

ผลของทรานเรสเวออะทอลและผลิตภัณฑ์จากอู่นแดงต่อการดูดซึม
และอัตราการตรึงเจอร์ของตับของหนูเมาส์ ความเป็นพิษ การ
ยับยั้งวัฏจักรเซลล์และการชักนำการตายแบบอะพอพโทซิสใน
เซลล์มะเร็งของคน

นางสาวนภาพร แก้วดวงดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาชีววิทยาสังแวดล้อม
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2549

**EFFECTS OF TRANS-RESVERATROL AND RED
GRAPE PRODUCTS ON ABSORPTION AND LIVER
ULTRASTRUCTURES OF MICE, CYTOTOXICITY,
CELL CYCLE ARREST, AND INDUCTION OF
APOPTOSIS IN HUMAN CANCER CELL LINES**

Napaporn Kaewdougdee

**A Thesis Submitted in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy in Environmental Biology**

Suranaree University of Technology

Academic Year 2006

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องุ่นและสารสกัดจากองุ่นแดงเป็นสารต้านอนุมูลอิสระและสารต้านมะเร็ง การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อตรวจหาปริมาณของสารประกอบฟีนอลิกของผลิตภัณฑ์องุ่นแดงสายพันธุ์ชินฟานเดลจากฟาร์มของมหาวิทยาลัยเทคโนโลยีสุรนารี ซึ่งได้แก่ ไวน์ น้ำองุ่นและสารสกัดเอทานอลจากกากองุ่นและศึกษาผลของผลิตภัณฑ์องุ่น และสารทรานเรสเวอะทอลต่อการดูดซึมของสารประกอบฟีนอลิกและอัลตราสตรักเจอร์ของเนื้อเยื่อตับในหนูเมาส์ ความเป็นพิษ การยับยั้งวัฏจักรเซลล์ และการชักนำให้เกิดการตายแบบอะพอพโตซิสของเซลล์มะเร็งของคนจากการตรวจหาปริมาณของสารประกอบฟีนอลิกในผลิตภัณฑ์องุ่น พบว่า สารประกอบฟีนอลิกที่พบในสารสกัดเอทานอลจากกากองุ่น ($4,407.33 \pm 13.65$ มิลลิกรัมต่อลิตร) มีปริมาณสูงกว่าไวน์แดง ($3,613.00 \pm 15.13$ มิลลิกรัมต่อลิตร) และน้ำองุ่น ($1,102.67 \pm 21.96$ มิลลิกรัมต่อลิตร) อย่างมีนัยสำคัญทางสถิติ หลังจากการป้อนด้วยน้ำองุ่นและสารสกัดเอทานอลจากกากองุ่นหนึ่งครั้ง พบการดูดซึมสูงสุดในพลาสมาของหนูเมาส์ ICR ที่ช่วงเวลา 12 ชั่วโมงเท่ากับ 0.22 ± 0.01 กรัมต่อลิตร และช่วงเวลา 6 ชั่วโมงเท่ากับ 0.22 ± 0.01 กรัมต่อลิตร ตามลำดับ แต่การวิเคราะห์หาปริมาณการดูดซึมของทรานเรสเวอะทอลในพลาสมาของหนูเมาส์ ICR โดยวิธี capillary electrophoresis ไม่พบระดับของทรานเรสเวอะทอลในพลาสมาของหนูเมาส์ทั้งในกลุ่มที่ได้รับทรานเรสเวอะทอลและทรานเรสเวอะทอลร่วมกับไวน์

การให้ผลิตภัณฑ์องุ่นและทรานเรสเวอะทอลกับหนูเมาส์ ICR ทุกวัน ครบ 6 เดือน มีผลต่อพยาธิวิทยาระดับอัลตราสตรักเจอร์ของเซลล์ตับต่ำ เมื่อเทียบกับกลุ่มควบคุมโดยพิจารณาจากการสะสมของไขมันและไกลโคเจน และความผิดปกติของออร์แกเนลล์ในเซลล์ตับ ความเป็นพิษของทรานเรสเวอะทอลและสารสกัดเอทานอลจากกากองุ่นต่อเซลล์มะเร็งตับอ่อน Panc 2.03 และเซลล์มะเร็งตับ SNU 1079 ขึ้นกับความเข้มข้นของสาร เมื่อทดสอบโดยวิธี MTS ทรานเรสเวอะทอลสามารถยับยั้งวัฏจักรเซลล์ของ Panc 2.03 และ SNU 1079 ในระยะ S และ G₁ ตามลำดับ ส่วนสารสกัดเอทานอลจากกากองุ่นยับยั้งวัฏจักรเซลล์ของ Panc 2.03 ในระยะ S และ

