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Comparative Study of Sedation, Pre-Anesthetic and Anti-Anxiety Effects of Hemp Seed Extract and Diazepam in Rats

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Abstract: Hempseed with scientific name *Cannabis sativa*, spreads across the world. Hempseed oil's fatty acids compounds were obtained from different parts of Iran with different weather and were tested. The herb contains canabidole, canabinol and canabidiolic acid. The herb is used for preparing some sedation drugs which are used in neural stresses, anxiety, migraine, asthmatic coughs as well as local anesthesia in dentistry. Stuart points out that all parts of hempseed are used in China traditional medicine. Its seed is used as tonic, strengthening, laxative, diuretic, calmative, gastric anti parasite and narcotic. Masson and Teobold say that hempseed has a great value for tetanus treatment. Hempseed is a real calmative for stomachache and digestive disorders as well as beneficial for cancers, ulcers and rheumatic pains. In the present study, 30 Wistar male rats of 200-230 g weight 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.

Key words: Sedation, pre-anesthetic, anti-anxiety, hemp seeds, diazepam, rats

INTRODUCTION

Now a days, medical herbs form an important part of traditional medicine in most countries as well as especial and valuable place in new treatment procedures. In the present study the attempt was to introduce the extract as a pre-anesthetic and anti anxiety medicine which is more effective and has fewer side effects compared with chemical drugs. Also, the herd is used for disease treatment in traditional medicine (Cetto, 1999; Pellow *et al*., 1985; Racine, 1972).

Hempseed with scientific name *Cannabis sativa*, spreads across the world. Hempseed oil's fatty acids compounds were obtained from different parts of Iran with different weather and were tested. The herb contains canabidole, canabinol and canabidiolic acid. The herb is used for preparing some sedation drugs which are used in neural stresses, anxiety, migraine, asthmatic coughs as well as local anesthesia in dentistry. Stuart points out that all parts of hempseed are used in China traditional medicine (Mirhaydar, 2002; Cetto *et al*., 2000; Perez *et al*., 1985). Its seed is used as tonic, strengthening, laxative, diuretic, calmative, gastric anti parasite and narcotic. Masson and Teobold say that hempseed has a great value for tetanus treatment. Hempseed is a real calmative for stomachache and digestive disorders as well as beneficial for cancers, ulcers and rheumatic pains (Zargari, 1989; Lemus *et al*., 1996).

Because of existing effective materials such as canabidole, canabinol and acid the herb has sedation and calmative effects. Considering the mentioned items and due to existing canabidiolic acid, the herb has sedation and anti-anxiety effects. In the present study, the effect of different doses has been evaluated (Mirhaydar, 2002; Dos Santos *et al*., 2005; Wilson *et al*., 1998).

MATERIALS AND METHODS

Understudied animals: In the present study, 30 Wistar male rats of 200-230 g weight 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.

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Obtaining extract: A total of 1000 g dried hempseed was powdered in order to obtain extract from stem and 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.

Evaluating method as well as sedation and pre-anesthetic effects of hempseed: In order to evaluate the sedation and pre-anesthetic effects of hempseed extract compared with diazepam, 150 mg of extract per kg of body weight in 1st group, 300 mg of extract per kg of body weight in 2nd group, 450 mg of extract per kg of body weight in 3rd group, 1.2 mg diazepam per kg of body weight in 4th group, the same amount of methyl sulfoxide was injected intra peritoneal in 5th group and 6th group did not receive any drug. A total of 40 mg kg^{-1} BW of ketamine was injected intra peritoneal in all groups 30 min 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 hempseed extract. The system consists of two arms (50×10 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, hempseed extract with dosages of 150, 300, 450 and 1.2 mg kg^{-1} BW diazepam of diazepam and dimethyl sulfoxide (as placebo) were used as intra peritoneal injection. Methyl 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 second; time duration of their presence in maze is 5 min (Wilson *et al*., 1998). SPSS Software program was used in order to analysis statistical data as well as Tokay follow up test for determining a meaningful difference among dual groups. The $p<0.01$ has been considered as meaningful. 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. The results of dual Tokay follow up test show a meaningful difference between intra peritoneal injections of 400 mg kg^{-1} BW of hempseed extract and 1.2 mg kg^{-1} BW of diazepam. Based on Fig. 1 and 2, intra peritoneal injections of 450 mg kg^{-1} BW of hempseed extract has lower induction time and higher sleeping time compared with 1.2 mg kg^{-1} BW of diazepam so that there is a meaningful difference ($p<0.01$). In other words, the extract has better sedation and pre anesthetic effects compared with diazepam. But dosages of 150 and 300 mg kg^{-1} BW of the extract do not show a meaningful difference with diazepam. Dosages of 150 and 300 mg kg^{-1} BW of the extract have weaker and identical functions, respectively compared with diazepam.

