



Evaluation of Sedative and Pre-Anesthetic Effects of *Viola odorata* Linn. Extract Compared With Diazepam in Rats

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Abstract: Diazepam is a long-acting "classical" benzodiazepine. Diazepam has anticonvulsant properties. Diazepam inhibits acetylcholine release in mouse hippocampal synaptosomes. *Viola odorata* contains alkaloid, glycoside, saponins, methyl silylate, mucilage and vitamin C. the objective of present study was to evaluate of sedative and pre-anesthetic effects of *Viola odorata* linn. extract compared with diazepam in rats. In the present study, 30 wistar male rats weighting 300±10 g and about 3 month-old were used for laboratory experiments. In order to evaluate the sedation and pre-anesthetic effects of herbal extract compared with diazepam, 100 mg/kg of extract in first group, 200 mg/kg in second group, 400 mg/kg in third group, diazepam 1.2 mg/kg in group fourth, placebo 1.2 mg/kg (dimethyl sulfoxide) was injected intra peritoneal in fifth group, and sixth group did not receive any drug. Induction time and sleeping time were measured immediately following administration of ketamine. The results demonstrate that the injection of different dosages of the extract causes to increase sleeping time ($p < 0.01$). Also, we showed that the extract has better sedation and pre anesthetic effects compared with diazepam. But dosages of 100 and 200 mg/kg BW of the extract didn't show a significant difference with diazepam. Dosages of 100 and 200 mg/kg BW of the extract have weaker and identical functions, respectively, compared with diazepam. The significant of differences compared with extract dosage of 400 mg/kg BW suggests that the increase of extract dose leads to increase the sedation effect. In conclusion, we can declare that *Viola odorata* extract has better sedation and pre-anesthetic effects than diazepam but dose-dependently. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.

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1. Introduction

Viola odorata is a species of the genus *Viola* native to Europe and Asia, but has also been introduced to North America and Australasia. It is commonly known as wood violet (10), sweet violet, English violet, common violet, or garden violet. The sweet scent of this flower has proved popular throughout the generations particularly in the late Victorian period, and has consequently been used in the production of many cosmetic fragrances and perfumes (38). The French are also known for their violet syrup, most commonly made from an extract of

violets. In the United States, this French violet syrup is used to make violet scones and marshmallows. The scent of violet flowers is distinctive with only a few other flowers having a remotely similar odour. References to violets and the desirable nature of the fragrance go back to classical sources such as Pliny and Horace when the name 'Ion' was in use to describe this flower from which the name of the distinctive chemical constituents of the flower, the ionones – is derived. In 1923 Poucher writes that the flowers are widely cultivated both in Europe and Asia and the East for their fragrance, with both the

flowers and leaves being separately collected and extracted for fragrance, and flowers also collected for use in confectionary and the production of a galenical syrup (39). There is some doubt as to whether the true extract of the violet flower is still commercially available at all (23). It certainly was in the early 20th Century (39), but by the time Steffen Arctander was writing in the late 1950s and early 1960s production had "almost disappeared" (38). The violet leaf absolute however remains widely used in modern perfumery (2,22).

Common Violet is mainly used as an herbal remedy in cases of various respiratory ailments. It can be very beneficial in treatment of congestion, coughs and sore throat. Taken in large doses, root of Common Violet can be used as an emetic. Used in form of a decoction, it acts as a mild laxative. Recent studies have shown the presence of glycoside of salicylic acid in Common Violet leaves, which explains its efficient use in cases of headaches and body pains. Syrup made from Common Violet's flower has anti-septic, anti-inflammatory, laxative and expectorant properties. It can be helpful in cases of various respiratory conditions, but also in treatment of headaches, insomnia, dizziness and exhaustion (21).

Viola odorata contains alkaloid, glycoside, saponins, methyl silylate, mucilage and vitamin C (46). The plant has been reported to possess antioxidant (18) and diuretic (48) activities along with other beneficial effects but no study has been found regarding its blood pressure lowering or lipid-lowering activity. In present investigation, we report the sedative effects of *Viola odorata* along with possible mechanisms.

