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Anatomical Associations of Mandibular Nerve Branches in Thai Cadavers

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Abstract

The mandibular nerve (MN) branches supply the areas of infratemporal fossa and oral cavity. Clinically, the variations of these nerves must be concerned before maxillofacial surgeries. The variant MNs especially the lingual nerve (LN) and inferior alveolar nerve (IAN) have been reported in many races. However, this data has never been documented in Thai population. This study aimed to investigate anatomical associations of MN branches in Thai cadavers. Fifty-one hemi-sectioned heads of embalmed Thai cadavers were carefully dissected to observe 1) the bifurcation pattern between LN and IAN, 2) the communication of the MN branches, and 3) the distances from lower 2nd molar (mesial and distal aspect) to LN. The results showed that there are 2

types of LN-IAN bifurcation (upper [96.08 %] and lower [3.92 %] mandibular notch. In addition, we found 3 types of unusually communicating branches (type I [55.56 %]: between LN and IAN, type II [33.34 %]: between LN and trunk of MN, and type III [11.11 %]: between LN and auriculotemporal nerve, respectively. Moreover, the distances from mesial and distal lower 2nd molar to the LN are approximately 11.54 ± 2.53 and 11.35 ± 2.23 mm. These anatomical associations of mandibular nerve branches observed in Thai cadavers are the basic data that may be important before operation in maxillofacial region.

Keywords: mandibular nerve branches, lingual nerve, inferior alveolar nerve, Thai cadavers

Combined Extract of *Morus alba* and *Polygonum odoratum* Improves Memory Impairment in Experimental Menopause Rats Induced by Bilateral Ovariectomy

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Abstract

Introduction and Objective: The novel intervention against menopause related symptoms such as memory impairment is still required due to the limitation of the current therapy. Based on the cognitive enhancing effect of *Morus alba* and *Polygonum odoratum* and the synergistic effect concept of traditional medicine. Therefore, we investigated whether the combined extract of *Morus alba* and *Polygonum odoratum* improved memory impairment in ovariectomized rats.

Materials and Methods: Leaves of *M. alba* and *P. odoratum* were collected from

Amphoe Muang Khon Kaen, Thailand. They were prepared as water extract by decoction method and lyophilized as powder. The ratio of both extracts which provided highest contents of phenolics and flavonoids was selected for this study. To investigate the effect of the combined extract of *M. alba* and *P. odoratum* on memory impairment, female Wistar rats were divided into various groups; 1) control (naive intact) 2) sham operation plus vehicle 3) ovariectomized (OVX) plus vehicle 4) OVX plus positive control 5)-7) OVX plus the combined extracts of *M. alba* and *P. odoratum* at doses of 5, 150 and 300 mg.kg⁻¹ respectively. All

rats were subjected to the assigned interventions for 12 weeks. All interventions were started at 7 days after operation. The memory assessment was performed using Morris Water Maze test at 7, 14, 21, 28, 56 and 84 days after treatment. At the end of study hippocampus and prefrontal cortex were isolated and analyzed by means of the oxidative stress marker including malondialdehyde (MDA) level, the activities of superoxide dismutases (SOD), glutathione peroxidase (GPx), catalase and acetylcholinesterase suppression (AChEI) activity as Indices. In addition, the density of neuron in CA1, CA2, CA3 and dentate gyrus of hippocampus were also determined.

Results: All doses of the combined extract significantly decreased escape latency but increased retention time together with the increase neuron density in hippocampus especially in CA1, CA2 and CA3. Low and medium dose of the combined extract also suppressed AChE activity in OVX rats. The decreased MDA level was observed only in OVX rats which treated with medium of the combined extract. Therefore, the memory enhancing effect the combined extract might occur partly via the

increased neuron density in hippocampus. In addition, the low dose of the combined extract might also increase cognitive function in OVX rats via the suppression of AChE whereas the medium dose of the combination extract might improve cognitive function partly both via the suppression of AChE and via the decreased oxidative stress status in hippocampus. The possible underlying mechanism for the high dose of the combine extract was still unknown and required further investigation.

