Evaluation of the analgesic effect of Umbelliprenin and Umbelliprenin-morphine co-administration on the acute, chronic and neuropathic pain

Article in Indian journal of pharmaceutical education · February 2015
DOI: 10.5530/jipe.49.2.7

6 authors, including:

Mahmoud Hashemzaei
Zabol University Of Medical Sciences
51 PUBLICATIONS 376 CITATIONS

Kaveh Tabrizian
Zabol University Of Medical Sciences
46 PUBLICATIONS 514 CITATIONS

Mehrdad Iranshahi
Mashhad University of Medical Sciences
234 PUBLICATIONS 5,032 CITATIONS

Milad Iranshahi
Mashhad University of Medical Sciences
35 PUBLICATIONS 591 CITATIONS

Some of the authors of this publication are also working on these related projects:

Study the effect of natural products on autoimmune diseases View project

Lime juice Adulteration View project
Evaluation of the analgesic effect of Umbelliprenin and Umbelliprenin-morphine co-administration on the acute, chronic and neuropathic pain

Mahmoud Hashemzaei¹, Mohammad Amin SadeghiBonjar¹, Kaveh Tabrizian¹,², Mehrdad Iranshahi³, Milad Iranshahy³, Ramin Rezaee⁴

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
²Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran
³Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
⁴Department of Molecular Sciences, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

ABSTRACT

Background and purpose: Neuropathic Pain (NP) is a complex and chronic pain which is accompanied by nerve injury. Umbelliprenin (UMB) is a naturally occurring prenylatedcoumarin with anticancer, antioxidant, anti-inflammatory, antibacterial and antileishmanial activities. This study aimed to investigate the antinociceptive effects of UMB on acute, chronic and neuropathic pain and its combination therapy with morphine on the neuropathic pain.

Methods: Albino mice weighing 20-25 g were randomly divided into 13 groups (n=7), subjected to hot plate with groups including morphine 1 mg+UMB (0.01 μM/kg), morphine (1 mg/kg, i.p.), UMB (0.01 mM/kg), Imipramine 40 mg/kg and NS (normal saline) (0.9%) as vehicle), formalin test with groups including (NS, Imipramine (40 mg/kg, i.p.), morphine (9 mg/kg, i.p.) and UMB (0.01 μM/kg)) and morphine tests with groups including (NS (0.9%), imipramine (40 mg/kg, i.p.), morphine (1 mg/kg, i.p.) + UMB (0.01 mM/kg) and morphine (1 mg/kg, i.p.). The acute and neuropathic pain were evaluated using hot-plate and formalin and morphine tests. Results: Administration of UMB single dose (0.01 mM) reduced NP significantly (p<0.05) compared to the negative control and didn’t change acute pain against Diclofenac. Antinociceptive effects of UMB were comparable to Imipramine as a standard positive control. UMB potentiated morphine 1 mg/kg response on NP. Conclusion: This research indicates that UMB alone reduces NP and its combination with morphine potentiates morphine effects. Therefore, UMB-morphine co-administration is proposed to be used instead of conventional treatment.

Key words: Umbelliprenin, Neuropathic Pain, Morphine, Sciatic Nerve Ligation.

INTRODUCTION

Neuropathic Pain (NP) is caused by cancer, diabetes mellitus, Parkinson's disease, Alzheimer's disease. About more than 3-4.5% of global population is suffering from NP. Classical analgesics such as anticonvulsants, tricyclic antidepressants, local anesthetics, opioids analgesics have inconsistent benefit or adverse effects. About 10-30 % of patients suffering from syndromes of NP are drug resistant.¹ There is remarkable need for novel analgesic being more effective or safer.

UMB, a prenylated coumarin, is related to naturally occurring compounds, which are widely distributed in Ferula plant species such as Citrus limon.²,³ It has been also found in celery, Angelica archangelica, Coriandrum sativum. They are belonged to a very large class of sesquiterpene which possess anticarcinogenic, free radical scavenging, anti-inflammatory, antileishmanial properties and were shown to be able to inhibit red pigment production in Serratia marcescens, decrease matrix metalloprotease (MMP) activity, and inhibit lipooxygenase and acetyl cholinesterase.

