Original Research

Geraniin attenuates naloxone-precipitated morphine withdrawal and morphine-induced tolerance in mice

Ella Anle Kasanga[1], Eric Boakye-Gyasi[1], Robert Peter Biney[2], James Oppong Kyekyeku[3], Christian Agyare[4], Eric Woode[1]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Department of Pharmacology, School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [4]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ABSTRACT

Background and Aim: Potentially life-threatening and unpleasant side effects associated with some analgesics have fueled the drive for the search for more analgesics with better side effect profiles. Geraniin, the most dominant secondary metabolite in the aqueous extract of the aerial parts of *Phyllanthus muellerianus*, has been shown to possess antinociceptive properties mediated partly by opioidergic mechanisms. The purpose of this study is to determine whether geraniin exhibits tolerance and if it is able to ameliorate withdrawal signs in naloxone-precipitated morphine withdrawal. **Materials and Methods:** After chronic treatment of mice with geraniin orally, the formalin test was used to ascertain whether tolerance will develop to its antinociceptive effects and if there is morphine-induced tolerance cross-generalization with geraniin. The effect of geraniin on naloxone-precipitated morphine withdrawal signs in morphine-dependent mice was also investigated. **Results:** Geraniin (3-30 mg/kg) did not produce any tolerant effects after chronic administration and there was also no cross-generalization with the tolerant effects of morphine. Geraniin did not induce withdrawal signs but significantly reduced the number of jumps in morphine-dependent mice. **Conclusion:** Geraniin does not produce any tolerant effects like morphine and also reduced the signs associated with naloxone-precipitated morphine withdrawal in mice.

KEY WORDS: Dependence, geraniin, muellerianus, naloxone, withdrawal

Address for correspondence:
Eric Boakye-Gyasi, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. E-mail: ebgyasi.pharm@ knust.edu.gh

INTRODUCTION

Pain forms part of most diseases and it is usually the major factor of the disease that alerts the patient to seek medical treatment [1]. Despite the frequency of pain symptoms, individuals often do not obtain satisfactory relief of pain possibly because of inappropriate or insufficient use of existing therapies [2,3]. Insufficient use of existing therapies can be due to the numerous and life-threatening side effects associated with the use of most of these agents [4,5]. The opioids are extensively used in moderate to severe pain management. However, physical dependence and tolerance to opioids occur to some degree whenever opioids are administered for more than a few days [6]. Dependence can result in various physical and psychological signs which in humans may include restlessness, irritability, increased salivation, lacrimation and sweating, muscle cramps, vomiting, and diarrhea [7]. These drawbacks limit the use of opioids in

the management of pain and thus fueling the search for novel analgesics that do not have these side effects.

Although the mechanisms of the development of tolerance to and dependence on opioids have been vigorously investigated, it still remains unclear. Several cellular models including blockade of glutamate action at the N-methyl-D-aspartate (NMDA) receptors, phosphorylation and receptor conformation changes, decoupling of receptors from G-proteins and the receptor desensitization, μ-opioid receptor internalization and/or receptor downregulation and upregulation of the cyclic adenosine monophosphate pathway have been proposed to play important roles in the development of opioid tolerance and dependence [8,9]. Some medicinal plants and isolates, especially those with significant antioxidant activity, have proven beneficial in the attenuation of dependence and tolerance to opioid agents [10,11].

Geraniin ($C_{41}H_{28}O_{27}$) is a dehydroellagitannin which occurs as two isomers in solution [Figure 1]. It is pale amorphous compound with a molecular weight of 952.64 g/mol and density of 2.26 g/mL [12]. Geraniin has been shown to possess strong cellular proliferation effects using primary dermal fibroblasts and human adult high calcium low temperature (HaCaT) keratinocytes [12]. It has also been shown to possess antiviral [13] and anti-inflammatory properties [14]. Geraniin has been reported previously as a very potent antioxidant [15].

The antinociceptive properties and possible mechanism of action of geraniin in animal models have been recently reported [16] to have some opioidergic involvement. Considering the drawbacks of opioid analgesics, this study, therefore, seeks to determine if geraniin exhibits tolerance like the conventional opioids and if geraniin can have a potential use in the management of naloxone-precipitated morphine withdrawal.

