Fitoterapia 128 (2018) 148–161

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Antidepressant potential of novel flavonoids derivatives from sweet violet (*Viola odorata* L): Pharmacological, biochemical and computational evidences for possible involvement of serotonergic mechanism

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ARTICLE INFO

Keywords:
Flavonoids
Antidepressant
Forced swimming test
Serotonergic system
Molecular docking, *Viola odorata*

ABSTRACT

Plant-derived natural constituents are of great interest in modern drug discovery due to their natural diversity. *Viola odorata* L has been traditionally used for the treatment of neuropsychiatric disorders. The present study was undertaken to isolate phytoconstituents including three flavonoids 5,7-Dihydroxy-3,6-dimethoxyflavone[**1**] 5,7,4′-trihydroxy-3′,5'dimethoxyflavone [**2**] and 5,7,4′-trihydroxy-3′-methoxyflavone [**3**] from the whole plant of *Viola odorata* L and to investigate the antidepressant-like effects of these compounds and their possible mechanism of action using antagonists of the serotonergic, dopaminergic and adrenergic system. Classical animal models of depression including tail suspension test (TST) and forced swimming test (FST) using mice were used to evaluate the antidepressant-like effects. Mice were divided into various groups and were administered with either vehicle control, fluoxetine (FLX), or test compounds **1–3** intraperitoneally (i.p.). For experiments involving mechanism determination, mice were pretreated with 5-HT, dopamine and adrenergic antagonists. The brain 5-HT levels were determined following FST. Molecular docking studies were carried out to determine the binding affinity of compounds **1–3** to serotonergic receptors. The results indicated that compounds **1–3** at the dose of 1–30 mg/kg, i.p significantly decreased the immobility time in the FST and TST in mice. The reduction in immobility time was reversed by pre-treating the mice with pCPA (5-HT synthesis inhibitor) 100 mg/kg, i.p. and 5-HT receptor antagonists including WAY100635 (5-HT1a antagonist), ketanserin (a 5-HT2a antagonist) and ondansetron (5-HT3 antagonist) but not with prazosin (α1-adrenergic antagonist) and SCH23390 (D1 dopaminergic antagonist) or haloperidol (D2 dopaminergic antagonist). Moreover, in neurochemical assays, compounds **1–3** caused a significant increase in the 5-HT level in the brain tissue as compared to vehicle. These increases were reversed in the mice groups pretreated with pCPA. Furthermore, molecular docking results also depict that compounds **1–3** can interact with 5HT1A, 5HT2A, and 5HT3 receptors, and are more specific to the 5HT3 receptor subtype. In conclusion, the findings of this study clearly suggest that compounds **1–3** possess antidepressant-like effects which might be mediated via the serotonergic system.

1. Introduction

Major depression also known as clinical depression, represents a significant public health problem affecting 4.7% of people worldwide. This means that one out of every 20 people is suffering from major depression globally [11]. The high prevalence of suicide (15%) and other complications associated with depression have projected depression to be the second leading cause of death after cardiovascular diseases by the year 2020. Depression is characterized by loss of interest or pleasure, profound feelings of gloominess, despair, low self-worth and feelings of guilt, fatigue, sleep disturbances, loss of appetite, weight loss and poor concentration and suicidal ideation [19]. Depression can also appear as comorbid symptom with several psychiatric conditions including response to stress, substance abuse and chronic diseases

Abbreviations: TST, Tail suspension test; FST, Forced swimming test; 5-HT, 5-Hydroxytryptamine; pCPA, para-chlorophenylalanine; DMSO, Dimethylsulfoxide; FLX, Fluoxetine; OFT, Open field test; i.p, Intraperitoneal; DI, Dopamine type 1 receptor; D2, Dopamine type 2 receptor; α1, Alpha1; GPCRs, G protein-coupled receptors; SSRIs, Selective serotonin reuptake inhibitors; RIMAs, Reversible inhibitors of monoamine oxidase A; TCAs, Tricyclic antidepressants; SNRIs, Serotonin–noradrenaline reuptake inhibitors

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https://doi.org/10.1016/j.fitote.2018.05.016
Received 12 April 2018; Received in revised form 1 May 2018; Accepted 12 May 2018

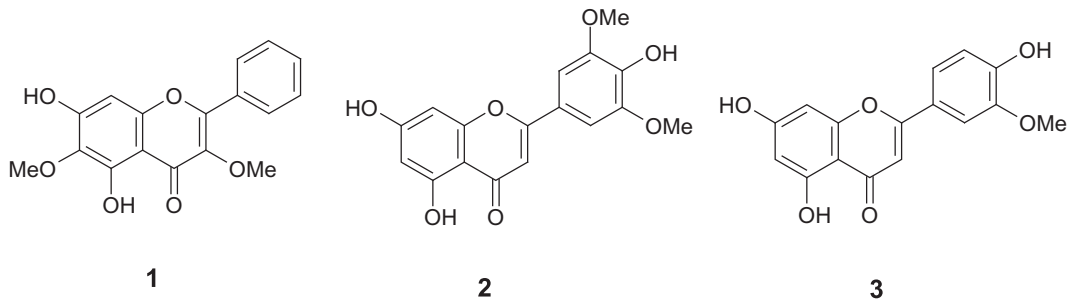

Fig. 1. Chemical structure of 5,7-Dihydroxy-3,6-dimethoxyflavone (1), 5,7,4′ -trihydroxy-3′,5′-dimethoxyflavone (2) and 5,7,4′-trihydroxy-3′-methoxyflavone (3).

including cancer, diabetes mellitus and hypertension [18]. Although, a plethora of research has been done to determine the etiology of depression, the exact cause of major depression is yet to be known. However, various factors including individual genetic makeup and stress may affect brain chemistry, and thus reduce the ability to maintain mood stability [33].

