Study of sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (Achillea millefolium) extract compared with Diazepam in rats

Ali Rezaie1*, Changhiz Ahmadizadeh2

1- Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2- Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran

* Corresponding Author’s Email: arezaiel@gmail.com

Abstract: The genus Achillea comprises of ~85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 Achillea species and 23 of them are endemic. The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (Achillea millefolium) extract compared with diazepam in rats. In the present study, 90 wistar male rats were numbered in groups consisted of 15 animals and were placed in especial cages. In order to evaluate the sedation and pre-anesthetic effects of yarrow extract compared with diazepam, 100 mg/kg of polar extract in first group, 100 mg/kg of semi-polar extract in second group, 100 mg/kg of non-polar extract in third group, 2 mg/kg diazepam in group four, 2 mg/kg amount of dimethyl sulfoxide was injected intra peritoneal in fifth group, and sixth group did not receive any drug. Elevated plus maze was used in order to evaluate anti-anxiety effects of yarrow extract. The results of dual Tokay follow up test show a significant difference between intra peritoneal injections of 100 mg/kg BW of polar extract than semi-polar and non-polar than diazepam. Intra peritoneal injection of polar fraction of yarrow extract has showed better and significant effects than semi-polar, non-polar and diazepam. In conclusion, can state that, polar-fraction of yarrow has more sufficient sedative, pre-anesthetic and anti-anxiety effects than diazepam and other under studied groups. Authors suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.


Keywords: Yarrow, Sedation, Anti-Anxiety, Polar, Semi-Polar, Non-Polar Fraction, Dimethylsulfoxide, Rat.

1. Introduction

The genus Achillea (Asteraceae), named after the mythological Greek warrior Achilles, who used Achillea species for healing wounded-soldiers during the Trojan War [Cheers, 1999]. The genus Achillea comprises of ~85 species, most of which are endemic to Europe and the Middle East. Turkish flora possesses 42 Achillea species and 23 of them are endemic [Duman and Achillea, 2000]. These species have some interesting properties and are used in cosmetics, fragrances and agriculture, for example, plant protection [Senatore et al., 2005]. Some Achillea species have been known to be ethnopharmacologically used in folk remedies for various purposes such as hemorrhoid and wound healing [Baytop, 1999]. Herbal teas prepared from some Achillea species are very often used in folk medicine as diuretic, for abdominal pain, against diarrhea, flatulence and emmenagog, moreover for wound healing purposes [Fujita et al., 1995; Honda et al., 1996; Yesilada et al., 1993]. Achillea biebersteini is locally named yarrow, and other species widely used as a folk remedy to treat abdominal pain, wounds and stomachache as well [Sezik et al., 2001; Baytop, 1997]. A. biebersteini Afan. [Asteraceae, Section: Filipendulinae (D.C.) Boiss] (syn. A. micrantha) is a perennial herb, villose, stems erect, simple or branched from the base; 30–60 cm high; leaves up to 10 cm, oblong-lanceolate in outline, pinnatisect into numerous narrow segments, segments divided into minute linear-lanceolate mucronate lobes; the heads are radiate, in large dense compound corymb; involucr 4-5 mm, oblong-ovoid; flowering period, April-May. Several biological activity studies have been performed on various Achillea species, including antibacterial, antioxidant, anti-inflammatory and antispasmodic activities [Karamenderes and Apaydin, 2003; Candan et al., 2003; Al-Hindawi et al., 1989; Skociubus et al., 2004].

Benzodiazepines possess sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions [Page et al., 2002; Olkkola and Ahonen, 2008], which are useful in a variety of indications such as alcohol dependence, seizures, anxiety, panic, agitation and insomnia. Most are administered orally; however, they can also be given intravenously, intramuscularly or rectally [RPSGB, 2009]. In general, benzodiazepines are well-tolerated and are safe and effective drugs in the short term for a wide range of conditions [Perugi et al., 2007; Tesar, 1990]. Tolerance can develop to their effects and there
is also a risk of dependence, and upon discontinuation a withdrawal syndrome may occur. These factors, combined with other possible secondary effects after prolonged use such as psychomotor, cognitive, or memory impairments, limit their long-term applicability [Faught, 2004; Allgulander et al., 2003]. The effects of long-term use or misuse include the tendency to cause or worsen cognitive deficits, depression and anxiety [McIntosh et al., 2005, Ashton, 2005].