MATERIALS AND METHODS

Source of Geraniin

Geraniin (96% w/w HPLC grade) (CAS number: 60976-49-0) [Figure 1], isolated from the aqueous extract of the aerial parts of *Phyllanthus muellerianus* (Kuntze) Exell., was a kind donation by Prof. Andreas Hensel, Institute of Pharmaceutical Biology and Phytochemistry, University of Muenster, Muenster, Germany. This study was conducted in June 2015.

Animals

ICR mice (30 ± 5 g) were obtained from the Noguchi Memorial Institute for Medical Research, Ghana and housed in the vivarium of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, KNUST. They were housed in stainless steel cages in groups of 5 animals per cage with soft wood shavings as bedding. Food (normal mice chow: Agricare Ltd., Kumasi, Ghana) and tap water were *ad libitum*. Mice were also maintained under standard laboratory conditions (temperature 24-25°C, relative humidity 60-70%, and 12 h light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Animal Care and Use Committee, 1998). All protocols used were approved by the Departmental Ethics Committee (No.: FPPS/PCOL/0115/2015).

Drugs and Chemicals

Morphine hydrochloride and naloxone hydrochloride were obtained from Bodene (PTY) Limited Trading; Port Elizabeth, South Africa; formalin was purchased from British Drug Houses, Poole, England and diazepam (DZP) from Kilitch Drugs, Maharashtra, India. Doses of drugs used in this study are based on previous preliminary works carried on in the laboratory. Normal saline was used as vehicle throughout this study.

### Assessment of Tolerance Liability

The formalin test [16] was used to ascertain whether, after chronic treatment, tolerance develops to the antinociceptive effects of geraniin and morphine as well as whether morphine-induced tolerance cross-generalize with geraniin. Mice were divided randomly into six groups ($n = 5$) and for the first 8 days were treated as follows:
- Groups I-III - Vehicle (10 ml/kg, p.o.)
- Groups IV - geraniin (20 mg/kg, p.o.)
- Groups V-VI - Morphine (6 mg/kg, i.p.).

On day 9, animals in Group I were given vehicle (10 ml/kg, p.o.), Groups II and IV received geraniin (10 mg/kg), and Groups III and V were also given morphine (3 mg/kg). To ascertain the possibility of morphine-induced tolerance cross-generalizing with geraniin, Group VI also received geraniin (10 mg/kg). Summary treatments on day 9 were as follows:
- Group I - Vehicle treatment for 8 days and vehicle again on day 9
- Group II - Vehicle treatment for 8 days and geraniin (10 mg/kg) on day 9
- Group III - Vehicle treatment for 8 days and morphine (3 mg/kg) on day 9
- Group IV - Geraniin (20 mg/kg) treatment for 8 days and geraniin (10 mg/kg) on day 9
- Group V - Morphine (6 mg/kg) treatment for 8 days and morphine (3 mg/kg) on day 9
- Group VI - Morphine (6 mg/kg) treatment for 8 days and geraniin (10 mg/kg) on day 9.

60 min after geraniin and 30 min after morphine administration, formalin was injected into the right paw and the behavior of the mice was recorded for 60 min. A nociceptive score was determined for each 5 min time block by measuring the amount of time spent in the biting/licking of the injected paw. Tracking of the behavior was done using public domain software JWatcher™, Version 1.0. The average nociceptive score for each animal per time block was calculated by multiplying the frequency and time spent in biting/licking and data were expressed as the mean ± standard error of the mean (SEM) of scores between 0 and 10 min (first phase) and 10 and 60 min (second phase) after formalin injection.
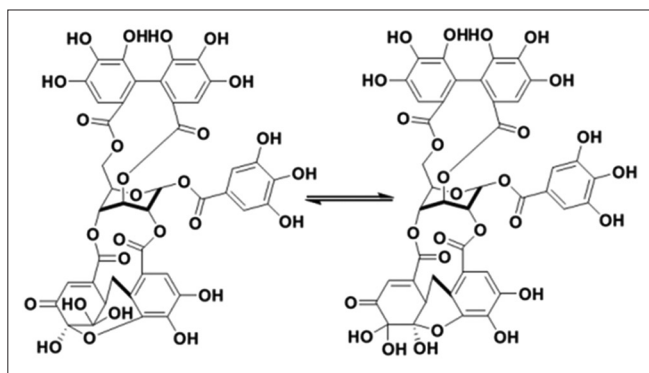
Figure 1: Chemical structures of the two isomers of geraniin in solution (adapted from Agyare *et al.,* 2011)

Ability of Geraniin to Induce Withdrawal Syndromes of Dependence

A method previously described by [17] was used to determine whether geraniin induces withdrawal signs similar to that produced by morphine administration. Mice were grouped ($n = 5$) and received the following drug treatments.