Diazepam is a long-acting "classical" benzodiazepine. Other classical benzodiazepines include chlordiazepoxide, clonazepam, lorazepam, oxazepam, nitrazepam, temazepam, flurazepam, bromazepam, and clorazepate (8). Diazepam has anticonvulsant properties (11). Diazepam has no effect on GABA levels and no effect on glutamate decarboxylase activity, but has a slight effect on gamma-aminobutyric acid transaminase activity. It differs from some other anticonvulsive drugs with which it was compared (7). Benzodiazepines act via micromolar benzodiazepine binding sites as Ca^{2+} channel blockers and significantly inhibit depolarization-sensitive Calcium uptake in rat nerve cell preparations (47).

Diazepam inhibits acetylcholine release in mouse hippocampal synaptosomes. This has been found by measuring sodium-dependent high affinity

choline uptake in mouse brain cells in vitro, after pretreatment of the mice with diazepam in vivo. This may play a role in explaining diazepam's anticonvulsant properties (35).

Diazepam binds with high affinity to glial cells in animal cell cultures (19). Diazepam at high doses has been found to decrease histamine turnover in mouse brain via diazepam's action at the benzodiazepine-GABA receptor complex (36). Diazepam also decreases prolactin release in rats (20).

It may also be used before certain medical procedures (such as endoscopies) to reduce tension and anxiety, and in some surgical procedures to induce amnesia (12,13). It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnesic properties (30). The pharmacological action of diazepam enhances the effect of the neurotransmitter GABA by binding to the benzodiazepine site on the GABA_A receptor (via the constituent chlorine atom) leading to central nervous system depression (41).

Adverse effects of diazepam include anterograde amnesia (especially at higher doses) and sedation, as well as paradoxical effects such as excitement, rage or worsening of seizures in epileptics. Benzodiazepines also can cause or worsen depression. Long-term effects of benzodiazepines such as diazepam include tolerance, benzodiazepine dependence and benzodiazepine withdrawal syndrome upon dose reduction; additionally, after cessation of benzodiazepines, cognitive deficits may persist for at least six months and may not fully return to normal; however, it was suggested that longer than six months may be needed for recovery from some deficits.^[4] Diazepam also has physical dependence potential and can cause serious problems of physical dependence with long term use. Compared to other benzodiazepines, though, physical withdrawal from diazepam following long term use is usually far more mild due to its long elimination half-life. Nevertheless, urgent action by national governments to improve prescribing practices has been recommended (15).

Advantages of diazepam are a rapid onset of action and high efficacy rates, which is important for managing acute seizures, anxiety attacks and panic attacks; benzodiazepines also have a relatively low toxicity in overdose (41). Diazepam is a core medicine in the World Health Organization's "Essential Drugs List", which is a list of minimum medical needs for a basic health care system (50). Diazepam was first synthesized by Leo Sternbach (45), is used to treat a wide range of conditions, and

has been one of the most frequently prescribed medications in the world since its launch in 1963.

2. Materials and methods

2.1. Understudied animals

In the present study, 30 wistar male rats weighting 300 ± 10 g and about 3 month-old were used for laboratory experiments. Animals were kept in standard condition, at $20-25^{\circ}\text{C}$, 70% humidity and light cycle of 12 hours lighting and 12 hours darkness. Standard plates were used in order to feeding by method of Ad-Libitum i.e. 24 hours feeding. Especial dishes were used for water. The rats were numbered in groups consisted of 5 animals and were placed in especial cages.