The meaningfulness of differences compared with extract dosages of 300 and 450 mg kg^{-1} BW suggests that the increase of extract dose leads to increase the sedation

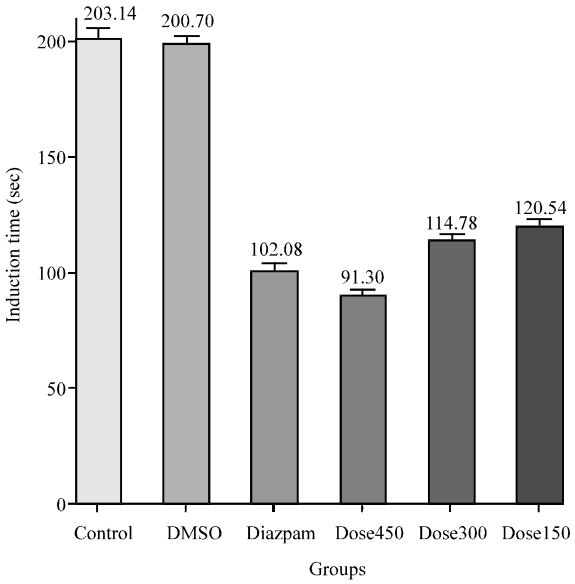

Fig. 1: Mean of induction time obtained from understudied groups plant hemp seed

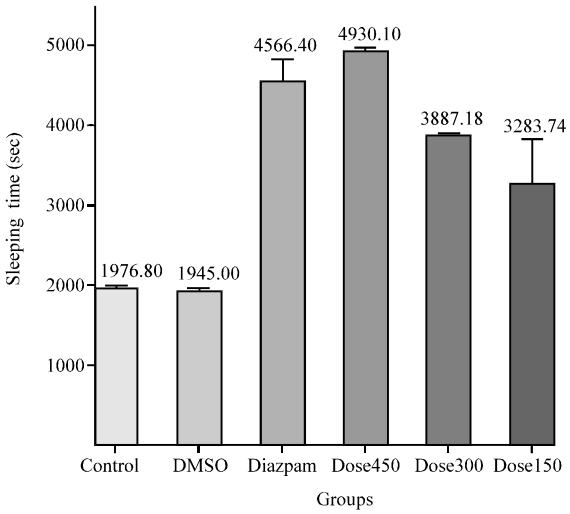

Fig. 2: Mean of sleeping time obtained from understudied groups plant hemp seed

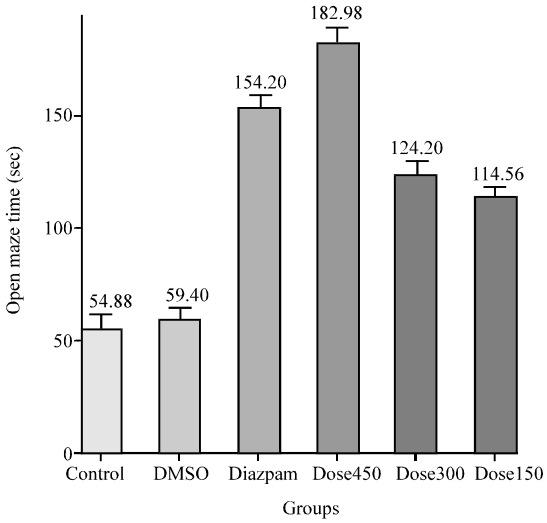

Fig. 3: Mean of maze data obtained from understudied groups plant hemp seed

and anti anxiety effect. Based on Fig. 3, the results show that hempseed extract in dosage of 450 mg kg^{-1} BW has a better anti anxiety effect compared with 1.2 mg kg^{-1} BW of diazepam. Also, they show a meaningful 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. But the extract dosages of 150 and 300 mg kg^{-1} BW demonstrate a meaningful difference, i.e. have a weak function ($p<0.01$).