For the first 3 days,
- Group I - morphine (50, 50 and 75 mg/kg s.c. at 1100, 1400 and 1700 h, respectively)
- Group II - vehicle (10 ml/kg s.c. at 1100, 1400 and 1700 h, respectively)
- Group III - geraniin (150, 150 and 225 mg/kg p.o. at 1100, 1400 and 1700 h, respectively).

On the 4th day, Group I received morphine (50 mg/kg, s.c.), Group II received vehicle (10 ml/kg, s.c.), and Group III received geraniin (150 mg/kg, p.o.). 2 h later, naloxone (5 mg/kg, s.c.) was administered to all the animals and mice were immediately placed in a glass cylinder (30 cm high, 20 cm in diameter). The number of jumping episodes (withdrawal symptoms) was recorded for 30 min.

Effect of Geraniin on Naloxone-precipitated Morphine Withdrawal Signs

To determine whether geraniin can ameliorate withdrawal signs produced by naloxone in morphine-dependent animals [17], mice were randomly assigned to 5 groups (I-V) ($n = 5$). Groups I-V received morphine (50, 50 and 75 mg/kg s.c. at 1100, 1400 and 1700 h, respectively) for 3 days.

On the 4th day, group I received vehicle (10 ml/kg, p.o.), Groups II-IV received geraniin (3-30 mg/kg, p.o.), and Group V were treated with DZP (5 mg/kg, i.p.). 30 min (for i.p.) or 1 h (for p.o.) later, animals in all the groups received morphine (50 mg/kg, s.c.). 2 h later they were all treated with naloxone (5 mg/kg, s.c.) to precipitate opioid-induced withdrawal. Animal behavior was recorded for 30 min and the number of jumps counted using the public domain software JWatcher™, version 1.0.

Effects of Geraniin on Motor Co-ordination

To rule out the possible effect of geraniin affecting motor function, the rota-rod test was performed. Naive mice were trained on 3 successive days on the rota-rod (Ugo Basile, model 7600, Comerio, Varese, Italy) at a speed of 25 rpm. On the test day (day 4), five groups of mice ($n = 5$) were administered geraniin (3-30 mg/kg, p.o.), D-tubocurarine (0.1 mg/kg, i.p.) or vehicle. The animals were then repeatedly tested for their motor co-ordination performance on the rota-rod (cut-off time 120 s) at 0.5, 1, 1.5, 2, 2.5, and 3 h after drug administration. The maximum time that the animals were able to spend on the rota-rod was recorded [18].

Data Analysis

All data are presented as mean ± SEM ($n = 5$). The time-course curves were subjected to two-way (treatment × time) repeated measures analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). Differences in AUCs were analyzed using one-way ANOVA for normally distributed data or Kruskal–Wallis test for data that was not normally distributed with drug treatment as a between subjects factor. Further comparisons between vehicle and drug-treated groups were performed using the Tukey's multiple comparisons test. $P \leq 0.05$ was considered statistically significant. Data analysis was done in September 2015.