Conclusion: The combination extract of *M.alba* and *P.odoratum* is the potential food supplement to attenuate memory impairment in menopause women. However, further researches concerning the précised underlying mechanism, chronic toxicity and clinical trial study are necessary.

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Key words: *Morus alba*, *Polygonum odoratum*, menopause, memory impairment, oxidative stress

HT7 Acupoint Stimulation Mitigates Memory Impairment and Improves pERK1/2 Activation in Alcoholic Rats

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Abstract

Rationale and objective: Currently, the therapeutic strategy against memory deficit induced by alcoholism is still limited and difficult to approach due to an expensive cost. Therefore, a cheap and effective strategy is required. On the basis of the memory-enhancing effect of the HT7 acupoint stimulation, we aimed to determine the effects of HT7 acupoint stimulation on memory, oxidative status, cholinergic function and histomorphology of hippocampus in animal model of alcoholism. Moreover, the ERK1/2 activation in hippocampus was also determined.

Materials and methods:

Male Wistar rats were assigned into various groups as following; 1) Control, 2) Ethanol 3) Ethanol+Sham acupuncture 4) Ethanol+Acupuncture HT7. Rats were induced alcoholism in a stepwise fashion. The first to fourth week, ethanol concentration was increased 5% per week. Then, the concentration of ethanol was raised to 30% until the end of study. Alcoholic rats were treated either acupuncture at HT7 or with sham acupuncture bilaterally for 1 minute each once daily at a period of 14 days. All rats were assessed spatial memory using Morris water maze test after single

intervention and at 7 and 14 days of intervention. At the end of study, hippocampai were isolated and determined MDA level and the activities of CAT, SOD, GSH-Px and AChE using colorimetric assays. The neuronal densities in various subregions of hippocampus were also determined using the cresyl violet staining method. Finally, the ERK 1/2 activation was determined by Western blot analysis method. All data were expressed as mean \pm SEM and analyzed via ANOVA followed by Tukey post hoc test. P-value less than 0.05 were regarded as significance.

Results: Acupuncture at HT7 acupoint attenuated the elevation of escape latency and the decreased retention time induced by alcohol in alcoholic rats. The decreased AChE activity and MDA level and the activities of CAT, SOD and GSH-Px in hippocampus together with the increased neuron density in all areas of hippocampus were also observed. In addition, our results showed that HT7 acupoint stimulation significantly increased density of pERK1/2

bands. Therefore, laser acupuncture at HT7 acupoint may improve memory deficit induced by alcohol via the decreased oxidative stress and the enhanced cholinergic function in hippocampus.

Conclusion: Acupuncture at the HT7 acupoint is an effective non-invasive intervention to mitigate memory deficit induced by alcoholism. Therefore, it may be served as alternative choice for treating memory deficit induced by alcoholism. However, further exploration in clinical trial is essential.

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Keywords: Acupuncture, HT7 acupoint, memory impairment, alcoholism, AChE, ERK1/2

Evaluation of Safety and Stability of “MP1”, the Novel Function Drink for Postmenopausal Women

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General description/Objectives: Recent findings show that MP1, a novel functional drink containing the extract of purple corn cob, can enhance cognitive function in animal model of menopause. However, the essential information concerning the maximum bearing dose and stability of MP1 is still unknown. Therefore, this study aimed to determine consumption safety and stability of MP1.

Materials and method: Microbial, pesticides, and heavy metal contamination were determined to assure the consumption safety whereas acute toxicity was performed to determine maximum bearing dose of MP1. In addition, the stability of product was performed by determining

the biological activities and biomarker of product at 1,2 and 3 months after preparation. Acute toxicity was performed by using female Wistar rats as experimental animal. The fixed dose test according to OECD guideline was performed by administering MP1 at doses of 2000 mg/kg and observed the mortality rate within 24 hr. 14 days later, the rats were sacrificed and vital organs were observed both at macro and microscopic levels. Blood chemistry and hemotological changes were also determined.

Results: No contaminations of microbial, pesticides, and heavy metal were observed. No neurotoxicity signs and pathological lesions were observed. LD50 was more than 2000 g/kg. In addition, the

stability test showed that at 4 °C without light, all biological activities of MP1 were changed less than 20% within 2 months.