2.2. Obtaining extract

500 g dried leafs was powdered in order to obtain extract from leaves. The powder was soaked in methanol and chloroform (70:30) for at least 24 hours; then, the obtained mixture was entered rotary operator system in vacuum pressure for obtaining raw extract. The resulted raw extract was dissolved in the least quantity of hot methanol followed by freezing at -15°C and was filtered immediately for obtaining fatless extract. The fat-removed extract was dissolved in chloromethane, dried by magnesium sulfate and removed solvent by operator rotary system under vacuum in order to water-remove and obtain pure extract. Then, the obtained extract was given a person who prescribes only the drugs and doesn't know anything about their nature.

2.3. Evaluating method as well as sedation and pre-anesthetic effects of *Viola Odorata* compared with diazepam

In order to evaluate the sedation and pre-anesthetic effects of herbal extract compared with diazepam, 100 mg/kg of extract in first group, 200 mg/kg in second group, 400 mg/kg in third group, diazepam 1.2 mg/kg in group fourth, placebo 1.2

mg/kg (dimethyl sulfoxide) was injected intra peritoneal in fifth group, and sixth group did not receive any drug. 40 mg/kg ketamine per body weight was injected intra peritoneal in all groups 30 minutes following mentioned drugs. Induction time and sleeping time were measured immediately following administration of ketamine.

3. Results

Following the injection of pre-anesthetic drugs, the injection of anesthetic inductive drugs, recording of induction time and sleeping time are considered as markers of the rate of sedation effects of a pre anesthetic drug. In present study, lower induction time and higher sleeping time was considered as appropriate pre-anesthetic and sedative action (table 1).

The results demonstrate that the injection of different dosages of the extract causes to increase sleeping time ($p < 0.01$). The results of dual Tokay follow up test show a significant difference between intra peritoneal injections of 100, 200, 400 mg/kg BW of herbal extract and 1.2 mg/kg BW of diazepam.

Based on diagrams 1 and 2, intra peritoneal injections of 100 mg/kg BW of herbal extract has lower induction time and higher sleeping time compared with 1.2 mg/kg BW of diazepam; so that there is a significant difference ($P < 0.01$).

On the other hands, the extract has better sedation and pre anesthetic effects compared with diazepam. But dosages of 100 and 200 mg/kg BW of the extract didn't show a significant difference with diazepam. Dosages of 100 and 200 mg/kg BW of the extract have weaker and identical functions, respectively, compared with diazepam. The significant of differences compared with extract dosage of 400 mg/kg BW suggests that the increase of extract dose leads to increase the sedation effect (table 1).