In the present study two methods, evaluating the sedation and pre anesthetic effect and evaluating of anti anxiety effects were used for comparing hempseed extract against diazepam. Some other researches were conducted in the past for example in 1914 and 1920, Hanzlik suggested that anti spasm effect had caused to its administration in angina, asthma, gastric muscular fibers' contraction and disorders related to unstriated muscles' spasms (Dunham and Miya, 1957). Kafaie and Razavi demonstrated in his studies that hempseed liquid extract has long time effects on spatial memory stabilization in rats; they concluded that high injection dosages of the herb's liquid extract causes to some disorders in memory and learning but it causes to memory strengthening in low or mediate dosages (Broudiscou and Lassalas, 2000; Kang *et al*., 2002; Fathiazad and Lotfipour, 2003). Lev-Ran evaluated the difference and quality of the life and mental health of people who uses hempseed compared with who do not use it; they reported that the mental ability is lower in people who do not use hempseed. Evaluation of the use of hempseed and disorders of its usage was conducted on last 12 months which are 4.1 and 1.5%, respectively. Hayat Gheybi conducted a study on the effect of Esfahan variety of hempseed powder on serum lipid of male rats; they concluded that the Esfahan variety of hempseed contains much amounts of narcotic compounds such as Tetrahydrocanabinols (THC) which does not modify lipid profile. It seems that longtime use of Esfahan hempseed leads to lipidic disorders in blood (Ferrini *et al*., 1974; Fisher, 1989; Rodgers *et al*., 1997).

Based on reported studies, different compounds were identified and isolated from hempseed which were mentioned previously. The presence of alkaloid and flavonoid compounds in the herb cause to increase sleeping time due to injection of anesthetic drug that conforms the present study. Based on observations, it can be said that sedation effect of the extract is related to these compounds (Dos Santos *et al*., 2005). Diazepam, on one hand is as a benzodiazepine drug has sedation and pre anesthetic effects on central neural system and on the other hand is considered as an anti anxiety drug. So, causes to some sedation and anti anxiety effects by interaction with GABA receivers presented in brain especially in reticular part of middle brain (Katzung, 2004).

Based on obtained results, among administered dosages, 450 mg kg^{-1} BW of the extract has a meaningful difference with diazepam during sedation process ($p<0.01$) and has better sedation and pre anesthetic effects compared with Diazepam, i.e. has shorter induction time and longer sleeping time compared with diazepam so can be used as pre anesthetic drug instead of diazepam. But 450 and 150 mg kg^{-1} BW of the extract show no meaningful difference over diazepam ($p<0.01$). Extract dosage of 150 mg kg^{-1} BW has a weaker function

compared with diazepam and the extract dosage of 300 mg kg^{-1} BW has a identical function compared with diazepam.

Based on different study in the present study, in order to obtain suitable dosages, the extract dosages of 1500, 300, 450 mg kg^{-1} BW were used. Also, in the second part of the study, based on obtained results, it has demonstrated that 450 mg kg^{-1} BW of the herb has better anti anxiety effect compared with 1.2 mg kg^{-1} BW of diazepam that is by administrating 450 mg kg^{-1} BW the rats remain more time on maze open arm compared with diazepam also, their traverse on open arms is greater which is as an anti-anxiety marker. Considered with the herb's flavonoid and alkaloid compounds and obtained results it can be concluded that hempseed has sedation, pre-anesthetic and anti-anxiety effects (Katzung, 2004; Archer, 1973).

Now a days, the studies on traditional medicine have been increased. But hempseed has remained unknown in spite of traditional uses from its stems and leaves. Considered with observations about the extract's sedation and anti anxiety effects, more studies are required about identification and extraction of the herb's constituents.

CONCLUSION

It can be concluded, generally that based on different studies the extract of hempseed may affect via effecting on benzodiazepine receivers connected to GABA receivers (considering its flavonoid content). Based on the obtained results by the present study it can be said that according to sedation process the extract dosage of 450 mg kg^{-1} BW among other dosages has had more meaningful results and has a better sedation, pre-anesthetic and anti-anxiety effect compared with diazepam ($p<0.01$).

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