated hydroxytoluene (BHT) as a

standard.[6] In brief, 100 µL of DPPH solution and the same volume of diluted OA extract were mixed in a 96-well microplate and held at room temperature for 30 min. BHT was used as a positive control. The absorbance was measured at 520 nm with a blank containing DPPH. The DPPH radical scavenging activity was calculated and expressed as IC50.

2.6 Western blot analysis of γ-GCL protein expression

Western blot analysis was used to determine the expression of γ-GCL and β-actin in liver tissue. RIPA buffer (#9806, Cell Signaling Technology, Inc. MA, USA) was used to lyse tissues with addition of protease inhibitor cocktail (M221: Amresco, OH, USA).

The protein samples were mixed with SDS loading buffer and subjected to separation by electrophoresis in 10% SDS polyacrylamide gel.

The separated proteins are transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked using 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST buffer). After blocking for one hour at room temperature and rinsing by TBST, the membrane was overnight incubated with the primary antibody in TBS. After washing with TBST, the membrane was incubated with the horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for one hour.

After rinsing the membrane to remove unbound antibody by TBST buffer, the membrane was incubated in enhanced chemiluminescence (ECL) substrate solution (Super Signal West Pico Chemiluminescent Substrate: ThermoScientific, IL, USA). Densities of each protein band were visualized and analyzed with the aid of Image Studio version 2.1 software (Odyssey Imaging System LT-COR® biosciences, USA).

2.7 Experimental animals and designs

Twenty-four adult male Sprague-Dawley rats (200-250 gm) were used and ethical committee clearance was obtained from Institutional care and use committee of Thammasat University (IACUC No. 009/2558). Experiments were conducted under Animals for Scientific Purposes Act, B.E. 2558 (A.D. 2015), Government of Thailand. All institutional and national rules for the care and utilization of laboratory animals were followed.

All animals were held in standard laboratory conditions for one week. The conditions were temperature-controlled environment (25 ± 1ºC), 55 ± 5% relative humidity, regular 12 hours light/12 hours dark cycles. They were fed with standard rat chow diet and water ad libitum.

The animals were divided into four groups (n = 6)

Group I: Control group (given distilled water)
Group II: OA extract at the dose of 100 mg/kg BW/day
Group III: OA extract at the dose of 200 mg/kg BW/day
Group IV: OA extract at the dose of 400 mg/kg BW/day

The rats received the extract according to their group for twenty-eight days.

2.8 Biochemical analysis

Blood was drawn from the experimental animals on the twenty-eighth day after they received the extract. The serum was collected to estimate the levels of blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) by the laboratory unit at Thammasat University Hospital.

2.9 Liver histology study

After twenty-eight days of the study, the rats were anesthetized and sacrificed. Their liver tissues were collected and fixed in 10% formalin for one week, and then embedded in paraffin. The paraffin blocks were cut into 5 µm thick serial sections, then stained with hematoxylin-eosin (H&E) and examined for histological study by photomicroscope.

2.10 Statistical analysis

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

Results

3.1 Phytochemical screening and total phenolic compound

The phytochemical screening of OA leaf extract revealed the presence of alkaloid, cardiac glycosides, tannin, steroid, and phenolic compounds. The phenolic content in OA was found to be 41.27 ± 1.86 mg of gallic acid equivalents per gram.

3.2 Antioxidant activity studies

Free radical scavenging activity assessed by DPPH assay gave IC_{50} of 36.91 ± 0.66 μg/mL (that of BHT was 12.34 ± 1.14 μg/mL). The protein expression of antioxidant enzymes, γ-glutamylcysteine ligase (γ-GCL) in the liver is shown in Figure 1.

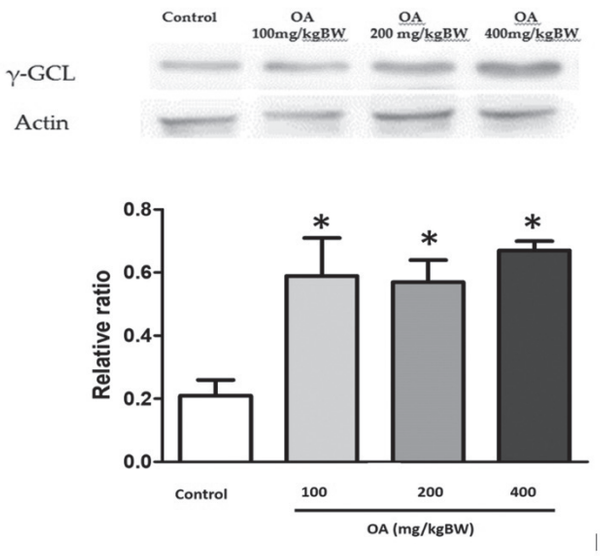

Figure 1 The protein expression of γ-GCL in the liver tissue of the control and OA treated rats. *p < 0.05.

