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Original Article

Study of Sedative, Pre-Anesthetic, and Anti-Anxiety Effects of *Verbascum thapsus L.* Extract Compared with Diazepam in Rats

Ali Rezaie[1*], Mehdi Ebrahimi[2], Eilyad Issabeagloo[3], Manish Kumar[4], Mehrdad Nazeri[5], Samad Rezaie[6], Solmaz Zakhireh[7]

1- Department of Clinical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2- DVM, Tabriz Branch, Islamic Azad University, Tabriz, Iran
3- Department of pharmacology, Faculty of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
4- Secretary, Academy for Environment and Life Sciences, Agra, (U.P.) INDIA
5- Young Researchers Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran
6- Department of Dentistry, Tehran medical Branch, Islamic Azad University, Tehran, Iran
7- Department of Basic Science, Faculty of Chemistry, Ahar Branch, Islamic Azad University, Ahar, Iran
Corresponding author: Arezaie1@gmail.com

ABSTRACT

Plants have been used as folkloric sources of medicinal agents since the beginning of mankind. As the age of modern medicine, single pure drugs emerged, and plant-derived active principles, their semi-synthetic and synthetic analogs have served as a major route to new pharmaceuticals. The major purpose of this study was to evaluation sedative, pre-anesthetic, and anti-anxiety effects of Verbascum thapsus L. extract compared with diazepam in rats. In the present study, 30 wistar male rats weighting 210±20 g and about 12 month-old were used for laboratory experiments. Animals were kept in standard condition, at 20-25°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. In order to evaluate the sedation and pre-anesthetic effects of Verbascum extract compared with diazepam, 1 g/kg of extract in first group, 2 g/kg in second group, 4 g/kg in third group, 1.2 mg/kg of diazepam in group forth, group fifth received 1.2 mg/kg placebo and group sixth did not receive any drug. Elevated plus maze was used in order to evaluate anti-anxiety effects of Verbascum extract. Our results showed that Verbascum thapsus L. extract has better sedative and pre-anesthetic effects at the dose of 200 mg/kg compared with diazepam. Also, anti-anxiety properties of this medicinal plant were more significant. In conclusion can be state that extract of this medicinal plant has better sedation, pre-anesthetic, and anti-anxiety effects than diazepam. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of it.

Key words: *sedation, anti-anxiety, pre-anesthetic, Verbascum thapsus L, diazepam, Rat.*

INTRODUCTION

Some species of Verbascum L. (Scrophulariaceae) have widely been used throughout centuries to treat internal and external infections. According to Meurer-Grimes *et al.* [1], Hildegard von Bingen has mentioned in her first book Physica "De wullena", with which she probably refers to *Verbascum thapsus* L. She mentions its use against hoarseness in a concoction of wine, fennel, and mullein leaves. Since then, many other internal and external uses of the leaves and flowers of several Verbascum L. species have been documented in many societies in Europe, Asia, Africa and northern America [1]. Verbascum L. species contain a wide range of compounds, such as glycosides [2-5], alkaloids [6], and saponins. In addition, species of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkoloids [7]. This group of compounds has not been previously investigated for antimicrobial activity. As it was stated in a report by McCutcheon et al. [8], extracts of *V. thapsus* revealed antiviral activity against Herpes virus type 1. Furthermore, aqueous extracts of *V. fruticulosum* L. demonstrated strong growth inhibition on the malaria parasite [9]. *Verbascum phlomoides* L., *Verbascum densiflorum* Bertol. and *Verbascum thapsus* L. species have been used for their ethnopharmacological effects among common people in Iran. Especially their flowers have been used. The drug, prepared from their flowers, has diuretic and expectorant effects. Leafs of plants have also been used for their diuretic, expectorant and sedative effects. Seeds of Verbascum species are poisonous because of the saponins contained. People use

these poisonous seeds for hunting fish. Verbascum species are called 'fishplant' in the nothern Anatolia because of that property.

Ethnobotanical information obtained from traditional healers may serve as an intial lead for bioactive compounds [10]. In addition, investigations confirm that higher plants used as antiinfective phytomedicines may serve as a valuable source for novel antibiotics. *Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss. are endemic to Uludag˘ Mount-Bursa, Turkey and Euro-Siberian elements. Antimicrobial effects of *Verbascum bombyciferum* Boiss. and *Verbascum olympicum* Boiss. have been previously investigated by Meurer-Grimes et al. [1].