Conclusion: MP1 is safe for consumption up to 2000 mg/kg . Stability is high up to 2 months. Therefore, it shows the high potential to be used in human. However, clinical trial is required to assure the safety for human application.

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Keywords: MP1, acute toxicity, neurotoxicity, stability

Laser Acupuncture at HT7 Acupoint Improves Behavioral Disorders and Oxidative Stress Status in Cortex, Striatum and Hippocampus in VPA-rat Model of Autism

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Abstract

Introduction and objective: The novel effective therapeutic strategy against autism, a severe neurological development disorder, is still requires. Recent findings show that oxidative stress plays a crucial role on the pathophysiology of autism and laser acupuncture at HT7 can improve oxidative status in many neurological disorders. Therefore, we aimed to assess the effect of laser acupuncture at HT7 on behavior disorders and oxidative stress status in cortex, striatum and hippocampus

of Valproic (VPA) - rat model of autism.

Materials and methods: Both male and female rat pups at 14 days old were divided into the following groups; 1) control 2) VPA 3) VPA+Laser acupuncture at HT7 4) VPA+Sham laser acupuncture. Rat pups were induced autistic-like behaviors by the subcutaneous administration of Valproic acid (VPA) at dose of 400 mg/kg BW on post natal day (PND) 14. Then, they were stimulated at HT7 once daily for 10 minutes during PND14-PND40. All rats were subjected to behavioral testing including negative

geotaxis, mid air righting, hot plate test, rotarod test, open-field activity, elevated plus-maze test, learning and memory test and social behavior on various postnatal days up to postnatal day 40. At the end of study, brain oxidative status including MDA levels and the activities of SOD, CAT and GSH-Px were determined in cortex, striatum and hippocampus.

Results: Laser acupuncture at HT7 significantly improved autistic-like behaviors. The decreased MDA levels in all areas mentioned above were observed while the increased GSH-Px activity was observed only in striatum and hippocampus and no changes in SOD and CAT activities were observed.

Conclusion: Laser acupuncture at HT7 mitigates autistic-like symptoms partly via the improved oxidative status. Further researches are necessary to understand the precise underlying mechanism.

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Keywords: laser acupuncture, autistic like symptoms, autism, valproic acid

Quercetin Loaded Nanofiber Improves Functional Recovery of Peripheral Nerve Injury in Diabetic Rats

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Abstract

General description/Objectives: Currently, the therapeutic efficacy of nerve injury in diabetic condition is not in satisfaction level. Based on the neuroprotective effect of quercetin and the beneficial effect of both delivery via nanofiber and the transdermal route, the beneficial effect of quercetin-loaded nanofiber on peripheral nerve injury in diabetic condition is focused. In this study, we aimed to determine the effect of quercetin-loaded nanofiber on functional recovery of peripheral nerve injury in STZ-diabetic rats.

Materials and Methods: Male Wistar rats were induced diabetes mellitus by a single shot injection of streptozotocin (STZ).

Right sciatic nerve was induced injury by crush injury. Diabetic rats with nerve injury were treated with 5%, 10% and 15% quercetin-loaded nanofiber (1x1 cm) once daily for 21 days. The evaluation of nerve function was performed using foot withdrawal reflex and De Medinacelli method every 3 days throughout the study period. At the end of the study period, they were determined nerve conduction velocity (NCV). The lesion nerve was isolated and determined the axon density, oxidative stress status and phosphorylation of ERK (pERK).

Results: Diabetic rats which received quercetin loaded nanofiber could improve sciatic function index via Demedicelli method and foot withdrawal latency to-

gether with the increase of NCV, axon density, oxidative stress status and pERK. Therefore, we did suggest that quercetin-loaded nanofiber decreased oxidative stress status and increased pERK leading to the improved nerve damage leading to the increased NCV and axon density resulting in the improved functional recovery of sciatic nerve.

Conclusion: The present study demonstrates the potential of quercetin-loaded nanofiber to facilitate functional recovery in diabetic condition. Therefore, it may be

served as the health product for caring diabetic neuropathy. However, further studies are necessary.