3.3 Biochemical analysis

Biochemical parameters: AST, ALT, ALP, BUN, and Cr are shown in Table 1.

Table 1 Serum biochemical parameters of the control and OA treated rats

	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dL)	Cr (mg/dL)
Control	120 ± 5.51	43.33 ± 1.45	80.33 ± 5.93	20 ± 1.16	0.37 ± 0.03
OA 100 mg/kg BW	84.67 ± 7.22	36.67 ± 2.67	68.67 ± 2.40	19 ± 2.00	0.23 ± 0.03
OA 200 mg/kg BW	81.33 ± 6.17	31.67 ± 1.86	64.33 ± 4.37	16.33 ± 0.33	0.20 ± 0.00
OA 400 mg/kg BW	83 ± 6.43	37.67 ± 1.67	69.67 ± 2.19	16.67 ± 0.88	0.27 ± 0.03

Note: AST; Serum aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, BUN; blood urea nitrogen and Cr; creatinine

3.4 Liver histology study

As shown in Figure 2, there is no change of normal hepatic lobular pattern, portal triads, central vein; hepatocellular trabeculae are regularly distributed. The hepatocytes have typical cuboidal shape, normal size, and cell membrane integrity as shown in Figure 3.

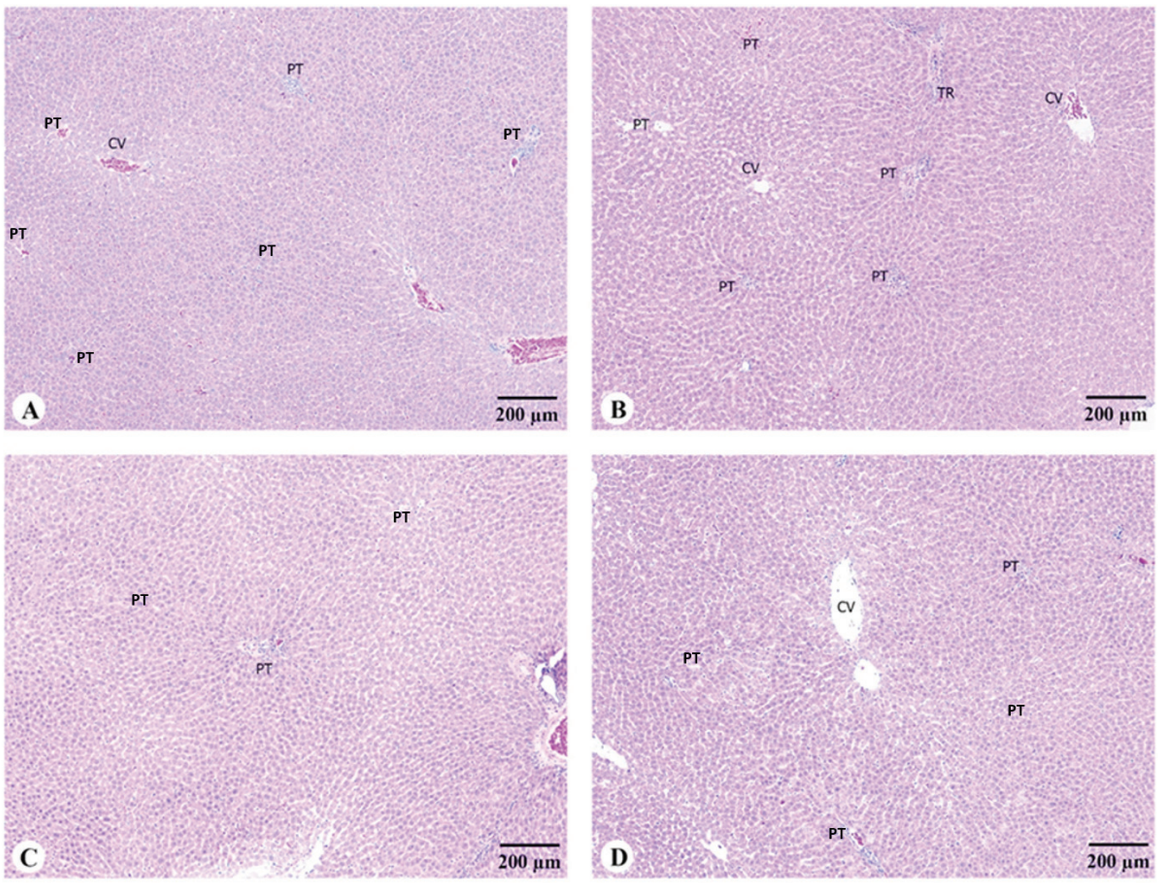

Figure 2 The Liver histological sections stained with hematoxylin and eosin of the control (A) and OA treated rats: 100 (B), 200 (C), and 400 (D) mg/kg BW. (the 4x magnification).

Note: CV; central vein, PT; portal triad, TR; trabeculae

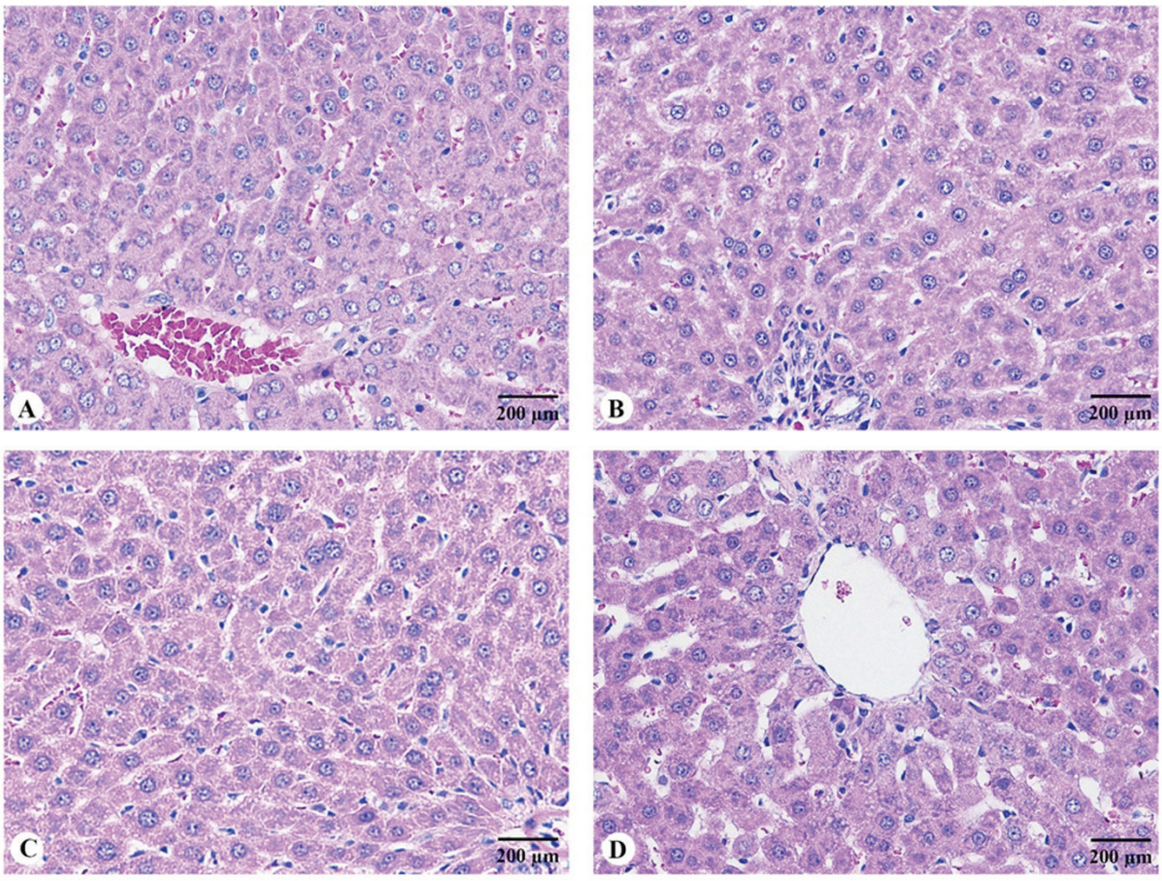

Figure 3 The Liver histological sections stained with hematoxylin and eosin of the control (A) and OA treated rats: 100 (B), 200 (C), and 400 (D) mg/kg BW (the 20x magnification).

