Studies on the anti-nociceptive activities of melatonin and its *N*-benzoyl derivative

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Introduction: Melatonin is a hormone secreted by the pineal gland in mammals. It is well documented for antioxidant, anti-inflammatory and anti-nociceptive activities. However, the use of melatonin as an anti-nociceptive agent limited due to its short half-life and low bioavailability. Many efforts have been reported to modify the structure of drugs candidates as esters or amides derivatives to improve physicochemical properties and therapeutic effects. *N*-benzoyl substitution of melatonin also increase similarity to indomethacin, an anti-inflammatory drug, and might be increase anti-inflammation activity. This study aimed to synthesize *N*-benzoyl derivative of melatonin and also investigate its anti-nociceptive effects in mice. **Materials and Method:** *N*-benzoyl melatonin was synthesized via reacted melatonin with benzoyl chloride and using 4-dimethylaminopyridine as catalyst. The reaction processed under room temperature for 24 hours then purified by column chromatography. Nuclear magnetic resonance (NMR) was use to confirm the structure. The anti-nociceptive activities were carried out using acetic acid induced writhing test and tail flick test to determined dose-dependent respond of melatonin and its *N*-benzoyl derivatives. Moreover, we also determined time course effect via tail flick test. **Results:** According from synthesis reaction, we obtained *N*-benzoyl melatonin as a yellowish crystal with 35% yield. The result of ^{1}H NMR showed five extra proton signals at chemical shift 7.28 to 7.45 ppm. In addition, carbons on the benzene ring show at 132.01, 128.50, 128.15 and 127.74 ppm including with quaternary carbon at chemical shift 173.59 ppm. In tail flick experiment, melatonin at treated doses of 10, 25 and 50 mg/kg significantly increased tail flick latency to 4.7, 5.0 and 5.3 seconds, respectively compare to vehicle treatment (3.0 seconds). With the same doses test, *N*-benzoyl significantly increased tail flick latency to 4.6, 5.7 and 6.6%, respectively. Moreover, *N*-benzoyl melatonin also showed longer acting than melatonin in time course study. Effect of melatonin derivative on acetic acid-induced writhing also investigated. *N*-benzoyl melatonin showed effect for inhibited number of tail flick similar to melatonin (%inhibtion of 10, 25, 50 mg/kg *N*-benzoyl-melatonin was 34, 7, 38.5, 47.5 %, respectively v.s. %inhibition of melatonin was 28.1, 45.8, 49.0%, respectively) **Conclusion:** *N*-benzoyl melatonin, appeared to have higher potency and longer acting for anti-nociceptive than melatonin, suggesting that *N*-benzoyl-melatonin has potentially to developed as new analgesic drug.

Keywords: Melatonin, Benzoyl derivatives, Anti-nociceptive, Tail flick, Writhing test,

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Expression profile of hepatic cytochrome 450 proteins in high fat and high fructose fed mice

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Introduction: Cytochrome P450 (CYP) is a superfamily of drug metabolized enzymes mainly located in the liver that plays an important role in catalyzing biotransformation of endogenous compounds and other substances such as drugs and environmental chemicals. The fast-paced life adapts people dietary habit to fast food with full of high fat and high fructose, which is one factor implicating metabolic disorders and can cause hepatic pathology such as non-alcoholic fatty liver disease. This pathology of liver results in modification of hepatic metabolism function. Therefore, the present study aims to investigate the expression profile of hepatic CYP450 protein of high fat- and high fructose-fed mice. **Materials and Method:** Seven-week-old male ICR mice (n=5) were intragastrically given 65% hydrogenated soil bean oil 1 mL/day/mouse and freely accessed to 20% fructose in drinking water daily for 2, 4, or 8 weeks while the regular diet-fed mice (n=5) were employed as a control in this study. The mice were sacrificed at 24 h after the last treatment and the hepatic microsome was prepared. The animal handling was approved by the Animal Ethics Committee for Use and Care of Khon Kaen University, Thailand (Approval No. AEKKU 92/2555). The expression of CYPs protein including CYP1A1/2, CYP2B9/10, CYP1B1, and CYP3A11 were performed using Western blotting analysis technique followed by statistically analyzed by ANOVA and tukey's *post hoc* test. **Results:** In high fat- and high fructose-fed mice, there was no significantly change of hepatic CYP1A1/2 protein expression profile compared to the mice fed with regular diet, while the protein expression levels of CYP2B9/10 was reduced significantly after administration of high fat and high fructose diet for 4 and 8 weeks. The significant increase of CYP1B1 protein expression was observed after 8 weeks of the treatment while that of CYP3A11 protein was elevated after the 4- to 8-week treatment. These observations supported that continuous consuming of the high fat- and high fructose dietary could modify the expression levels of hepatic CYP450 proteins. **Conclusion**: Alteration of the expression profiles of hepatic CYP2B9/10, CYP1B1, and CYP3A11 proteins was significantly related to the duration of high fat- and high fructose-intake. The change of CYP activity may possibly affect drug or xenobiotic metabolism which can lead to ineffective treatment or increasing risk of toxicity. These findings suggested the high-fat high-fructose-fed mice as a useful animal model for studying metabolic condition via regulation of CYP enzyme that related to drug metabolism or drug interaction.

Keywords: Cytochrome P450 activities, High fat and high fructose diet, CYP1A1/2, CYP1B1, CYP2B9/10, CYP3A11.

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