Benzodiazepines are widely used for sedation during diagnostic procedures. Controversy exists regarding the respiratory depressant effects of sedative and hypnotic doses of benzodiazepines. Although studies [11-13] designed to assess the effects of diazepam on ventilatory function have yielded conflicting results, the respiratory response to diazepam appears to be affected by the dose and rate of drug administration, as well as by methodologic, environmental, and individual patient factors. Despite marked variability in the respiratory response to diazepam [14-16], large bolus (hypnotic) doses of diazepam produce significant depression of the ventilatory response to carbon dioxide (CO,) [17-18]. Although some investigators [19, 20, 11, and 13] have reported that CO, responsiveness is not affected by sedative doses of diazepam, others [12 21-24] have demonstrated that even premedicant doses of diazepam can depress respiratory drive. The major purpose of this study was to evaluation sedative, pre-anesthetic, and anti-anxiety effects of *Verbascum thapsus* L. extract compared with diazepam in rats.

MATERIALS AND METHODS

Understudied animals

In the present study, 30 wistar male rats weighting 210±20 g and about 12 month-old were used for laboratory experiments. Animals were kept in standard condition, at 20-25°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 6 animals and were placed in especial cages. This experimental study was carried out in Islamic Azad University Research Center and all procedures and works on animals was conducted under Animal Rights Monitoring Committee of Islamic Azad University Research Center.

Obtaining extract:

Dried leaves were 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.

Evaluating method as well as sedation and pre-anesthetic effects of *Verbascum* compared with diazepam

In order to evaluate the sedation and pre-anesthetic effects of *Verbascum* extract compared with diazepam, 1 g/kg of extract in first group, 2 g/kg in second group, 4 g/kg in third group, 1.2 mg/kg of diazepam in group forth, group fifth received 1.2 mg/kg placebo and group sixth did not receive any drug. 40 mg/kg thiopental 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.

Elevated plus maze was used in order to evaluate anti-anxiety effects of *Verbascum* extract. The system consists of two arms (10×15 cm) which are open and against each other and two arms (40×10×50 cm) which are closed and against each other. They are related to each other by a central plate (10×10 cm) in a semi dark and silent. They are placed in 50 cm distance from the earth. In order to determine anti-anxiety effects of the drugs, the duration of remaining the rats on open

arms is considered as non-anxiety marker and the duration of remaining the rats on closed arms is considered as anxiety marker. More duration of remaining the rats on open arms demonstrates the strong anti-anxiety effects of considered drug. SPSS software program was used in order to analysis statistical data as well as Tokay follow up test for determining a significant difference among dual groups. $P<0.05$ has been considered as significant. Also, data were reported as mean ± SD.

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. The results demonstrate that the injection of extract at the dose of 1 g/kg showed week results than diazepam but, injection of the extract at the dose of 2 g/kg causes to increase sleeping time also, 4 g/kg considered as lethal dose ($p<0.01$). (Diagram 1,2; table 1)

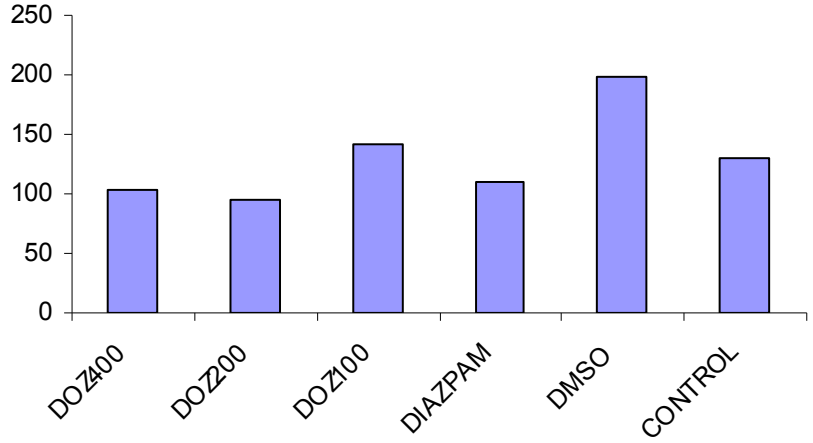

Diagram 1: mean value of data obtained from induction time in understudying group.

