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Study of Sedation and Pre-Anesthetic Effects of Polar, Semi-Polar and Non-Polar Fractions of Hop (*Humulus lupulus L.*) Extract Compared with Diazepam in Rats

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Abstract: *Humulus lupulus* is a medicinal plant which in Farsi is called "razak". The purpose of this research is, studying the sedative effects of polar, semi polar and non-polar fractions extracted from Hop (*Humulus lupulus*.L) in comparison with diazepam in the animal model of Rat. For conducting this research polar, semi polar and non-polar fractions extracted from Hop Based on the polarity of solvent. Then study continued with injecting the obtained extracts and other medicines to different groups of Wistar breed of rats. First group was injected with 100mg/kg of Polar fraction extract, the second group, with 100mg/kg of Semi-polar extract, the third group, with 100 mg/kg of non-polar extract of *Humulus lupulus* the fourth group with 2 mg/kg of Diazepam the fifth group with the same volume of DMSO used as solvent of injectable medicines and the sixth group was the control group and did not receive any drug. The method of injection was intraperitoneal (IP) form. Statistical diagrams and results showed a significant decreasing of anesthetic induction time and increasing of sleeping time of Ketamine induced anesthesia, after IP injection of the Polar fraction extract of *Humulus lupulus*. The obtained results showed that the polar-fraction extract of *Humulus lupulus* has more sufficient sedative effects than diazepam and other under studied groups.

Key words: *Humulus lupulus* · Sedation · Polar · Semi-Polar · Non-Polar Fraction · Dimethylsulfoxide · Rat

INTRODUCTION

The generic name Humulus, coined in the middle Ages, is said to derive from the Slavic word chmele (hops) or from the old Germanic word Humel or Humela (fruit-bearing). Speculation that the name comes from the Latin word humus (earth) is unfounded. The species name lupulus is the diminutive of lupus (wolf), which refers to the mistaken idea that hop tendrils strangle plants. The origin of the English name hop is uncertain, but may originate from the Norwegian word hupp, which means ‘tassel’ or ’tuft‘ or from the Anglo-Saxon hoppan (to climb) [1, 2].

The Hop was known in antiquity, but does not appear to have been used either as a medication or as an ingredient in producing beer. The 8th century Arab physician Mesuë recommended the syrup as a good medication for bilious fever and for purifying the blood. It subsequently appeared in herbal books during the middle Ages. The Hop is a four to eight meters long perennial climbing plant that likes to twine up hedges, fences and the edges of forests [3]. In spring, thin, rough stalks with anchor-like, astonishingly adhesive hairs rise from the branched rootstock. In contrast to most European climbing plants, the hop twists in a clockwise direction. The heart-shaped, three-to five-lobed leaves are sharply dentate and opposing. The hop is dioecious, i.e. there are male and female plants. Male flowers form axillary, hanging panicles with whitish-green, five-tipped bracts. They do not develop lupulin glands and are therefore not cultivated. The female plants form cone-shaped catkins which form small, oval multiple fruits composed of yellowish-green scales. The interior surface of the scale bears small, yellowish-reddish glands that give the hop its characteristic bitter taste. The hop is pollinated by means of wind dispersal [4-7].

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Hops (strobiles of *Humulus lupulus* L., Cannabaceae) are native to Eurasia and they have been cultivated for more than 1000 years [8]. Distinct lineages native to temperate regions of North America also exist. In addition, the species has been introduced as a cultivar to temperate regions of South America, South Africa and Australia. The numerous common names for this plant highlight the historical significance of the species, particularly in North temperate cultures [3, 9, 10].

Benzodiazepines possess sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant and amnesic actions [11, 12], 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 [13]. In general, benzodiazepines are well-tolerated and are safe and effective drugs in the short term for a wide range of conditions [14, 15]. 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 [16, 17]. The effects of long-term use or misuse include the tendency to cause or worsen cognitive deficits, depression and anxiety [18, 19].

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 [20].

Intravenous diazepam or lorazepam are first line treatments for status epilepticus [21, 22]; However, lorazepam has advantages over diazepam, including a higher rate of terminating seizures and a more prolonged anticonvulsant effect [23]. 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]. Diazepam is used for the emergency treatment of eclampsia, when IV magnesium sulfate and blood pressure control measures have failed [25, 26]. Benzodiazepines do not have any pain-relieving properties of them and are generally recommended to be avoided in individuals with pain [27]. 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 [28, 29]. Tolerance often develops to the muscle relaxant effects of benzodiazepines such as diazepam [19]. Baclofen [30] 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 [31].

The aim of this study was to investigation of the sedation and pre-anesthetic effects of polar, semi-polar and non-polar fractions of Hop (Humulus lupulus L.) extract compared with diazepam in rats.

MATERIALS AND METHODS

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* 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.

Obtaining Extract: Five hundred g of fresh leaves of hop was powdered by liquid nitrogen and was dissolved in the 4 liter non-polar solvent like petroleum ether for 48 hours 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 hours, obtained extract is semi-polar fraction. Finally, the remnant leaves were dissolved in the 4 liters high polar solvent like methanol for 48 hours, obtained extract is polar fraction. Achieved fractions de-solved by rotary evaporator and readied for use.

Evaluating Method as Well as Sedation and Pre-anesthetic Effects of Hop Compared with Diazepam: In order to evaluate the sedation and pre-anesthetic effects of hop 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 intraperitoneal 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.