Despite the extensive research done to determine the causes of depression, the pathophysiological pathways and mechanisms involved in depression are not fully investigated. Various studies have shown that monoamines including noradrenaline, dopamine, and serotonin are implicated in the pathophysiology of depression. Thus, drugs that inhibit the reuptake of monoamines and increase the concentration of monoamines in the synaptic cleft have been shown to be clinically effective antidepressants [1]. Furthermore, drugs inhibiting the enzyme monoamine oxidase and thus, increasing the level of monoamines in the presynaptic neurons have also been shown to possess antidepressant effects [24]. Thus, these observations led to the most relative pharmacological theory of depression, known as the “monoamine amine deficiency theory” or “monoamine-deficiency hypothesis”. According to this theory, the underlying pathophysiological mechanism for the development of depression is the depletion of monoamine neurotransmitters including noradrenaline, dopamine, and serotonin (5-HT) in the central nervous system [26].

5-HT has been implicated in pain, sleep, cognition, feeding, sexual behavior, temperature regulation, and in various disorders associated with anxiety, mood, and psychosis [13]. Thus, the 5-HT system is considered to a promising target for the development of drugs for psychological disorders including depression [29]. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs used for the alleviation of depression due to their higher efficacy, safety and tolerability [4]. These drugs include citalopram, escitalopram, fluoxetine, paroxetine, sertraline, and vilazodone. In the treatment of depression, 5-HT mediates a number of pathways through interaction with multiple 5-HT receptors. There are seven types of 5-HT receptors including 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7, all of which except 5-HT3, belong to the superfamily of seven-transmembrane-domain, G protein-coupled receptors (GPCRs). 5-HT3 is a ligand ion channel and is permeable to Na+, K+, Ca2+ (and other cations). Thus in this context, the 5-HT system is a promising system for the development of compounds with multiple and complementary mechanism of action. Therefore, the modulation of the whole serotonergic neurotransmission via pharmacological agents may be useful for the development of newer and effective antidepressant agents with lesser side-effects.

Recently, there has been renewed interest in natural products due to their natural diversity for the development of therapeutic agents for various disorders including depression [19]. Medicinal plants have long been used for the treatment of various disorders from the very beginning of the human history [37]. *Viola odorata* L, also known as sweet violet, Gule- Banafsh, and English violet is a small perennial herbaceous herb and belongs to the family Violaceae which consists of about twenty-three genera and 930 species. There are total 500 reported species of viola, out of which 17 are found in South Asia including Pakistan, India, China, Sri Lanka and Nepal and in Australia [14]. In folk medicine Viola species have been used as antipyretic, laxative, emollient, expectorant, purgative, anti-asthmatic, anti-cancer, against jaundice, hepatitis, skin diseases and for management of constipation [28,34]. *Viola odorata* has been used in the management of bronchial asthma, bronchitis, hypertension, as an antipyretic, antimicrobial and as an analgesic and for treating anxiety [34].

The qualitative phytochemical investigations showed that *Viola odorata* contains flavonoids, glycosides, alkaloids, tannins, and coumarins [34]. In the current study we are reporting for the first time, the isolation and structure elucidation of three flavonoids [**1–3**]; and their antidepressant-like effects in mouse models of depression. Furthermore, their interaction with various 5-HT receptors and possible mechanism was also investigated Fig. 1.

2. Experimental procedures

2.1. Materials, drugs, chemicals, and instrumentation

All chemicals used in the study were of analytical grade purity and were purchased from Aldrich Chemical Co. Ltd. (St Louis, MO, USA). Silica gel with particle size 5–40 μM was obtained from Merck and Sephadex (LH − 20) gels were used for column chromatography whereas pre-coated silica (0.2 mm) aluminum backed Merck plates were used for thin layer chromatography. UV-Spectra were obtained using double beam (UV/Visible) Spectrophotometer (Hitachi model U-2000). Mass spectra were obtained with Thermo Finnigan (Waltham, MA, USA) PolarisQ Ion Trap system using a direct exposure probe. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively on a Varian Gemini spectrometer (Palo Alto CA, USA). Melting points (m.p.) of the compounds were determined with Stuart SMP10 melting point apparatus (Stone, Staffordshire, UK). Fluoxetine, ketanserin, WAY100635, ondansetron, SCH233390, haloperidol, prazosin and tween 20 and DMSO were purchased from Sigma-Aldrich, USA).

2.2. Plant extraction and fractionation

The whole plant (10 kg) of *Viola odorata* was collected from upper Dir, Kalam, and Swat regions of Khyber Pakhtunkhwa, Pakistan in June–July 2015 and was identified by Professor Muhammad Ibrar, taxonomist in the Department of Botany, University of Peshawar, Peshawar Khyber Pakhtunkhwa (KPK), Pakistan. The freshly collected air-dried plant material (10 kg) was ground into fine powder and extracted with 70% methanol (3 times, 10 days, 50 L). The combined methanolic extract was evaporated under vacuum to get a semi-solid extract (350 g). The resulting extract was then dissolved in 10% aqueous methanol and extracted with chloroform. The chloroform extract (75 g) was subjected to silica gel column chromatography (Φ28 × 8 cm) with elution started from chloroform and the polarity gradually increased with methanol until the eluent was 100% methanol