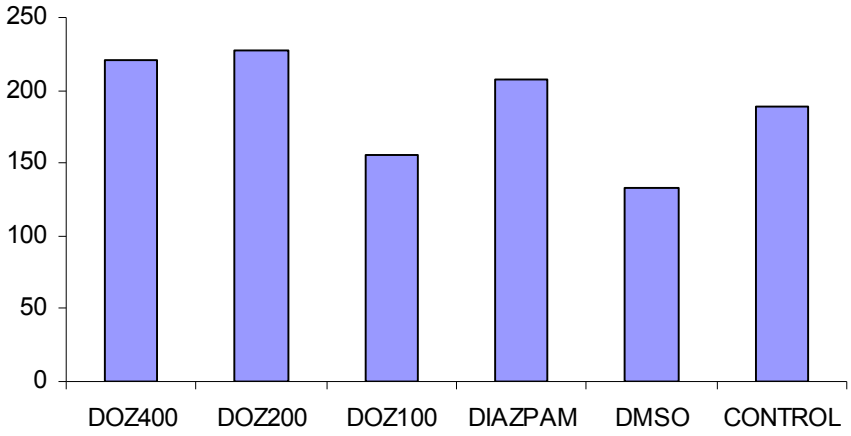

Diagram 2: mean value of data obtained from sleeping time in understudying group.

Table 1: mean values of Induction time (sec) and sleeping time (min) in experimental groups.

Group	Received medicines	Induction time (sec)	Sleeping time (min)
1	100 mg/kg extract, 40 mg/kg thiopental	141	156
2	200 mg/kg extract, 40 mg/kg thiopental	95	227
3	400 mg/kg extract, 40 mg/kg thiopental	104	221
4	1.2 mg/kg diazepam, 40 mg/kg thiopental	110	208
5	1.2 mg/kg DMSO (as placebo), 40 mg/kg thiopental	199	133
6	Received no treatment	130	189

Based on diagram 3, the results show that *Verbascum* extract in dosage of 200 mg/kg BW has a better anti-anxiety effect compared with 1.2 mg/kg BW of diazepam. Also, they show a significant difference statistically; in other words it causes to decrease the anxiety and increase of the time

spent on maze arms as well as increases the numbers of traverse on open arms (table 2 and diagram 3,4). Note that data related to open maze is indicator of anti-anxiety effects.

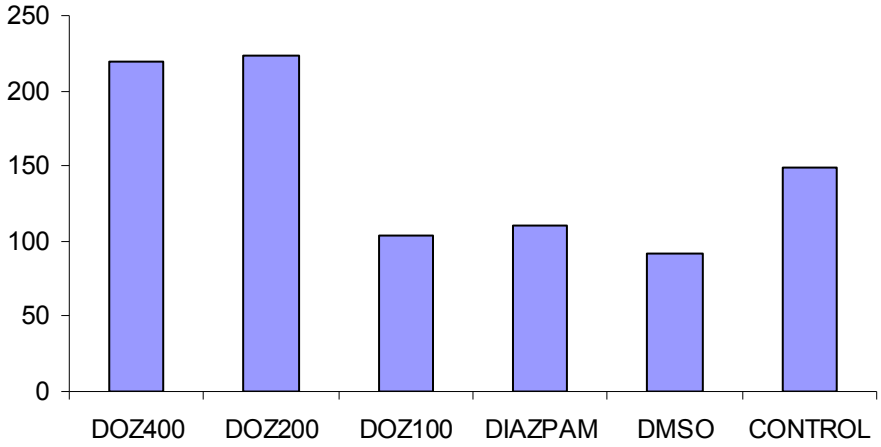

Diagram 3: mean value of data obtained from open maze test from understudying group.