RESULTS

The Assessment of Tolerance Liabilities

A two-way ANOVA showed a significant difference in the observed effects ($F_{5,24} = 104.5$, $P < 0.0001$) [Figure 2]. Morphine (3 mg/kg, i.p.) significantly attenuated nociceptive responses in both phases ($F_{2,12} = 51.15$, $P < 0.0001$ Phase I; $F_{2,12} = 82.99$, $P < 0.0001$ Phase II) of the formalin test in chronic vehicle-treated animals as seen in the AUC graphs. However, the same dose of morphine administered at day 9 in animals chronically treated with morphine (6 mg/kg, i.p.) failed to show such effect indicating the development of tolerance [Figures 2 and 3]. In contrast, oral administration of geraniin (10 mg/kg) showed a comparable antinociceptive activity ($F_{3,16} = 53.72$, $P < 0.0001$ Phase I; $F_{3,14} = 137.2$, $P < 0.0001$ Phase II) in mice treated chronically with either geraniin (20 mg/kg, p.o.) or normal saline, indicating lack of tolerance development [Figures 2 and 3]. Furthermore, geraniin (10 mg/kg, p.o.) still demonstrated appreciable antinociceptive activity in mice chronically treated with morphine (6 mg/kg, i.p.), indicating that no cross-tolerance exists with morphine [Figures 2 and 3].
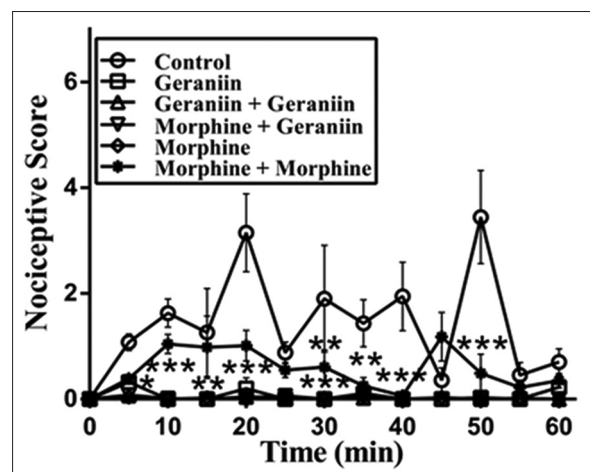
Figure 2: Effect of geraniin (10 mg/kg) and morphine (3 mg/kg) challenge on the time course effect of the total nociceptive score of formalin-induced licking of mice chronically treated with vehicle, geraniin (20 mg/kg) or morphine (6 mg/kg) for 8 days. Each point represents the mean ± standard error of the mean. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ compared to respective controls (two-way ANOVA followed by Dunnett's multiple comparisons test)

Evaluation of Dependence Liability

Withdrawal signs notably jumping, writhing, and diarrhea were observed after naloxone was administered to the morphine-treated animals. However, these effects were not observed in the geraniin or normal saline treated animals as shown in Figure 4. The incidence of jumping episodes was quantified and used to assess the extent of withdrawal [Figure 4]. Two-way ANOVA revealed a significant incidence of jumping episodes for morphine $F_{2,12} = 48.39$, $P < 0.0001$ [Figure 4a]; $F_{2,12} = 48.83$, $P < 0.0001$ [Figure 4b] compared to the normal saline treated animals. There was, however, no significant difference between the numbers of jumps for the geraniin treated and normal saline treated animals as depicted in the AUC graph [Figure 4].

Two-way ANOVA of the effect of geraniin showed a significant suppression in the jumping behavior of the mice after it was administered 1 h before the last dose of morphine ($F_{4,15} = 62.34$,

$P < 0.0001$) [Figure 5a]. From the AUC, geraniin at the highest dose used blocked the morphine-dependent withdrawal effect by 96.52% [Figure 5b]. DZP, used as a positive control also caused a significant reduction in the total number of jumps by 97.90% [Figure 5].

## Effects on Motor Co-ordination

Geraniin (3-30 mg/kg, p.o.) at all doses tested did not cause any significant change in the time spent on the rota-rod as shown in Figure 6a and b. The nondepolarizing neuromuscular blocker D-Tubocurarine (D-TC, 0.1 mg/kg), however, caused a significant reduction in the time spent on the rota-rod [Figure 6a and b].