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 [Bråthen et al., 2005].

Intravenous diazepam or lorazepam are first line treatments for status epilepticus [Riss et al., 2008; Walker, 2005]; However, lorazepam has advantages over diazepam, including a higher rate of terminating seizures and a more prolonged anticonvulsant effect [Prasad et al., 2005]. 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 [Isojärvi and Tokola, 1998]. Diazepam is used for the emergency treatment of eclampsia, when IV magnesium sulfate and blood pressure control measures have failed [Kaplan, 2004; Duley, 2005]. Benzodiazepines do not have any pain-relieving properties of themselves and are generally recommended to be avoided in individuals with pain [Zeilhofer et al., 2009]. 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 [Mezaki et al., 2005; Kachi, 2001]. Tolerance often develops to the muscle relaxant effects of benzodiazepines such as diazepam [Ashton, 2005]. Baclofen [Mañon-Espiallat and Mandel, 1999] or tizanidine is sometimes used as an alternative to diazepam. Tizanidine has been found to be equally effective as other antispasmodic drugs and have superior tolerability than baclofen and diazepam [Kamen et al., 2008].

The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of polar, semi-polar and non-polar fractions of yarrow (Achillea millefolium) extract compared with diazepam in rats.

2. Materials and methods

2.1. Understudied animals

In the present study, 90 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°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, 24 hours feeding. Special dishes were used for water. The rats were numbered in groups consisted of 15 animals and were placed in especial cages.

2.2. Obtaining extract

500 g of fresh leaves of yarrow was powdered by liquid nitrogen and was dissolved in the 4 liter non-polar solvent like petroleum ether for 48 hour by Soxhlet apparatus, obtained extract is non-polar fraction. Then, the remnants were dissolved in the 4 liter non-polar solvent like chloroform for 48 hour, obtained extract is semi-polar fraction. Finally, the remnant leaves were dissolved in the 4 liter high polar solvent like methanol for 48 hour, obtained extract is polar fraction. Achieved fractions de-solved by rotary evaporator and readied for use.

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

In order to evaluate the sedation and pre-anesthetic effects of yarrow extract compared with diazepam, 100 mg/kg of polar extract in first group, 100 mg/kg of semi-polar extract in second group, 100 mg/kg of non-polar extract in third group, 2 mg/kg diazepam in group four, 2 mg/kg amount of dimethyl sulfoxide was injected intra peritoneal in fifth group, and sixth group did not receive any drug. 100 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.

2.4. Evaluating method as well as anti-anxiety effects of yarrow extract compared with diazepam

Elevated plus maze was used in order to evaluate anti-anxiety effects of yarrow 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. Therefore, yarrow extract with dosages of 100, 200, 400 mg/kg BW and 1.2 mg/kg BW diazepam of diazepam and dimethyl sulfoxide (as placebo) were used as intra peritoneal injection. Dimethyl sulfoxide was placed in maze center 30 minutes following administration of the mentioned drugs. The time duration in which the rats remained in each of maze’s arms was recorded in terms of second; time duration of their presence in maze is 5 minutes. 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<0.01 has been considered as significant. Also, data were reported as mean ± SD.

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. The results of dual Tokay follow up test show a significant difference between intra-peritoneal injections of 100 mg/kg BW of polar extract than semi-polar and non-polar than diazepam (p<0.01) table 1.

