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Study of Sedation, Pre-Anesthetic and Anti-Anxiety Effects of Hop (*Humulus lupulus* L.) Extract Compared with Diazepam in Rats

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Abstract: *Humulus lupulus* (hops) is a climbing perennial vine that vigorously grows 20-35 feet each year. *Humulus lupulus* is a member of the hemp family which has grown wild since ancient times in Europe, Asia and North America. The female flowers mature in late Summer and are used to add bitterness, flavor and aroma to beer. In ancient times the young shoots were eaten as a vegetable and the dried flowers were used for their slight narcotic effect and sedative action in the treatment of mania, toothache, earache and neuralgia. One modern herbal medicine practitioners continue to use hops as a sedative and mild hypnotic as well as for its endocrine, free radical scavenging and antitumor properties. The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of hop (*Humulus lupulus* L.) extract compared with diazepam in rats. In the present study 30 wistar male rats weighting 300±10 g and about 3 months old were used for laboratory experiments. In order to evaluate the sedation and pre-anesthetic effects of hop extract compared with diazepam, 50 mg kg^{-1} of extract in 1st group, 100 mg kg^{-1} in 2nd group, 1.2 mg kg^{-1} in 3rd group, 2 mg kg^{-1} in Group 4th, 2 mg kg^{-1} amount of dimethyl sulfoxide was injected intra peritoneal in 5th group and 6th group did not receive any drug. Data showed that hop extract has better sedation, pre-anesthetic and anti-anxiety effects than diazepam. Researchers suggest that still need more studies on this plant component in order to understand the more sedative and anxiolytic effects of this plant.

Key words: Sedation, pre-anesthesia, anti-anxiety, Hop (*Humulus lupulus* L.) extract, diazepam, rats

INTRODUCTION

Humulus lupulus L. Cannabaceae (commonly named hops) is natural from Central Europe and it is widely cultivated throughout the temperate regions of the world (Heinrich *et al*., 2004; Zanoli and Zavatti, 2008). The hop is one of major raw material in brewing therefore, the economic value of the hop plant is derived from its worldwide application as an essential flavoring ingredient for the brewing of beer. The impact of hops on beer quality is manifold but by far most important are specific features attributed to beer flavor including bitter taste and hoppy aroma. Many hop bittering compounds were early discovered by Verzele and Keukeleire (1991). The hop bitter acids are alicyclic phenolic acids which are respectively di or tri-prenylated phloroglucinol derivatives and their oxidation products (Chen and Lin, 2004; Stevens and Page, 2004). In general, two major α and β acids are in hop. The two series comprise, in fact, three constituents differing in the nature of the side chain (De Keukeleire *et al*., 2003). They are α-acids with three major analogous (cohumulone, humulone and adhumulone) and β-acids also with three major analogous (colupulone, lupulone and adlupulone) with a six-membered ring structure (De Keukeleire *et al*., 2003). The β-acids differ structurally from the α-acids by having one more prenyl group. In addition, there are several homologues and analogues including posthumulone/postlupulone, prehumulone/prelupulone and adprehumulone (Ciochina and Grossman, 2006; Khatib *et al*., 2006). Cohumulinone and humulinone are two corresponding oxidation products from α-acids in five member ring structure. Likewise, there are cohulupone and hulupone the oxidation corresponding to colupulone and lupulone/adlupulone in five member ring structure (De Keukeleire, 2000). The α-acids and the corresponding iso-α-acids as well as the β-acids each occur in six different congeners differing in the carbon skeleton of the alkanoyl side chain. During the brewing process the water insoluble α-acids of the hop extract are converted into the more soluble iso-α-acid. Isomerization of α-acids generated cis/trans iso-α-acids in a five member ring structure. A remarkable instability of α-acids and trans-iso-α-acids during beer storage was found to be