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Keywords: Quercetin-loaded nanofiber, nerve injury, diabetes

Evaluation of Consumption Safety and Anti-diabetic Retinopathy of the Combination Extract of Purple Waxy Corn and Ginger

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General description/Objectives: To date, the cheap and effective interventions against diabetic retinopathy, the challenges in the developing countries are required. Based on the crucial roles of oxidative stress and aldose reductase on diabetic complications and the antioxidant and aldose reductase suppression activities of purple waxy corn and ginger, we aimed to anti-retinopathy effects of the combined extract of purple waxy corn and ginger.

Materials and methods: Male Wistar rats, weighing 200-250 g, were induced diabetic condition via the single injection

of streptozotocin (55 mg/kg.BW⁻¹). The rats which showed the blood glucose levels >250 mg.dL⁻¹ were recruited for further study. Diabetic rats were orally given the extract at doses of 50, 100 and 200 mg/kg.BW⁻¹ for 10 weeks. Then, they were determined histopathology of retina. The changes of MDA together with the activities of SOD, CAT, GPx and AR in eyes were determined using biochemical assay. Moreover, acute toxicity study of the extract was determined.

Results: The results Evaluation of consumption safety and anti-diabetic retinopathy of the combination extract

of purple waxy corn and ginger that the extract was safe up to 5 g/kg BW. In addition, all doses of the combined extract decreased lens opacity, MDA and AR in the eyes of diabetic rats. The elevation of CAT and GPx activities were also observed. The anti-retinopathy property of the combined extract was also confirmed by the increased number of neuron in ganglion cell layer and thickness of total retina and retinal nuclear layer in diabetic rats.

Conclusion: The combination extract of purple waxy corn and ginger is a potential functional food to protect against diabetic retinopathy. However, further studies concerning chronic toxicity and clinical trial are still essential.

Keywords: Purple waxy corn, ginger, diabetic retinopathy, toxicity

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Flower Extract of *Nelumbo nucifera* Exhibits Aphrodisiac Effect in Stress Male Rats via the Alteration of Dopaminergic Function and NO-PDE5 Pathway

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Abstract

Introduction and objective: Nowadays, sexual dysfunction induced by stress is increased but the therapeutic efficacy is still limited. Based on the traditional reputation for enhancing male sexual function of *Nelumbo nucifera* Gaertn (sacred lotus), a plant in a family of Nelumbonaceae, This study was set up to determine the aphrodisiac effect of *N.nucifera* and its underlying mechanism in stress male rats.

Materials and methods: Adults male Wistar rats were divided into 1) naïve control 2) vehicle (distilled water) treated

group plus stress 3) Sildenafil citrate (5 mg kg⁻¹) plus stress 4) Tianeptine (15 mg kg⁻¹) plus stress and 5) *N.nucifera* extract at doses of 10, 100 and 200 mg kg⁻¹. All rats were daily given the extract via oral route once daily 30 min prior to the exposure to 12-hr immobilization stress for 14 days of experimental period. Sexual behaviors including latency and number of mounting, intromission and ejaculation were determined every 7 days throughout the 14 days of study period. At the end of experiment, PDE-5 activity and eNOS in cavernosum, MAO-B activity in medial preoptic area

and nucleus accumbens, TH-IR neurons in ventral tegmental area and serum levels of both testosterone and corticosterone were evaluated.

Results: All doses of *N.nucifera* extract significantly decreased mounting latency mount latency and intromission latency but increased ejaculation frequency. In addition, all doses of *N. nucifera* also decreased corticosterone level and the suppression of PDE-5 activity. Moreover, *N.nucifera* suppressed MAO-B in MPOA and NAc, increased high density of tyrosine hydroxylase immunoreactive neurons in NAc and VTA. In addition, it also increased density of eNOS band in corpus cavernosum. Taken all data together, it was suggested that *N.nucifera* flower extract improved mounting via the increased dopamine function

and decrease corticosterone whereas the improved intromission might occur via the multi-factors such as the improved dopamine function, decreased PDE-5 activity and increased eNOS. However, further researches are necessary to understand the possible active ingredient, toxicity and detail mechanism of action. In conclusion, the current findings support the traditional medicinal uses of this plant as an aphrodisiac herb.

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Keywords: *Nelumbo nucifera* Gaertn, aphrodisiac, immobilization stress