ยับยั้งเซลล์ SNU 1079 ในระยะ S และ G₂ ฤทธิ์ความเป็นพิษของทรานเรสเวอะอะทอล และสารสกัดเอทานอลจากกากองุ่นต่อเซลล์มะเร็งเกิดจากการชักนำให้เกิดการตายแบบอะพอพโตซิสภายในเซลล์ที่สามารถตรวจสอบโดยวิธีการย้อมด้วย DAPI และ annexin-V FITC การแตกหักของดีเอ็นเอ และการลดการแสดงออกของโปรตีน pro-caspase 3 และ โปรตีน Bcl-2 ข้อมูลจากการวิจัยครั้งนี้เสนอแนะว่ากลไกที่ชักนำให้เกิดการตายในเซลล์มะเร็งทั้งสองชนิดอาจจะเกิดผ่านทาง การควบคุมของโปรตีนที่เกี่ยวข้องกับการชักนำให้เกิดการตายแบบอะพอพโตซิส และ/หรือ ผ่านทางการยับยั้งของวัฏจักรเซลล์

สาขาวิชาชีววิทยา

ปีการศึกษา 2549

ลายมือชื่อนักศึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษา _____

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

NAPAPORN KAEWDOUNGDEE : EFFECTS OF TRANS-RESVERATROL AND RED GRAPE PRODUCTS ON ABSORPTION AND LIVER ULTRASTRUCTURES OF MICE, CYTOTOXICITY, CELL CYCLE ARREST, AND INDUCTION OF APOPTOSIS IN HUMAN CANCER CELL LINES THESIS ADVISOR : ASST. PROF. BENJAMART CHITSOMBOON, Ph.D., 181 PP.

TRANS-RESVERATROL/ABSORPTION/ULTRASTRUCTURE/CYTOTOXICITY/
CELL CYCLE/ARREST/APOPTOSIS/CANCER

Trans-resveratrol and red grape products have been known to be antioxidants and anticarcinogens. The present study investigated the total phenolic compound (TPC) contents of Zinfandel grape products, wine, juice and pomace, from the Suranaree University of Technology farm. The effects of grape products and trans-resveratrol on absorption of TPC *in vivo*, ultrastructure of mouse liver tissue, cytotoxicity, cell cycle arrest and apoptotic induction on human cancer cell lines were investigated. The TPC content of ethanolic grape pomace extract ($4,407.33 \pm 13.65$ mg/L) was significantly higher than those of red wine ($3,613.00 \pm 15.13$ mg/L) and grape juice ($1,102.67 \pm 21.96$ mg/ml). After single oral administration, the highest absorptions of TPC content in plasma of ICR mice were 0.22 ± 0.01 g/L at 12 h, and 0.22 ± 0.01 g/L at 6 h post administration of juice and ethanolic grape pomace extract, respectively. In contrast, the recoveries of trans-resveratrol absorptions as analyzed by capillary electrophoresis were not detected in the plasmas of both trans-resveratrol and trans-resveratrol-spiked wine treated groups. Exposure of ICR mice to grape

products and trans-resveratrol daily for six months reduced ultrastructural pathologic of hepatocytes, included minimal glycogen, fat accumulation, and organelle abnormality, compared to their corresponding vehicle controls. Trans-resveratrol and ethanolic grape pomace extract exhibited cytotoxic effects on pancreatic Panc 2.03 and cholangiocarcinoma SNU 1079 cells in a dose dependent manner assessed by MTS assay. Trans-resveratrol treatment of Panc 2.03 and SNU 1079 cells resulted in S and G₁ phase arrests in the cell cycle, respectively. Ethanolic grape pomace extract treatment of Panc 2.03 cells resulted in S phase arrest, while the same treatment of SNU 1079 cells resulted in both S and G₂ phase arrests of the cell cycle. The cytotoxic activity was mediated via apoptosis as demonstrated by DAPI and annexin V-FITC staining, DNA fragmentation, and decreased pro-caspase 3 and Bcl-2 protein expressions. These data suggest a possible mechanism of cytotoxicity in both cancer cell lines, at least in part, through the regulation of apoptosis-related proteins and/or cell cycle dysregulation.

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Academic Year 2006

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ACKNOWLEDGEMENTS

I would like to thank Asst. Prof. Dr. Benjamart Chitsomboon, my thesis advisor, for her encouragement, valuable suggestions and advice.

I would like to thank Asst. Prof. Dr. Chariya Hahnvajanawong who is very kindly provided me with the extract for this project and her valuable guidance. I would also like to thank Assoc. Prof. Dr. Banchob Sripa for his valuable comments on the experiments throughout the project and Prof. Dr. Kovit Pattanapanyasat, his generosity for allowing me to use his laboratory facilities to conduct my research.