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independent from the nature of the alkanoyl side chain (Intelmann *et al*., 2009). The most important chemical conversion that occurs in Hops is the thermal isomerization of the α-acids or humulones to the iso-α-acids or isohumulones via an acyloin-type ring contraction. Isohumulones are optically active molecules which occur as cis and transisomers and gives rise to two epimeric isohumulones which are distinguished as cis-isohumulones and transisohumulones, depending on the spatial arrangement of the tertiary alcohol function at C (4) and the prenyl side chain at C (5) (Bohr *et al*., 2008). Tetrahydro-iso-α-acids are obtained by hydrogenation of the double bonds in the side chains of the iso-α-acid and hexahydro-iso-α- acids are accessible by a combination of the reduction of the side-chain carbonyl group and hydrogenation of the double bonds (De Keukeleire, 2000). Iso-α and reduced iso-α-acid contribute to bittering values and antimicrobial property in beer and are easily deprotonated, being commonly referred to as acids due to their beta triketo moiety (Garcia-Villalba *et al*., 2006; Hall *et al*., 2008) having pronounced bacteriostatic activity; they strongly inhibit the growth of Gram-positive bacteria (De Keukeleire, 2000).

Bitter acids can be used as potential cancer chemopreventive agents (Gerhauser, 2005) and in recent years, hops have gained considerable interest due to the biological and potential cancer chemopreventive activities of some of their constituents (Bohr *et al*., 2008). Humulone possess antioxidative, anti-inflammatory and other biologically active activities such as antitumorpromoting effects on mouse skin carcinogenesis (Lee *et al*., 2007; Van Cleemput *et al*., 2009). The main constituents found in essential oils of *H. lupulus* are humulene and myrcene (Zanoli *et al*., 2007; Chadwick, 2006). Prenylated flavonoids, other major components of this species may be divided into two major groups prenylated chalcones and prenylated flavanones such as 6-prenylnaringenin, 8-prenylnaringenin and 8-geranylnaringenin (Nikolic *et al*., 2005; Vogel and Heilmann, 2008). Anti-proliferative and apoptosis-inducing effects had been attributed to side chain variants of prenylflavanones (Diller *et al*., 2007; Magalhaes *et al*., 2008; Mendes *et al*., 2008). Resveratrol and its piceid derivative were also identified in hop (Schwekendiek *et al*., 2007). Content of α-acids, β-acids, desmethylxanthohumol and xanthohumol can vary with cultivation and climatic conditions (De Keukeleire *et al*., 2007). Proanthocyanidins also named condensed tannins (Li and Deinzer, 2006; Callemien and Collin, 2008) phenolic acids (ferulic and chlorogenic acids) (Zanoli *et al*., 2007; Li and Deinzer, 2006; Callemien and Collin, 2008) and flavonoid aglycones and glycosides (Segawa *et al*., 2006; Arraez-Roman *et al*., 2006) are also found in *H. lupulus*. Proanthocyanidins exhibited a wide range of biological activities such as antioxidants offering protection against cardiovascular and neurodegenerative diseases and immune disorders (Garcia-Villalba *et al*., 2006). In hop strobilus (*H. lupulus*) collected in estonia, xanthohumol, humulol, cohumulone, humulone, prehumulone, colupulone, lupulone, prelupulone and sesquiterpenoic acid were found (Helmja *et al*., 2007). Gamma-Aminobutyric Acid (GABAA) receptors had been widely studied since they are the site of action of a number of clinically important drugs including benzodiazepines, barbiturates and anesthetics (Morris *et al*., 2006). Benzodiazepines are the first-line drugs for the treatment of anxiety disorders, acting at the GABAA receptors which remain primary targets for novel anxiolytic compounds. These compounds are thought to produce their pharmacological effects by binding to a benzodiazepine recognition site on the GABAA receptor complex, facilitating the inhibitory activity of GABA. The aim of this study was to investigation of the sedation, pre-anesthetic and anti-anxiety effects of hop (*Humulus lupulus* L.) extract compared with diazepam in rats.

MATERIALS AND METHODS

Understudied animals: In the present study, 30 Wistar male rats weighting 300±10 g and about 3 months old were used for laboratory experiments. Animals were kept in standard condition, at 20-25°C, 70% humidity and light cycle of 12 h lighting and 12 h darkness. Standard plates were used in order to feeding by method of *ad libitum*, i.e., 24 h 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: About 500 g dried hop leafs was powdered in order to obtain extract from leaves. The powder was soaked in methanol and chloroform (70:30) for at least 24 h 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 does not know anything about their nature.