RESULTS

Following the injection of pre-anesthetic drugs, the injection of anesthetic inductive drugs, recording of

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

Group	Received treatment (mg/kg)	Induction time (Mean ± SE)	Sleeping time (Mean ± SE)
Group 1	100 mg/kg P.E.+ ketamine 100	82.33	7400.00
Group 2	100 mg/kg S.P.E, ketamine 100	448.67	1416.00
Group 3	100 mg/kg N.P.E, ketamine 100	279.33	4620.00
Group 4	Diazepam 2, ketamine 100	535.33	3300.00
Group 5	DMSO 2, ketamine 100	802.00	2736.00
Group 6	Without pre-anesthetic, ketamine 100	800.00	1991.33

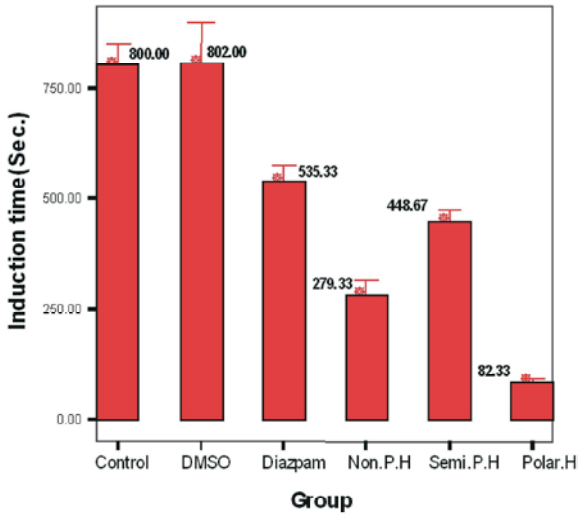

Diagram 1: Mean value of data obtained from induction time in understudying groups.

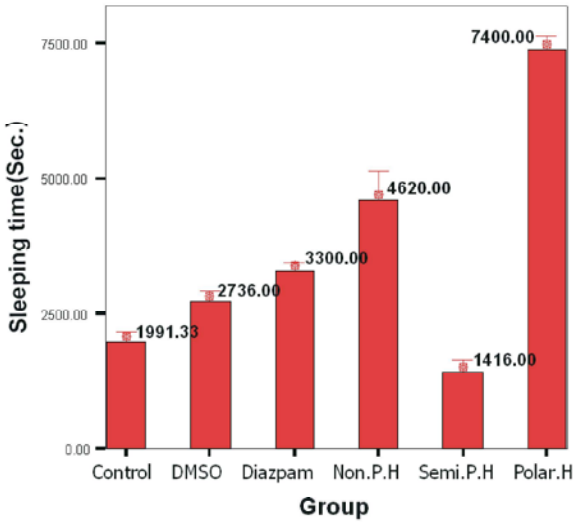

Diagram 2: Mean value of data obtained from sleeping time in understudying groups.

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 (p<0.01) between intra peritoneal injections of 100 mg/kg BW of polar extract than semi-polar and non-polar than diazepam (Table 1).

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

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 [32]. 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 ã-aminobutyric acid (GABA) ergic system to produce the biological effect [33]. However, only a low content of flavonoids was found in this hydroethanolic extract. *H. lupulus* is traditionally used as sleeping aids and probably acts via a central adenosine mechanism, which is possibly the reason for its sleep-inducing and maintaining activity [34]. Hops showed significant inhibition of GAD activity [32]. *H. lupulus* extracts induced the response of the ionotropic (GABAA receptors) [35] and its fraction containing α-acids: in dose-dependently prolonged pentobarbital induced sleeping time [36]. Xanthohumol had been reported as modulator of the GABAA receptor response [37].

Numerous other studies have been published citing hops to be active in specific bioassays. Hop extracts and/or compounds have been reported to be "active" or the equivalent by the authors of the given studies in the following assays: various antioxidant

and/or chemoprevention [1, 2, 38-41], antimicrobial, particularly against Gram positive bacteria [42-48] and cytotoxicity [49-52]. Many constituents have been reported from hops [3], among which in addition to the aforementioned estrogenic properties, many have been shown to possess other types of biological activity [52-57] such as potential cancer chemopreventive activity [58, 59] and suppression of COX-2 gene transcription [60]. The major chalcone xanthohumol, as well as the flavanones isoxanthohumol and 6PN and 8PN show various forms of antioxidant activity *in vitro* in the micromolar range and are weakly cytotoxic to certain cancer cell lines [58-63]. In addition, the metabolism of prenylated chalcones and flavanones from hops in vitro and *in vivo* has been investigated [4-7]. A research group had attributed the sedative effect of hops to 2-methyl-3-butene-2-ol, derived from hop constituents during storage but probably also formed in vivo by metabolization of α-acids. This compound, when intraperitoneally injected in rats, reduced motility without inducing a myorelaxant effect [64, 65]. However, according to Schiller this compound cannot be the constituent responsible for the sedating activity of hop preparations. Anxiolytic activity of hops had been attributed to three categories of constituents found in its extracts. Though the alpha-bitter acids proved to be the most active constituents, the beta-bitter acids and the hop oil clearly contributed to the sedating activity of hop extracts [34, 64]. According to Zanoli and Zanatti, α-acids fraction can be considered as the major responsible constituent for the enhanced pentobarbital effect and for the antidepressant property observed after the administration of hop extract. The â-acids fraction exerted an antidepressant activity as well, but reduced pentobarbital hypnotic activity [64, 66]. Hydroethanolic extract analyzed in this study exhibited anxiolytic activity (data not shown), which could be attributed to the high content of oxidized bitter acids, that seems to be high enough to contribute to anxiolytic activity of this extract and thus could be attributed to oxidized alfa-acids, such as, cohumulinone 5 and humulinone 7 derivatives presents in major concentration. Their biological effect could be explained by a reduction in the GABAergic activity, although the involvement of other neurotransmitter systems cannot rule out.

In conclusion, can state that, polar-fractions of Humulus lupulus has more sufficient sedative and pre-anesthetic 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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