DISCUSSION

This study has been able to show that tolerance does not develop to the antinociceptive effects of geraniin. Furthermore, geraniin
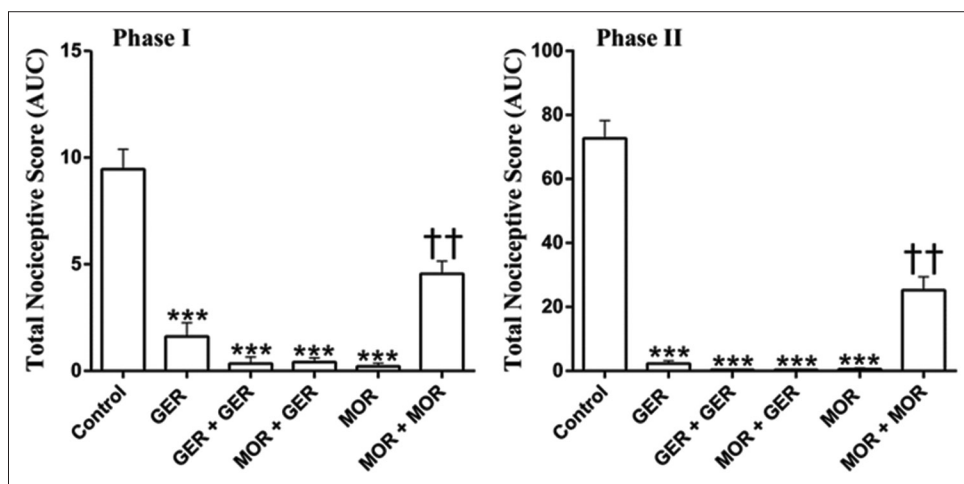
Figure 3: Effect of geraniin (10 mg/kg) and morphine (3 mg/kg) challenge on mice chronically treated with vehicle, geraniin (20 mg/kg) or morphine (6 mg/kg) for 8 days on the total nociceptive score of formalin-induced licking test in mice area under the curve for (a) phase I and (b) phase II of formalin-induced pain, respectively. Each column represents the mean ± standard error of the mean. ***$P \leq 0.001$ compared to vehicle control, ††$P \leq 0.01$ compared to MOR (one-way ANOVA followed by Tukey's multiple comparisons test). Control = animals treated with vehicle for 8 days and vehicle again on day 9, GER = animals treated with vehicle for 8 days and geraniin (10 mg/kg) on day 9, MOR = animals treated with vehicle for 8 days and morphine (3 mg/kg) on day 9, GER+GER = animals treated with geraniin (20 mg/kg) for 8 days and geraniin (10 mg/kg) on day 9, MOR+MOR = animals treated with morphine (6 mg/kg) for 8 days and morphine (3 mg/kg) on day 9, MOR+GER = animals treated with morphine (6 mg/kg) for 8 days and geraniin (10 mg/kg) on day 9
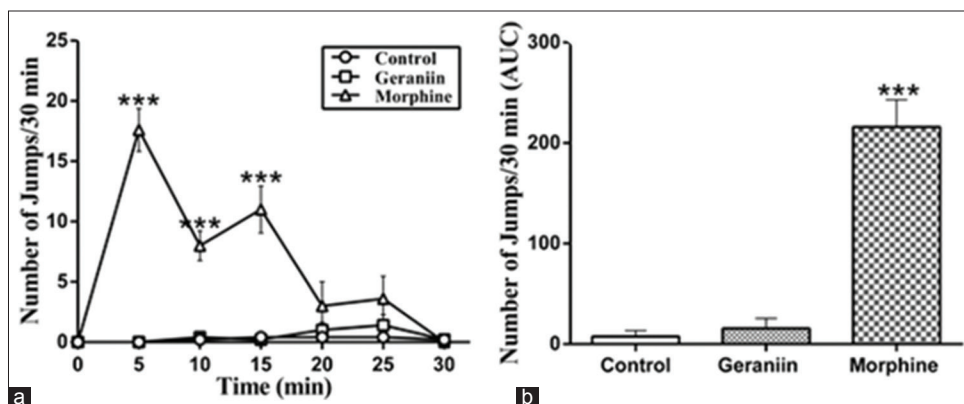
Figure 4: Effect of geraniin, vehicle and morphine in inducing withdrawal signs in mice depicted as (a) a time course curve and (b) an area under the curve graph, respectively. Data represented as mean ± standard error of the mean. ***$P \leq 0.001$ compared to the control

did not cause dependence in mice after multiple administrations like morphine and was, however, effective in reducing signs of withdrawal (jumping episodes) in morphine-dependent mice.