Special thanks are extended to the members of the laboratory at the The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University, School of Medicine, Baltimore, MD, USA for their support and patience; Asst. Prof. Dr. Anirban Maitra for his help in experimental designs, wonderful suggestions, and for correcting my manuscript; and Collin Karikari, Dr. George fieldman, Dr. Indrajit Roy for helping me with the apoptosis assay, cell cycle assay, sharing with me their wide technical expertise and their comments on my manuscript. I also express my appreciation for their assistance, and kindness from Assoc. Prof. Dr. Supalax Srijaranai, Dept. of chemistry, Faculty of Science, KKU, Assoc. Prof. Chanarong Arunyanak, Dept. of anatomy, Faculty of Medicine, KKU, Mrs Punneeporn Wasinrapee, Thailand MOPH-US CDC Collaboration, and Dr. Busarawan Sriwattana, Department of Medical Sciences, Ministry of Public Health. I wish to express to my appreciation to my friends for their friendship and sincere encouragement.

I am particularly indebted to the Environmental Biology Graduate Program at the Institute of Science, Suranaree University of Technology. Finally, I am very grateful to the Bansomdejchaopraya Rajabhat University research grant, my parents, Ms. Chulawan Kaewdongdee and Mrs. Chuntima Rueangsukudom for their financial support and understanding that helped me to overcome many difficult moments.

Napaporn Kaewdongdee

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LIST OF ABBREVIATIONS

| | |
|-----------------|--|
| ACN | Acetonitrile |
| BD | Becton Dickinson |
| BSA | Bovine serum albumin |
| bp | Base pair |
| b.wt. | Body weight |
| CE | Capillary electrophoresis |
| CHD | Coronary heart disease |
| cm | Centimeter |
| cm ² | Centimeter square |
| CO ₂ | Carbon dioxide |
| DNA | Deoxyribonucleic acid |
| DAPI | 4' 6'-Diamidine-2'-phenylindole Dihydrochloride |
| DMRT | Duncan multiple range test |
| DMSO | Dimethyl sulfoxide |
| DTT | Dithiotrietol |
| DW | Distilled water |
| ECL | Enhanced chemiluminescence system |
| EDTA | Ethylenediaminetetraacetic acid |
| EDTA-K3 | Vacutainer [®] EDTA-K3 |

LIST OF ABBREVIATIONS (Continued)

| | |
|---------------|---|
| ELISA | Enzyme Linked Immunoabsorbant Assay |
| ER | Endoplasmic reticulum |
| <i>et al.</i> | <i>Et. Alii</i> (Latin), and others |
| etc. | And others |
| FBS | Fetal Bovine Serum |
| Fig. | Figure |
| FITC | Fluorescein isothiocyanate |
| g | Gram |
| GAE | gallic acid equivalent |
| h | Hour |
| HEPES | N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid |
| HPLC | High-performance liquid chromatography |
| ICR | Institute cancer research |
| i.d. | Internal diameter |
| k | Kilo (10^3) |
| kDa | Kilodalton |
| kg | Kilogram |
| kV | Kilovolt |
| L | Liter |
| LOD | Limit of detection |

LIST OF ABBREVIATIONS (Continued)

| | |
|--------------------|--|
| LOQ | Limit of quantitation |
| m | Milli (10^{-3}) |
| M | Molar |
| 2 ME | 2-Mercaptoethanol |
| min | Minute |
| mg | Milligram |
| mg/L | Milligram per liter |
| mg/ml | Milligram per milliliter |
| ml | Milliliter |
| mM | Milli Molar |
| mm | Millimeter |
| MTS | 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt |
| MWs | Molecular weight |
| n | Nano (10^{-9}) |
| NaHCO ₃ | Sodium Bicarbonate |
| NaOH | Sodium Hydroxide |
| ND | Not detected |
| nm | Nanometer |
| NP 40 | Non-idet P 40 |

LIST OF ABBREVIATIONS (Continued)

| | |
|------------------|---|
| OD | Optical Density |
| OsO ₄ | Osmium tetroxide |
| PAGE | Polyacrylamide gel electrophoresis |
| Panc 2.03 | Pancreatic cancer cell line |
| PBS | Phosphate buffered saline |
| PI | Propidium Iodide |
| ppm | Part per million |
| p.s.i. | Pound per square inch = 6894.76 pascal |
| RESV | Resveratrol |
| rpm | Revolution per minute |
| RPMI 1640 | Roswell Park Memorial Institute |
| RSW | Resveratrol spiked wine |
| RT | Room temperature |
| SD | Standard deviation |
| SDS | Sodium dodecyl sulfate |
| SNU 1079 | Seoul National University 1079 |
| TEM | Transmission electron microscope |
| TEMED | N, N, N', N'-tetramethyl ethylene diamine |
| TPC | Total phenolic compound |
| TTS | Total solid |
| UV | Ultraviolet |

LIST OF ABBREVIATIONS (Continued)

| | |
|-------|------------------------------|
| v/v | Volume: Volume |
| w/v | Weight per volume |
| % | Percent |
| °C | Degree Celsius |
| μ | Micro (10^{-6}) |
| μg | Microgram |
| μg/ml | Microgram per milliliter |
| μl | Microliter |
| μm | Micrometer |
| x g | x gravitational acceleration |
| x | Times |