Anti-inflammatory and antinociceptive effects of UMB analogues have been studied.⁴,⁵ UMB inhibits iNOS mRNA and COX-2 mRNA expression which seem to be related to its anti-inflammatory effects.
in human lymphocytes and reduces transcription of related proteins.\textsuperscript{5,7-10} Furthermore, coumarins have anti-
nociceptive effect via opioidic pathway.\textsuperscript{2,11} The roots of \textit{Ferula persica} possessing UMB, are used for the treatment
of diabetes in folk medicine.\textsuperscript{12} UMB has strong lipoxygenase inhibitory properties and also has shown anti-
inflammatory effect in the carrageen an hind paw edema model in mice.\textsuperscript{5}

In this study, we attempted to determine the acute and chronic antinociceptive effect of UMB alone, and in
combination with morphine to investigate whether it can potentiate morphine anti-neuropathic pain.

\section*{METHODS}

\subsection*{Animals and surgery}

Male Albino mice produced from Faculty of Pharmacy of Zabol University of Medical Sciences weighing 20-25 g were randomly divided into 13 groups (n=7), subjected to hot plate test including morphine 1 mg + UMB (0.01 \text{μM/kg}), morphine (1 mg/kg, i.p.), UMB (0.01 \text{mM/kg}), Imipramin 40 mg/kg and NS (normal saline) (0.9%) as vehicle), formalin test including NS, Imipramine (40 mg/kg, i.p.), morphine (9 mg/kg, i.p.) and UMB (0.01 \text{μM/kg}) and morphine tests with 4 groups including NS (0.9%), imipramine (40 mg/kg, i.p.), morphine (1 mg/kg, i.p.) + UMB (0.01 \text{mM/kg}) and morphine (1 mg/kg, i.p.). The animals were kept under constant temperature (22-25\degree C), 12 hour light/
dark cycle and had free access to food and water. All procedures were in accordance with the guidelines from the declaration of Helsinki principles in the study of experimental animals.

\subsection*{Surgery}

The animals were anesthetized by ketamine (40 mg/kg, i.p.) (Merck; Germany) and xylazine (10 mg/kg, i.p.) (Merck; Germany). Sciatic nerve was ligated in the hind limb using a piece of copper wire.\textsuperscript{13}

\subsection*{Chemicals}

UMB was synthesized via the reaction of 7-hydroxy-
coumarin (1mol/l) and trans-trans-farnesyl bromide
(1.5mol/l) in acetone at room temperature.\textsuperscript{11} The reaction was accomplished in the presence of DBU (1, 8-diazabicyclo [5.4.0] undec-7-ene) (2M). 24 h later, the mixture was concentrated under reduced pressure and was purified by column chromatography (petroleum ether/ethyl acetate 9: 1 v/v) as white crystals.\textsuperscript{11}

\subsection*{Analgesic measurement}

\textit{Hot-plate test}

The Hot-plate test was performed to determine the
effect of UMB on NP\textsuperscript{12} with minor modifications. The animals were placed on a circular surface (diameter 19 cm) maintained at 55 ± 0.2\degree C and surrounded by a Plexiglas wall (12 cm high). The apparatus (Har-
vard; England) was equipped with a timer and a thermo
coupler to maintain a constant temperature. Licking the forepaws, lifting hind paws or jumping from the surface
was considered as the end point of response latencies.\textsuperscript{18} 45 seconds were indicated as cut-off time. 2 weeks after nerve ligation, pain intensity was measured.

\subsection*{Formalin test}

The formalin test was performed to determine acute and chronic pain of UMB single dose (0.01 \text{mM/kg}, i.p.). The mice were considered to evaluate neuropathic pain at different times after formalin injection (0, 30, 60, 90 and 120 min). All mice received an intraplantar injection of formalin (1% in saline) in the left hind paw. The duration of paw flinches, licking, and biting, 0-5 min after injection of formalin (first phase) and between 20 and 40 min (second phase) was recorded.\textsuperscript{14}

\subsubsection*{Morphine (1 mg/kg) UMB (0.01 mM/kg)}

This test was performed to elucidate whether UMB is able to change morphine (1 mg/kg, i.p.) effect on NP or not. The mice were randomly assigned in groups of NS (0.9%), imipramine (40 mg/kg, i.p.), morphine (1 mg/kg, i.p.) + UMB (0.01 mM/kg) and morphine (1 mg/kg, i.p.) to evaluate neuropathic pain at different times after formalin injection (0, 30, 60, 90 and 120 min).