The usefulness of opioid analgesics in clinical settings is often hampered by the development of tolerance that necessitates dose increase regardless of disease progression [19]. Tolerance produced by sustained morphine treatment has been associated with regulation of Transient Receptor Potential Vanilloid 1 (TRPV1) receptor, which is induced by chronic morphine application via mitogen activated protein kinases (MAPK) signaling pathways [19]. Other mechanisms that have also been suggested to contribute to morphine induced-tolerance include activation of the NMDA receptors and calcium regulated intracellular protein kinase C mechanisms [20,21]. Results from this study have shown that, unlike morphine, geraniin, a plant isolate with antinociceptive effect involving opioidergic mechanisms [16], at doses tested, does not induce tolerance after chronic administration in the formalin test of nociception. Moreover, in animals that showed significant tolerance to the analgesic effects of morphine, geraniin was still able to exhibit analgesic effects. These observed effects of geraniin could be due to interactions or blockade of the TRPV1 receptor or the MAPK signaling pathway and or blockade of the NMDA receptor or alterations in glutamate levels.

Development of physical dependence which is manifested by a unique withdrawal syndrome with diverse behavioral and physiological signs is also a limiting factor to the usefulness of most of the opioid analgesics including morphine [22]. Withdrawal is typically observed following abrupt termination of morphine intake or precipitated by administration of a narcotic antagonist such as naloxone [23]. Several withdrawal behaviors have been reported in rodents including jumping, exploratory rearing, body shakes, ptosis, weight loss, and diarrhea [22]. However, jumping has been widely considered the most sensitive and reliable index of withdrawal intensity and thus commonly used to assess the extent of morphine withdrawal [24]. Jumping is the most suitable sign of measuring withdrawal as jumps are easily counted and jumping rate increases when dependence increases or dose of antagonist increased [25].

Physical dependence signs associated with opioid withdrawal have been attributed to a rebound hyperactivity of both the dopaminergic and adrenergic systems in the central nervous system (CNS) [11]. Adenylate cyclase supersensitivity has also been observed in different brain areas including the nucleus accumbens, amygdala, and the locus coeruleus of morphine-dependent animals [26].

Benzodiazepines, via Gamma aminobutyric acid type A receptors, have been shown to have an inhibitory effect on the
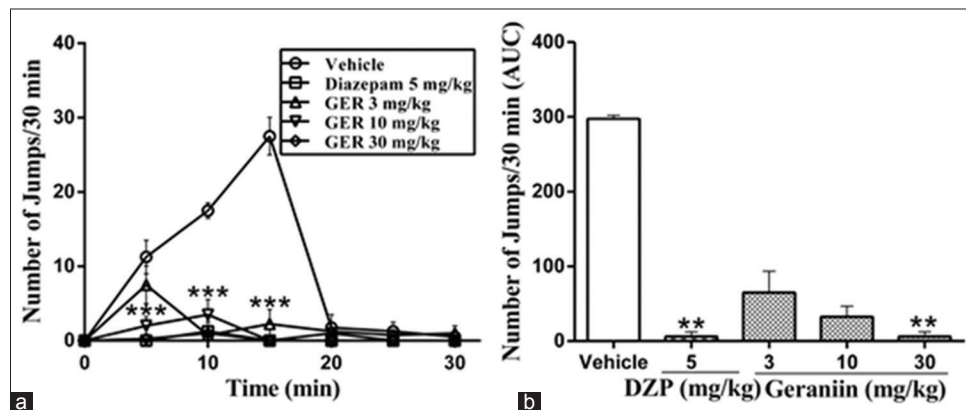
Figure 5: Effect of geraniin, diazepam and vehicle on the naloxone-precipitated withdrawal syndrome of morphine dependence in mice depicted as (a) the time course curve and (b) area under the curve, respectively. Data represented as mean ± standard error of the mean. **$P \leq 0.01$, ***$P \leq 0.001$ compared to the control
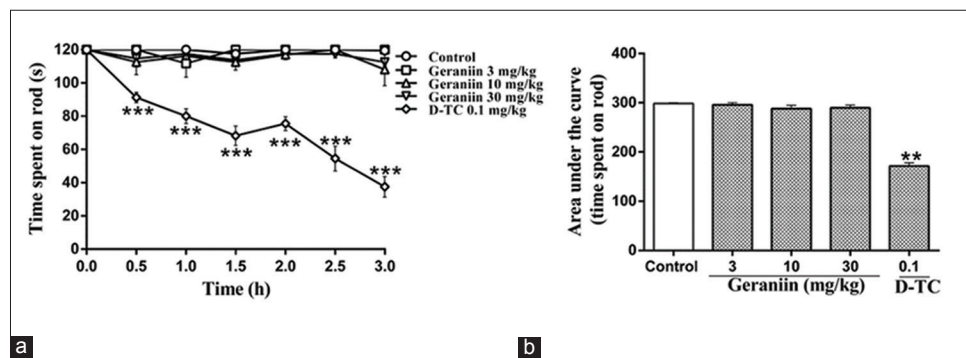
Figure 6: Effects of geraniin (3-100 mg/kg), D-Tubocurarine (D-TC, 0.1 mg/kg) and vehicle on time spent on rods shown as (a) time course curves and (b) area under the curve. Time course curve analyzed by a two-way ANOVA followed by Dunnett's multiple comparison test while area under the curve was analyzed by Kruskal–Wallis test followed by Dunn's multiple comparison test. **$P \leq 0.01$, ***$P \leq 0.001$