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

		Mean±SE	
Groups	Received treatment (mg kg^{-1})	Induction time	Sleeping time
1	Hop 50, ketamine 100	5.0	91
2	Hop 100, ketamine 100	4.0	100
3	Diazepam 1.2, ketamine 100	9.0	55
4	Diazepam 2, ketamine 100	8.5	56
5	DMSO 1.2, ketamine 100	11.0	46
6	Without pre-anesthetic, ketamine 100	12.0	45

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, 50 mg kg^{-1} of extract in 1st group, 100 mg kg^{-1} in 2nd group, 1.2 mg kg^{-1} in 3rd group, 2 mg kg^{-1} in Group 4th, 2 mg kg^{-1} amount of dimethyl sulfoxide was injected intra peritoneal in 5th group and 6th group did not receive any drug. About 100 mg kg^{-1} ketamine per body weight was injected intra peritoneal in all groups 30 min following mentioned drugs. Induction time and sleeping time were measured immediately following administration of ketamine (Table 1).

Elevated plus maze was used in order to evaluate anti-anxiety effects of hop 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, hop extract with dosages of 100, 200, 400 and 1.2 mg kg^{-1} BW diazepam of diazepam and dimethyl sulfoxide (as placebo) were used as intra peritoneal injection. Dimethyl sulfoxide was placed in maze center 30 min 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 2nd time duration of their presence in maze is 5 min. 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 AND DISCUSSION

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 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 mg kg^{-1} BW of hop extract and 1.2 mg kg^{-1} BW of diazepam.

Based on Fig. 1 and 2, intra peritoneal injections of 100 mg kg^{-1} BW of hop extract has lower induction time and higher sleeping time compared with 1.2 mg kg^{-1} BW of diazepam so that there is a significant difference

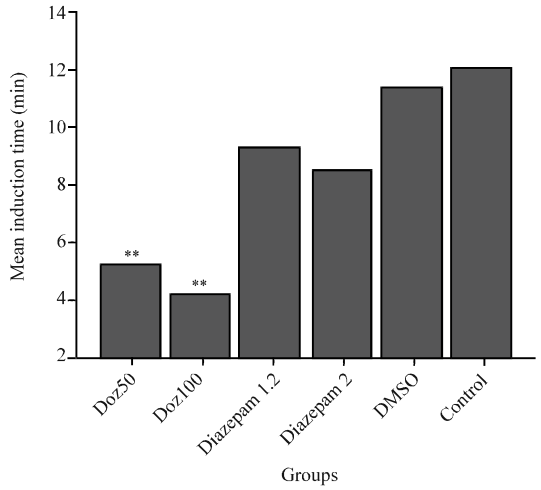

Fig. 1: Mean value of data obtained from induction time in understudying group. **Indicates significant difference in $p<0.01$ level

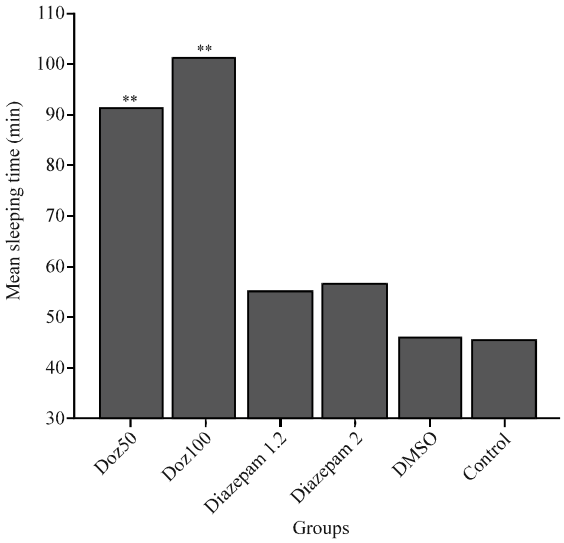

Fig. 2: Mean value of data obtained from sleeping time in understudying group. **Indicates significant difference in $p<0.01$ level

($p<0.01$). In other words, the extract has better sedation and pre anesthetic effects compared with diazepam. But dosages of 50 and 100 mg kg^{-1} BW of the extract do not show a significant difference with diazepam. Dosages of 50 and 100 mg kg^{-1} BW of the extract have weaker and identical functions, respectively compared with diazepam. The significant of differences compared with extract dosages of 200 and 400 mg kg^{-1} BW suggests that the increase of extract dose leads to increase the sedation and anti-anxiety effect. Based on Fig. 3 and 4, the results show