Discussion

The presence of alkaloid, cardiac glycosides, tannin, steroid and phenolic compounds of OA leaf extract corresponds with the previous studies from Indian OA.[16] Alkaloid, tannin, and phenolic compounds possess a wide range of biological properties including antioxidant properties.[21, 22] The radical-scavenging activity of the phenolic compound is attributed to a replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds as a result of their hydrogen donating ability.[22] However, our OA extract does not contain flavonoid and saponin which were reported in previous studies. Therefore, comparative studies involving phytochemical contents from different regions should be done to determine the variation of bioactive composition among different regions.

In DPPH assay, OA extract markedly abates DPPH, although it is less effective than BHT, a commercial antioxidative substance. This result reveals that OA extract has free radical scavenging activity from the ability to give hydrogen to free radical DPPH•. The results are well correlated with an intense protein band of antioxidant enzymes, γ-GCL in the liver. γ-GCL is a cytosolic enzyme required for γ-glutamylcysteine synthesis which competently disposes of H_2O_2 by acting as glutathione peroxidase-1 cofactor.[11] Therefore OA extract, with a strong γ-GCL protein band, could play a part in cellular redox control in antioxidant activity.

Moreover, treatment with OA extract is not toxic, which is revealed by normal serum biochemical parameters along with normal histology of liver tissue. In conclusion OA extract, possesses antioxidant effects due to the presence of bioactive compounds such as alkaloid, tannin and phenolic compounds, as well as an ability to promote γ-GCL function in GSH synthesis with no harmful effects.

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Compliance with Ethics Requirements: Ethical committee clearance was obtained from Institutional care and use committee of Thammasat University (IACUC No. 009/2558). Experiments were conducted under Animals for Scientific Purposes Act, B.E. 2558 (A.D. 2015), Government of Thailand. All institutional and national rules for the care and utilization of laboratory animals were followed.

Conflict of interest: All authors report no conflicts of interest relevant to this article.

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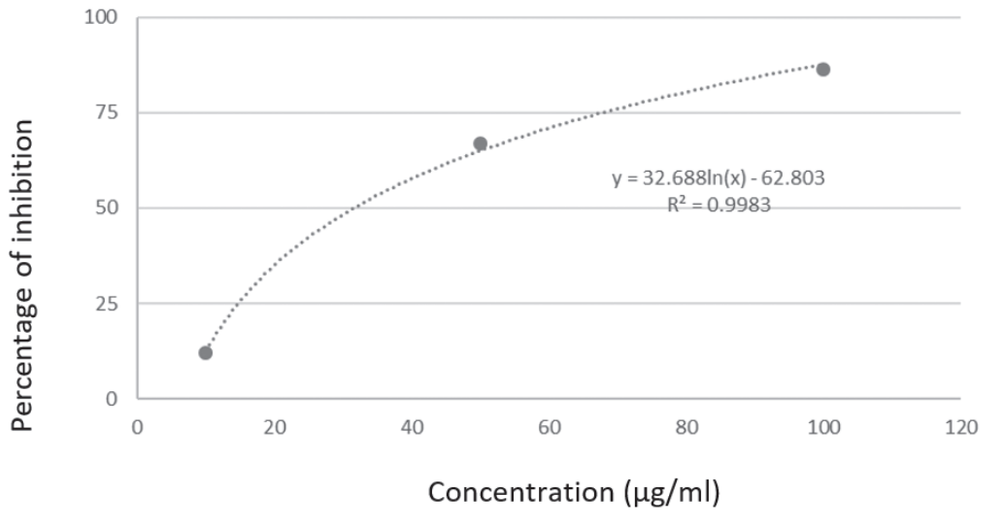
Supplementary data



Treatment	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
OA	10	11.93 ± 0.30	36.91 ± 0.66
	50	66.84 ± 3.29	
	100	86.49 ± 0.80	
BHT			12.34 ± 1.14

Graph of DPPH radical scavenging activity of OA as the sample and BHT as the positive control. The data of percentage inhibition were expressed as mean ± SD with triplicate (n = 3)