dependence of morphine [22] and this was illustrated in this study when DZP at a dose of 5 mg/kg significantly reduced the jumping episodes in morphine-dependent mice. Proposed reasons for the effectiveness of benzodiazepine in suppressing the development of physical dependence on morphine include their continuous activation of benzodiazepine binding sites during chronic morphine treatment and the inhibition of the increase in Ca^{2+} level which results from chronic morphine treatment [22].

In this study, geraniin showed inhibitory effect against withdrawal signs of morphine dependence without impairing motor function. It is possible that geraniin was able to interfere with dopaminergic and/or adrenergic activities in the CNS. With geraniin also being a polyphenolic compound, these results are in agreement with previous reports citing the inhibitory effects of polyphenolic compounds on naloxone precipitated morphine withdrawal [27,28]. Even though, the extent of its inhibitory effect was comparable to that of DZP at the highest dose, the mechanism of action cannot be emphatically stated to be via the same pathway as DZP.

Some oxidant species including nitric oxide, superoxide anions, and malondialdehyde have been implicated in the development of opiate abstinence symptoms [29,30]. As such, some agents with significant antioxidant activity have proven effective in reducing opioid withdrawal signs [11]. Geraniin has been reported previously as a very potent antioxidant [15] which could also contribute to the effectiveness of geraniin in attenuating naloxone-precipitated morphine withdrawal signs in mice.

In conclusion, results presented from this study indicate that geraniin does not appear to have any tolerant effect, it does not induce withdrawal signs and also alleviates signs associated with naloxone-precipitated morphine withdrawal.