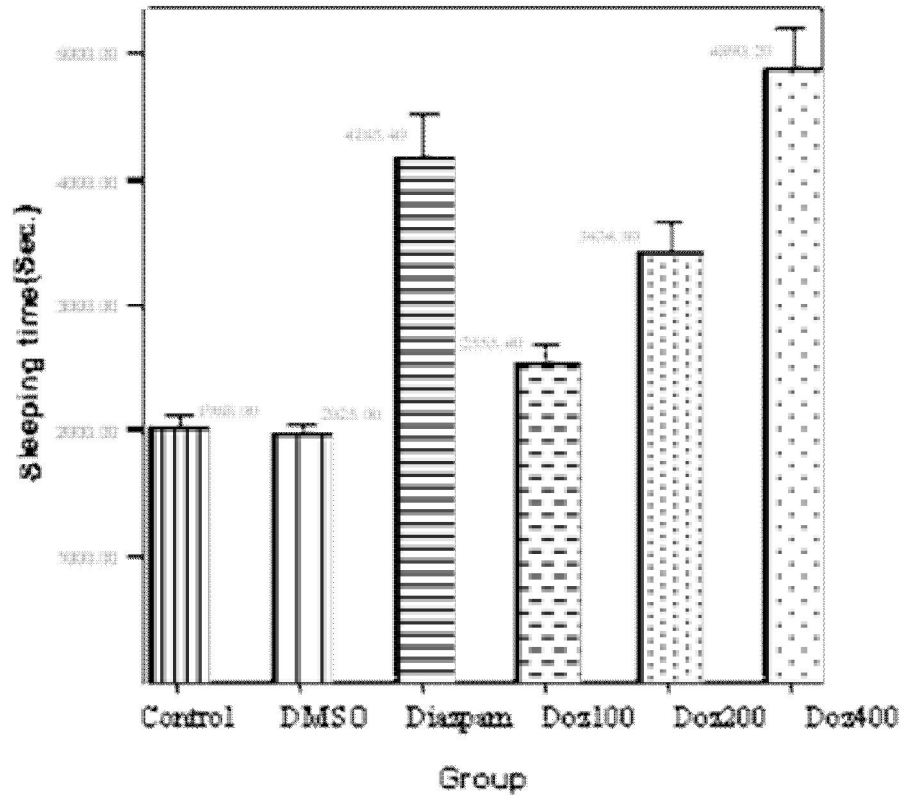


Diagram 1: mean value of data obtained from induction time in understudying group.

Diagram 2: mean value of data obtained from sleeping time in understudying group.

Table 1: group's classification and measured induction time and sleeping time

Group	Received medication (mg/kg)	Induction time (Mean \pm SE) sec.	Sleeping time (Mean \pm SE) sec.
Group 1	Extract 100, ketamine 40	140.54 \pm 3.60	2555.40 \pm 41.48
Group 2	Extract 200, ketamine 40	119.90 \pm 8.73	3434.80 \pm 87.62
Group 3	Extract 400, ketamine 40	75.88 \pm 5.13	4890.20 \pm 119.95
Group 4	Diazepam 1.2, ketamine 40	89.00 \pm 8.12	4185.40 \pm 127.96
Group 5	DMSO 1.2, ketamine 40	165.90 \pm 3.42	1988.00 \pm 22.63
Group 6	Without pre-anesthetic, ketamine 40	162.70 \pm 2.35	2025.00 \pm 2979

4. Discussion and conclusion

Diazepam was the second benzodiazepine to be invented by Dr. Leo Sternbach of Hoffmann-La Roche at the company's Nutley, New Jersey, facility (42) following chlordiazepoxide (Librium), which was approved for use in 1960. Released in 1963 as an improved version of Librium, diazepam became incredibly popular, helping Roche to become a pharmaceutical industry giant. It is 2.5 times more potent than its predecessor, which it quickly surpassed in terms of sales. After this initial success, other pharmaceutical companies began to introduce other benzodiazepine derivatives (43).

The benzodiazepines gained popularity among medical professionals as an improvement upon barbiturates, which have a comparatively narrow therapeutic index, and are far more sedating at therapeutic doses. The benzodiazepines are also far less dangerous; death rarely results from diazepam overdose, except in cases where it is consumed with large amounts of other depressants (such as alcohol or other sedatives) (6). Benzodiazepine drugs such as diazepam initially had widespread public support, but with time the view changed to one of growing criticism and calls for restrictions on their prescription (33).

Diazepam was the top-selling pharmaceutical in the United States from 1969 to 1982, with peak sales in 1978 2.3 billion tablets (43). Diazepam, along with oxazepam, nitrazepam and temazepam, represents 82% of the benzodiazepine market in Australia (32). While psychiatrists continue to prescribe diazepam for the short-term relief of anxiety, neurology has taken the lead in prescribing diazepam for the palliative treatment of certain types of epilepsy and spastic activity, for example, forms of paresis. It is also the first line of defense for a rare disorder called stiff-person syndrome (14). In recent years, the public perception of benzodiazepines has become increasingly negative (4).

Diazepam is mainly used to treat anxiety, insomnia, and symptoms of acute alcohol withdrawal. It is also used as a premedication for inducing sedation, anxiolysis or amnesia before certain medical procedures (e.g., endoscopy) (9,16).