Also, both of the semi-polar and non-polar fractions of extract of yarrow has showed significant difference than diazepam from aspect of induction time (P<0.01). From aspect of sleeping time, non-polar fraction had showed more sedative effect than diazepam. About semi-polar fraction, there was no significant difference in compared with diazepam from aspect of sleeping time, diagrams 1 and 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Received treatment (mg/kg)</th>
<th>Induction time (Mean ± SD)</th>
<th>Sleeping time (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>100 mg/kg P.E. + ketamine 100</td>
<td>83.68±2.93</td>
<td>4885.60±69.16</td>
</tr>
<tr>
<td>Group 2</td>
<td>100 mg/kg S.P.E, ketamine 100</td>
<td>106.60±3.57</td>
<td>3806.00±73.77</td>
</tr>
<tr>
<td>Group 3</td>
<td>100 mg/kg N.P.E, ketamine 100</td>
<td>114.14±2.24</td>
<td>3418.60±59.19</td>
</tr>
<tr>
<td>Group 4</td>
<td>Diazepam 2, ketamine 100</td>
<td>98.94±1.9</td>
<td>4366.60±65.72</td>
</tr>
<tr>
<td>Group 5</td>
<td>DMSO 2, ketamine 100</td>
<td>206.44±1.82</td>
<td>1871.40±19.67</td>
</tr>
<tr>
<td>Group 6</td>
<td>Without pre-anesthetic, ketamine 100</td>
<td>202.34±2.44</td>
<td>1876.00±23.02</td>
</tr>
</tbody>
</table>
Based on dual Tokay follow up test, during the spent time in the open arm of apparatus by mice that indicating the high rate of anti-anxiety effect of drug are as follow:

Intraperitoneal injection of polar fraction of yarrow extract has showed better and significant effects than semi-polar, non-polar and diazepam (P<0.01), indicate that polar fraction has more anti-anxiety effect than two others, table 2.

Also, Intraperitoneal injection of all fractions of yarrow extract has showed significant difference than diazepam (P<0.01) (diagrams 3 and 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Received treatment (mg/kg)</th>
<th>Open maze (Mean ± SD)</th>
<th>Close maze (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>100 mg/kg P.E.+ ketamine 100</td>
<td>175.52±5.27</td>
<td>44.94±1.69</td>
</tr>
<tr>
<td>Group 2</td>
<td>100 mg/kg S.P.E, ketamine 100</td>
<td>114.90±4.00</td>
<td>75.48±2.14</td>
</tr>
<tr>
<td>Group 3</td>
<td>100 mg/kg N.P.E, ketamine 100</td>
<td>106.62±2.48</td>
<td>133.84±5.01</td>
</tr>
<tr>
<td>Group 4</td>
<td>Diazepam 2, ketamine 100</td>
<td>144.58±4.01</td>
<td>57.56±1.54</td>
</tr>
<tr>
<td>Group 5</td>
<td>DMSO 2, ketamine 100</td>
<td>54.44±1.26</td>
<td>248.98±8.43</td>
</tr>
<tr>
<td>Group 6</td>
<td>Without pre-anesthetic, ketamine 100</td>
<td>60.14±2.25</td>
<td>274.66±4.02</td>
</tr>
</tbody>
</table>

4. Discussion and conclusion

Anxiolytic plants may interact with either glutamic acid decarboxylase (GAD) or GABA transaminase (GABA-T) and ultimately influence brain GABA levels and neurotransmission [Awad et al., 2007]. 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 [Herrera-Ruiz et al., 2008]. However, only a low content of flavonoids was found in this hydroethanolic extract. Yarrow is traditionally used as a sedative and probably acts via a central adenosine mechanism, which is possibly the reason for its sleep-inducing and maintaining activity [Schiller et al., 2006]. Yarrow showed significant inhibition of GAD activity [Awad et al., 2007]. Yarrow extracts induced the response of the ionotropic (GABAA receptors) [Aoshima et al., 2006] and its fraction containing α-acids: in dose-dependently prolonged pentobarbital induced sleeping time [Zanoli et al., 2005]. Xanthohumol had been reported as modulator of the GABAA receptor response [Meissner & Haberlein, 2006].