ACKNOWLEDGMENT

REFERENCES

1. Schim JD, Stang P. Overview of pain management. Pain Pract 2004;4 Suppl 1:S4-18.
2. Savage SR, Kirsh KL, Passik SD. Challenges in using opioids to treat pain in persons with substance use disorders. Addict Sci Clin Pract 2008;4:4-25.
3. Chen CH, Tang ST, Chen CH. Meta-analysis of cultural differences in Western and Asian patient-perceived barriers to managing cancer pain. Palliat Med 2012;26:206-21.
4. Mirshafiey A, Cuzzocrea S, Rehm BH, Matsuo H. M2000: A revolution in pharmacology. Med Sci Monit 2005;11:PI53-63.
5. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 1999;340:1888-99.
6. Collett BJ. Opioid tolerance: The clinical perspective. Br J Anaesth 1998;81:58-68.
7. Trang T, Sutak M, Quirion R, Jhamandas K. The role of spinal neuropeptides and prostaglandins in opioid physical dependence. Br J Pharmacol 2002;136:37-48.
8. Babhadiashar N, Vaseghi G, Rafieian-Kopaei M, Andalib S, Eshraghi A, Masoudian N. Neural mechanisms underlying morphine withdrawal in addicted patients: A review. Rev Clin Med 2015;2:151-7.
9. Martín F, Laorden ML, Milanés M. Morphine withdrawal regulates phosphorylation of cAMP response element binding protein (CREB) through PKC in the nucleus tractus solitaries A2 catecholaminergic neurons. J Neurochem 2009;110:1422-32.
10. Mansouri MT, Naghizadeh B, Ghorbanzadeh B. Ellagic acid enhances morphine analgesia and attenuates the development of morphine tolerance and dependence in mice. Eur J Pharmacol 2014;741:272-80.
11. Tabatabai SM, Dashti S, Doosti F, Hosseinzadeh H. Phytotherapy of opioid dependence and withdrawal syndrome: A review. Phytother Res 2014;28:811-30.
12. Agyare C, Lechtenberg M, Deters A, Petereit F, Hensel A. Ellagitannins from *Phyllanthus muellerianus* (Kuntze) Exell: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. Phytomedicine 2011;18:617-24.
13. Ishimaru K, Shimomura K. *Geranium thunbergii*: *In vitro* culture and the production of geraniin and other tannins. In: Bajaj, YP, editor. Medicinal and Aromatic Plants VIII. Berlin, Heidelberg: Springer Berlin Heidelberg; 1995. p. 232-47.
14. Boakye YD, Agyare C, Abotsi WK Ayande PG, Ossei PP. Anti-inflammatory activity of geraniin and aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell. Planta Med 2013;79:1142.
15. Boakye YD, Agyare C. Antimicrobial and antioxidant activities of geraniin and aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell. Planta Med 2013;79:1161-2.
16. Boakye-Gyasi E, Kasanga EA, Biney RP, Mensah KB, Agyare C, Woode E. Anti-nociceptive effects of geraniin and an aqueous extract of the aerial parts of *Phyllanthus muellerianus* (Kuntze) Exell. In murine models of chemical nociception. Iran J Pharm Sci 2016; [In Press].
17. Hosseinzadeh H, Nourbakhsh M. Effect of *Rosmarinus officinalis* L. Aerial parts extract on morphine withdrawal syndrome in mice. Phytother Res 2003;17:938-41.
18. Gareri P, Condorelli D, Belluardo N, Citraro R, Barresi V, Trovato-Salinato A, *et al*. Anti-absence effects of carbenoxolone in two genetic animal models of absence epilepsy (WAG/Rij rats and lh/lh mice). Neuropharmacology 2005;49:551-63.
19. Chen Y, Geis C, Sommer C. Activation of TRPV1 contributes to morphine tolerance: Involvement of the mitogen-activated protein kinase signaling pathway. J Neurosci 2008;28:5836-45.
20. Mao J, Price DD, Mayer DJ. Thermal hyperalgesia in association with the development of morphine tolerance in rats: Roles of excitatory amino acid receptors and protein kinase C. J Neurosci 1994;14:2301-12.
21. Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 1991;251:85-7.
22. Suzuki T, Tsuda M, Narita M, Funada M, Mizoguchi H, Misawa M. Diazepam pretreatment suppresses morphine withdrawal signs in the mouse. Life Sci 1996;58:349-57.
23. Iliya H, Boakye-Gyasi E, Annan K, Ekuadzi E, Woode E. Analgesic activity and effect of *Maerua angolensis* stem bark extract and fractions on morphine dependence in mice. Pharm Innov 2015;4:62-8.
24. Kest B, Palmese CA, Hopkins E, Adler M, Juni A. Assessment of acute and chronic morphine dependence in male and female mice. Pharmacol Biochem Behav 2001;70:149-56.
25. Alemy S, Karami M, Hossini E, Ebrahimzadeh MA, Majd NS. Antinociceptive activity and effect of methanol extract of *Salvia limbata* on withdrawal syndrome in mice. Eur Rev Med Pharmacol Sci 2012;16:38-42.
26. Capasso A, Piacente S, Pizza C, Sorrentino L. Flavonoids reduce morphine withdrawal *in-vitro*. J Pharm Pharmacol 1998;50:561-4.
27. Ghannadi A, Hajhashemi V, Abrishami R. Effects of the Persian *Carum copticum* fruit extracts on morphine withdrawal syndrome in mice. Res Pharm Sci 2012;7:127-31.
28. Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ. A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine

on neuronal function. Brain Res 1991;548:100-10.

29. Zarrindast MR, Gholami A, Sahraei H, Haeri-Rohani A. Role of nitric oxide in the acquisition and expression of apomorphine-or morphine-induced locomotor sensitization. Eur J Pharmacol 2003;482:205-13.

30. Pinelli A, Cighetti G, Trivulzio S. Plasma malondialdehyde levels and opiate withdrawal signs observed in rats treated with morphine plus naloxone: Effects of a-lipoic acid administration. Fundam Clin Pharmacol 2008;22:439-45.