Intravenous diazepam or lorazepam are first line treatments for status epilepticus (41,49); However, lorazepam has advantages over diazepam, including a higher rate of terminating seizures and a more prolonged anticonvulsant effect (40). Diazepam is rarely used for the long-term treatment of epilepsy

because tolerance to its anticonvulsant effects usually develops within six to 12 months of treatment, effectively rendering it useless for that purpose (24,37). Diazepam is used for the emergency treatment of eclampsia, when IV magnesium sulfate and blood pressure control measures have failed (17,27). Benzodiazepines do not have any pain-relieving properties of them and are generally recommended to be avoided in individuals with pain (51). However, benzodiazepines such as diazepam can be used for their muscle-relaxant properties to alleviate pain caused by muscle spasms and various dystonias, including blepharospasm (25,34). Tolerance often develops to the muscle relaxant effects of benzodiazepines such as diazepam (3). Baclofen or tizanidine is sometimes used as an alternative to diazepam (31). Tizanidine has been found to be equally effective as other antispasmodic drugs and have superior tolerability than baclofen and diazepam (26).

The anticonvulsant effects of diazepam can help in the treatment of seizures due to a drug overdose or chemical toxicity as a result of exposure to sarin, VX, soman, lindane, chloroquine, physostigmine, or pyrethroids (5,37) Diazepam is sometimes used intermittently for the prophylaxis of febrile seizures which occur as a result of a high fever in children and neonates under five years of age (28,41). Long-term use of diazepam for the management of epilepsy is not recommended; however, a subgroup individuals with treatment resistant epilepsy benefit from long-term benzodiazepines and for such individuals clonazepam has been recommended due to its slower onset of tolerance to the anticonvulsant effects (41).

Siddiqi et al., 2012 showed that *Viola odorata* leaves extract (Vo.Cr), which tested positive for alkaloids, saponins, tannins, phenolics, coumarins and flavonoids, caused a dose-dependent (0.1-1.0 mg/kg) decrease in mean arterial blood pressure in anaesthetized rats. In isolated guinea-pig atria, Vo.Cr equally inhibited force and rate of spontaneous atrial contractions. On the baseline of rat thoracic aortae (endothelium-intact and denuded), the plant extract caused phentolamine-sensitive vasoconstriction. When tested on phenylephrine (PE, 1 μ M) and K⁺ (80 mM)-induced vasoconstriction, Vo.Cr caused a concentration-dependent relaxation and also caused a rightward shift of Ca⁺⁺ concentration-response curves as well as suppression of PE (1 μ M) control peaks in Ca⁺⁺-free medium, similar to that caused by

verapamil. In the presence of L-NAME, the relaxation curve of Vo.Cr was partially inhibited showing involvement of Nitric oxide (NO) mediated pathway. In Tyloxapol-induced dyslipidemia, Vo.Cr caused reduction in total cholesterol and triglyceride levels. In high-fat diet-induced dyslipidemia model, the plant extract caused a significant decrease in total cholesterol, LDL-C, atherogenic index and prevented the increase in average body weights, while it increased HDL-C (44).

Akhbari et al., 2012 demonstrated that essential oil composition of the leaves of *Viola odorata* L. growing wild in Kashan, central Iran, was extracted by hydro distillation-solvent extraction method and analysed using GC-MS technique. The analysis revealed the presence of 25 identified compounds, representing 92.77% of the oil with butyl-2-ethylhexylphthalate (30.10%) and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (12.03%) being the two main components. Several components were identified for the first time in this chemotype of *V. odorata* (1).

Karioti et al., 2011 revealed the characteristic constituents of *V. odorata* flowers are considered to be the anthocyanins; however, a detailed literature research showed that data concerning their chemical content are scarce. They used HPLC-DAD-ESI-MS analyses method by extensive preparative chromatographic investigations and 2D NMR analyses revealed the predominance of complex flavonol glycosides and permitted the complete characterisation of the content of *V. odorata* preparations (29). In conclusion, we can declare that *Viola Odorata* extract has better sedation and pre-anesthetic effects than diazepam but dose-dependently. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.

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