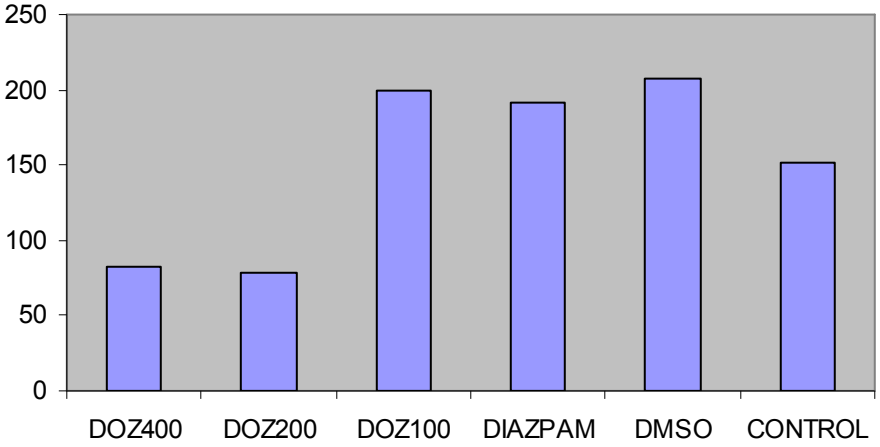

Diagram 4: mean value of data obtained from close maze test from understudying group.

Table 2: mean values of open-maze and close-maze in experimental groups.

Group	Received medicines	Open-maze (sec)	Close-maze (sec)
1	100 mg/kg extract, 40 mg/kg thiopental	104	199
2	200 mg/kg extract, 40 mg/kg thiopental	223	78
3	400 mg/kg extract, 40 mg/kg thiopental	219	82
4	1.2 mg/kg diazepam, 40 mg/kg thiopental	110	191
5	1.2 mg/kg DMSO (as placebo), 40 mg/kg thiopental	92	208
6	Received no treatment	149	152

DISCUSSION AND CONCLUSION

Diazepam is classified as a central nervous system (CNS) depressant, acting primarily on the limbic system by means of inhibition. This preferential depressant action on the subcortical structures of the CNS is accomplished without significantly altering respiratory, autonomic, or extrapyramidal activity [25].

The subcortical levels (primarily limbic, thalamic, and hypothalamic) of the CNS are depressed by diazepam and other benzodiazepines thus producing the anxiolytic, sedative, skeletal muscle relaxant, and anticonvulsant effects seen. The exact mechanism of action is unknown, but postulated mechanisms include: antagonism of serotonin, increased release of and/or facilitation of gamma-aminobutyric acid (GABA) activity, and diminished release or turnover of acetylcholine in the CNS. Benzodiazepine specific receptors have been located in the mammalian brain, kidney, liver, lung, and heart. In all species studied, receptors are lacking in the white matter [26].

Anxiolytic plants may interact with either glutamic acid decarboxylase (GAD) or GABA transaminase (GABA-T) and ultimately influence brain GABA levels and neurotransmission [27]. Flavonoids have recently increased in importance because they have been identified as a new type of ligand with in vivo anxiolytic properties. The flavones chrysin and apigenin, obtained from medicinal plants, have shown an anxiolytic effect in rodents exposed to behavioral tests. Apparently, these compounds modulate the γ-aminobutyric acid (GABA) ergic system to produce the biological effect [28]. However, only a low content of flavonoids was found in this hydroethanolic extract.

Crude extracts from *Verbascum pseudonobile* used in traditional medicine have been screened for potential anticancer bioactive agents, using evaluation of DNA-interaction activity. The extracts proved active in DNA interaction. It was found that there was correlation in DNA-intercalation and the hemolytic effect in plant extracts [29].

Ucar-Turker et al reported that the extracts of *Verbascum thapsus* showed antitumor activity against Agrobacterium tumefaciens-induced tumors on potato disc method as modified by McLaughlin's group. No tumor formation was observed with camptothecin (tumor suppressant), while the tested saponins had moderate tumor inhibition. Thus, saponins are believed to be responsible for these beneficial effects [30].

In 1982, it was given a definition for expression of activity, that is, the word cytotoxicity must be used only for in vitro activity, the words antineoplastic and antitumor must be used only for in vivo test using animal [31].

Development of novel clinically useful anticancer agents would be dependent on the screening system and the sample sources for the bioassay. Improving of simple anticancer pre-screen using convenient and inexpensive cytotoxic assay systems can offer numerous advantages as alternatives to extensive animal testing in the search for new anticancer drugs. The search for potential anticancer agents from natural sources mainly has been carried out with the guidance of bioassay confirmed by Borenfreund *et al* and the screening protocols for each tumor system have been well-established. These screening systems led to fractionate from the plants [32-35].

In conclusion can be state that extract of this medicinal plant has better sedation, pre-anesthetic, and anti-anxiety effects than diazepam. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of it.

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