Achillea species have been so far reported to contain diterpenes, sesquiterpenes, flavonoids, lignans, essential oil and rarely triterpenes [Ahmed et al., 2002; Barrero et al., 1990; Mockute and Judzentiene, 2003; Marchart and Kopp, 2003; Aljancic et al., 1996; Maffei et al., 1994; Oksuz et al., 1993; Kusmenoglu et al., 1995]. For instance, A. vermicularis was shown to have guaianolide- and germacrene-type sesquiterpenes as well as flavonoids, whereas A. setacea was reported to contain sesquiterpenes, essential oils and flavonoids [Marchart and Kopp, 2003; Oksuz et al., 1993; Unlu et al., 2002]. In addition to extracts, essential oils of the Achillea species were also analysed. The oil of A. pachycephala was found to contain 1,8-cineole and camphor as the major constituents, whereas 1,8-cineole and Artemisia ketone were major in A. oxyodonta. On the other hand A. biebersteinii was rich in camphor and borneol followed by 1,8-cineole. It was stated that all the oils were rich in oxygenated monoterpenes [Esmaeili et al., 2006]. Non-volatile components of A. biebersteinii afforded in addition to β-sitosterol, stigmasterol two sesquiterpene lactones, germacranolide [Badahah and El-Orfy, 2004]. Essential oil of A. millefolium consists of a number of monoterpenes such as α-pinene, β-pinene, 1,8-cineole, camphor and borneol in addition to some sesquiterpene lactones of germacrene-derivatives [Mockute and Judzentiene, 2003]. Major component in the essential oils of both A. setacea and A. teretifolia was elucidated to be 1,8-cineole [Unlu et al., 2002] whereas α-pinene, 1,8-cineole and camphor as well as germacrene D and bisabolene as the major constituents of ten other Achillea species (A. biserrata, A. clypeotala, A. crithmifolia, A. filipendula, A. macrophylla, A. pannonica, A. pyrenaica, A. biebersteinii, A. taygetea and A. tenuifolia) [Maffei et al., 1994]. Various biological activity studies were also completed on Achillea species. The antimicrobial and antioxidant activities of the essential oil and the methanolic extract of A. biebersteinii were studied in vitro by Baris et al. [Baris et al., 2006]. The essential oil showed antimicrobial activity against 8 bacteria sp., 14 fungi sp. and the C. albicans, whereas the methanolic extract remained inactive.

Baretta et al., (2012) showed that Achillea millefolium exerted anxiolytic-like effects in the elevated plus-maze and marble-burying test after acute and chronic (25 days) administration at doses
that did not alter locomotor activity. This behavioral profile was similar to diazepam. The effects of Achillea millefolium in the elevated plus-maze were not altered by picrotoxin pretreatment but were partially blocked by flumazenil. Furthermore, Achillea millefolium did not induce any changes in [(3)H]-flunitrazepam binding. Their results indicate that the orally administered hydroalcoholic extract of Achillea millefolium L. exerted anxiolytic-like effects that likely were not mediated by GABA(A)/BDZ neurotransmission and did not present tolerance after short-term, repeated administration, that is compatible with our research’s result.

Grundmann et al., (2009) demonstrated that anxiolytic activity of kaempferol (active component of yarrow) was partially antagonized by concomitant administration of flumazenil, but not by WAY-100635. In conclusion, our study clearly demonstrates that AV extract possesses anxiolytic-like activity and that at least one of its flavonoids, kaempferol, can elicit the same kind of neuropharmacological activity.

Molina-Hernandez et al., (2004) sowed that diazepam (2.0 mg/kg; i.p.) reduced conflict behavior both during late proestrus (p < 0.05) or diestrus (p < 0.05). Doses of 8.0 mg/kg (p < 0.05), 10.0 mg/kg (p < 0.05) or 12.0 mg/kg (p < 0.05) of Achillea millefolium reduced conflict behavior during late proestrus. Conversely, during diestrus, only the dose of 12.0 mg/kg (p < 0.05) of Achillea millefolium L. reduced conflict behavior. They concluded the anticonflict-like actions of Achillea millefolium L. may vary according to the estrous cycle phase.

In conclusion, can state that, polar-fraction of yarrow has more sufficient sedative, pre-anesthetic and anti-anxiety effects than diazepam and other under studied groups. 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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