\subsection*{Statistical analysis}

Data was analyzed using Graph Pad Prism 5.00. One-
way ANOVA followed by Newman-Keuls test to situ-
ations the potential differences was done. We used unpaired T test for comparison between control animals and the sciatic nerve ligated group. Statistically signifi-
cant differences considered as p<0.05. Data is repre-
sented as mean ± SEM.

\section*{RESULTS}

\subsection*{Effects of UMB single dose on neuropathic pain 14 days after sciatic nerve ligation using hot plate test at 0, 30, 60, 90 and 120 min.}

There were significant differences between UMB (0.01 \text{mM/kg}) + morphine (1 mg/kg), Imipramine (40 mg/
kg), UMB (0.01 \text{mM/kg}) and morphine (1 mg/kg), at 0 min (Figure 1: A). 30 min later, there were significant differ-
ences between all groups and the control group and also there was significant difference between morphine
1 mg/kg and UMB + morphine 1 mg/kg (p<0.05) (Figure 1: B). After 60, 90 and 120 min, there were significant
differences between Imipramine, UMB, and morphine 1 mg/kg + UMB (0.01 mM/kg) and morphine 1 mg/kg and NS groups (p<0.001) (Figure 1: C, D and E).

**Effects of single dose of UMB on licking and latency response on the acute phase of the formalin test.**

There were significant differences between UMB (p<0.05), morphine (p<0.001) and Diclofenac (p<0.01) and the control group (Figure 2: A & B).
Effects of UMB single dose on licking and latency response in the chronic phase in the formalin test.

There were significant differences between UMB, morphine and Diclofenac and the control group (p<0.001) (Figure 2: C & D).

DISCUSSION

The results indicated that UMB reduced chronic and acute pain. Moreover, NP due to sciatic nerve ligation in mice model was alleviated by UMB (0.01 mM/kg i.p.) UMB (0.01 mM/kg i.p.) in combination with morphine (1 mg/kg) could potentiate its antinociceptive effect. This was the first study on the effect of UMB on the antihyperalgesia, antinociceptive, anti-neuropathic pain and its combination with morphine.

Several studies showed that coumarin has antinociceptive and antihyperalgesia effects. UMB is chemically belonged to 7-hydroxycoumarins (7-HC) which have in vivo and in vitro anti-inflammatory analgesic and anti-pyretic effects, seems to be related to their ability in COX-2 inhibition. UMB has surprising inhibitory activity on soybean lipooxygenase enzyme, the key enzyme in inflammatory process, with IC<sub>50</sub> value of 0.0725 μM, compared to caffeic acid, with IC<sub>50</sub> value of 600 μM. It has been shown that 0.01 mM i.p. administration of UMB can significantly ameliorate inflammation, which is comparable to Indomethacin.

Following the formalin injection, hyperalgesia is divided into acute (the first 3-5 min) and chronic phase (15-20 min after formalin injection). UMB subsided both acute and chronic pain significantly. Nitric Oxide (NO) is one of the most detrimental especially chronic pain. Formalin rises nitrite and nitrate levels in plasma via activation of iNOS. In addition, NO can activate lipoxigenases and cyclooxygenase enzymes activity and consequently increases inflammatory and pain transduction via releasing substance P, PG and leukotrienes. Induction of pain in formalin test is facilitated by i.p. injection of the substances that increase NO level. Furthermore,
adminstration of iNOS inhibitors (p.o.,i.p. or intrathe- 
caal) can alleviate nociception especially in the second 
phase of formalin test and it is consistent with our study. 
Lipoxigenase metabolites increase sensitivity to pain in 
the second phase of formalin test by increasing pro-
duction of hydroperoxyeicosatetraenoic acid (HPETE<sub>3</sub>)
from arachidonic acid by 5, 12, and 15 lipoxigenase. 
HPETE<sub>3</sub> immediately after production, are converted 
to leukotrienes which the process can be inhibited by 
UMB. Using up to 20 μg/ml of UMB, decreased the 
production of NO and expression of inducible nitric 
oxide synthase (iNOS).<sup>6</sup>

NP is a relatively common pain that is associated 
with nervous system lesions or dysfunction.<sup>18</sup> The results 
showed that UMB could alleviate NP following sciatic 
nerve ligation. It was shown that iNOS and NO are 
involved in inflammation, neurodegeneration, and NP 
and one of underlying mechanism of UMB is iNOS 
inhibitory effects.<sup>19-21</sup>

UMB (0.01 mM) with morphine (1 mg/kg) potentiated 
morphine antinociceptive effects. UMB shows its 
antinociceptive effects partly via μ receptors stimulation.