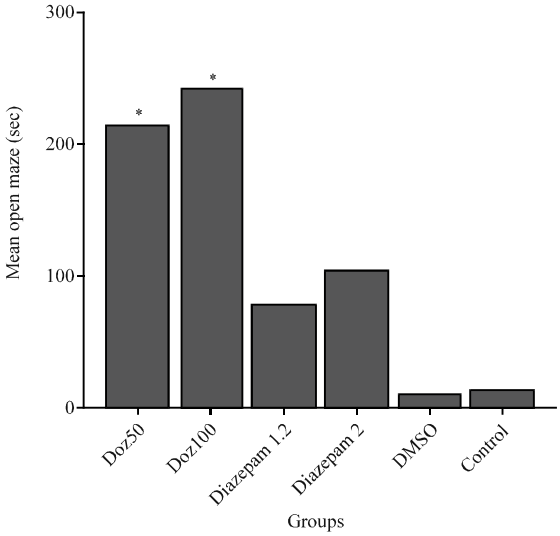

Fig. 3: Mean value of data obtained from open maze time in understudying group; **Indicates significant difference in $p<0.05$ level

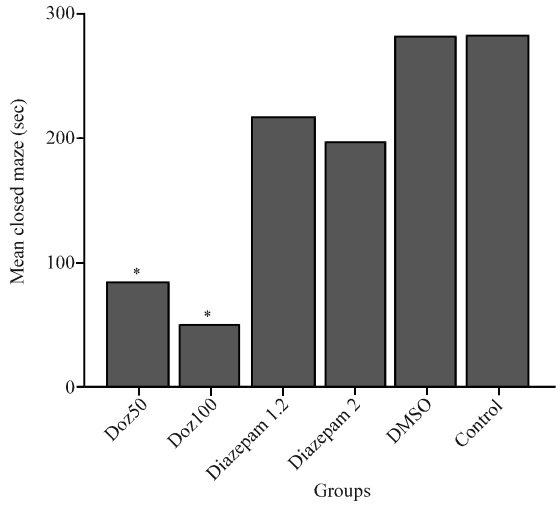

Fig. 4: Mean value of data obtained from closed maze time in understudying group; **Indicates significant difference in $p<0.05$ level

that hop extract in dosage of 400 mg kg^{-1} BW has a better anti-anxiety effect compared with 1.2 mg kg^{-1} 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 open maze arms as well as increases the numbers of traverse on open arms (Table 2). But the extract dosages of 100 and 200 mg kg^{-1} BW demonstrate a significant difference, i.e. have a weak function ($p<0.01$).

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. *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 andmaintaining activity (Schiller *et al*., 2006). Hops showed significant inhibition of GAD activity (Awad *et al*., 2007). *H. lupulus* 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 and Haberlein, 2006). 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 (Zanoli *et al*., 2007; Heinrich *et al*., 2004).

However, according to Schiller *et al*. (2006) this compound cannot be the constituent responsible for the sedating activity of hop preparations. Anxiolytic activity of hops had been attributed to three

Table 2: Group's classification and measured induction time and sleeping time by focus on maze pattern

		Mean±SE	
Groups	Received treatment (mg kg^{-1})	Induction time	Sleeping time
1	Hop 50	216	85
2	Hop 100	241	51
3	Diazepam 1.2	77	215
4	Diazepam 2	104	196
5	DMSO 1.2	10	282
6	Without pre-anesthetic	15	285

categories of constituents found in its extracts. Though the α-bitter acids proved to be the most active constituents, the β-bitter acids and the hop oil clearly contributed to the sedating activity of hop extracts (Schiller *et al*., 2006; Zanoli *et al*., 2007). According to Zanoli and Zavatti (2008), 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 β-acids fraction exerted an antidepressant activity as well but reduced pentobarbital hypnotic activity (Zanoli *et al*., 2007; Zanoli and Zavatti, 2008). Hydroethanolic extract analyzed in this study exhibited anxiolytic activity 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.

CONCLUSION

The results of this study show that hop extract has better sedation, pre-anesthetic and anti-anxiety effects than diazepam. Researchers 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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