It was elucidated that coumarins pose their effects via 
stimulation of opioid receptors.<sup>15,22</sup> Coumarins have 
inhibitory effects on NO and β-EP levels in brain. It 
could be concluded that its potentiating effect on mor-
phine is via direct activation of μ receptors.<sup>15,22</sup>

Matrix Metalloproteinase Proteins (MMPs) are criti-
cal enzymes in tumor, neovascularization and inflamation. 
Another hypothetical mechanism of UMB is reduction of pro-inflammatory proteins and decreasing 
of MMPs.<sup>3</sup> Each component with MMP inhibitory 
effect such as Diclofenac sodium poses anticancer due 
to anti-inflammatory effect.<sup>3</sup> Many other substances 
that can decrease MMPs in cells also ameliorate inflama-
tions that are in accordance with our findings.<sup>3,23</sup>

CONCLUSION

Taken together, UMB has strong antihyperalgesia, anti-
inflammatory effect especially in the late phase of for-
malin test. Moreover, it can potentiate morphine (1 mg/ 
kg) antinociceptive effects in combination with a single 
dose of UMB (0.01 mM i.p.).

REFERENCES

1. Blackburn-Munro G, Erichsen HK. Antiepileptics and the treatment of 
11(23): 2961-76.

from Ferula persica roots inhibits the red pigment production in Serrata 

Two matrix metalloproteinases inhibitors from Ferula persica var. persica. 

C, et al. Sesquiterpene coumarins from Ferula szowitsiana and in vitro 
antiinflammatory activity of 7-preneloyxocoumarins against promastigotes. 

5. Iranshahi M, Askari M, Sahebkar A, Adjpavlov-Litina D. Evaluation of 
antioxidant, anti-inflammatory and lipoxgenase inhibitory activities of 

6. Iranshahi M, Mahmoudi M, Zamanai Taghizadeh Rabe SH, Siadat Z. Anti-
inflammatory effect of Umbelliprenin from Ferula szowitsiana mediated by 
the inhibition of nitric oxide production by inflammatory macrophages. Clin 

products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide 
synthase (iNOS) in cultured mouse macrophage cells. J Ethnopharmacol. 


9. Moalem G, Tracey DJ. Immune and inflammatory mechanisms in neuropathic 

10. Le Bars D, Adam F. Nociceptors and mediators in acute inflammatory pain. 

11. Askari M, Sahebkar A, Iranshahi M. Synthesis and Purification of 
7-Prenyloxycoumarins and Herniarin as Bioactive Natural Coumarins. Iranian 

12. Afifi FU, Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on 

13. Malmberg AB, Basbaum AI. Partial sciatic nerve injury in the mouse as a 
model of neuropathic pain: behavioral and neuroanatomical correlates. Pain 

14. Hunskar S, Hole K. The formalin test in mice: Dissociation between 

15. Leal LKAM, Ferreira AAC, Bezerra GA, Matos FJA, Viana GSB. 
Antinociceptive, anti-inflammatory and bronchodilatator activities of 
Brazilian medicinal plants containing coumarin: a comparative study. J 

16. Barros TA, de Freitas LA, Filho JM, Nunes XP, Giuliatti AM, de Souza GE, 
et al. Antinociceptive and anti-inflammatory properties of 7-hydroxycoumarin 
in experimental animal models: potential therapeutic for the control of 

17. Singh VP, Patil CS, Kumar M, Kul kam SK. Effect of 5-lipoxgenase inhibitor 


19. Tal M. A novel antioxidant alleviates heat hyperalgesia in rats with an 

Dextromethorphan and its interaction with nitric oxide on sciatic nerve ligated 

galbanate, a novel inhibitor of nitric oxide production in mouse macrophage 

effect and mechanism of Ruta graveolens L. in mice. J Korean Soc Applied 

23. Saadat F, Zomorodian K, Pezeshki M, Khorrarnazadeh MR. The potential 
role of nonsteroidal antiinflammatory drugs (NSAIDs) in chemoprevention of 