original article

JPHS

Open Access

The effects of *Tilia platyphyllos* Scop. extract on seizure induced by picrotoxin and pentylentetrazole in mice.

Mahmoud Reza Heidari [1*], Seyed Mojtaba Sohrevardi [2], Ali Eskandarizadeh [3], Hesam Moghadammanesh [3,4], Mohammad Heidari [3,5], Sadegh Mohebbi [2,6]

1. *Pharmaceutics, Neuroscience and Physiology Research Centers, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*
2. *Pharmaceutics, Research Center, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*
3. *Neuroscience Research Centers, Faculty of Dentistry, Kerman University of Medical Sciences, Kerman, Iran*
4. *Physiology Research Center, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*
5. *Faculty of Pharmacy, Tehran University of Medical Sciences, Kerman, Iran*
6. *Food and Drug Organization, Ministry of Health, Tehran, Iran*

ABSTRACT

The effects of *Tilia plathphyllos* scop. in a nervous disorder, including seizure was reported in traditional literatures. In the present investigation, the effects of methanolic extract of *T. plathyphyllos* on seizure induced by picrotoxin and pentylentetrazole were studied in mice. In this experimental study, groups of 5 animals pretreated with doses of 100, 200 and 300 mg/kg of methanolic extract of flowering branches of *T. plathyphyllos*, via intraperitoneal injection. After 20 minutes each animal received picrotoxin 10 mg/kg or pentylentetrazole 80 mg/kg intraperitoneally, for induction of seizure. Latency of seizure, death time and percent of death were determined in treated and control groups. The latency of seizure induce by picrotoxin were increased in groups that pretreated with doses of 100 and 200 mg/kg of *T. plathyphyllos* extract, from 208 Sec to 298($p<0.05$) and 570 Sec ($p<0.01$) respectively. The latency of seizure induced by pentylentetrazole were increased with dose of 200 mg/kg, from 233 Sec to 351 Sec ($p<0.01$). The dose of 200 mg/kg of extract delayed the death time induced by picrotoxin from 1237 to 1498 Sec ($p<0.05$) and pentylentetrazole from 1973 to 2508 Sec ($p<0.01$). In additin the percentage of mortality from seizure induced by picrotoxin and pentylentetrazole from 100% were decreased to 80% ($p<0.05$) and 40% ($p<0.01$) respectively.
Methanolic extract of flowering branches of *T. plathyphyllos* delayed the onset of seizure, death time and decreased the percentage of mortality from picrotoxin and pentylentetrazole. Further studies are needed to use this plant as an antiseizure agent.

Key words: *Tilia plathyphyllos Scop*, Seizure, Picrotoxin, Pentylentetrazole

* ***Corresponding Author*** : Prof.Mahmoud Reza Heidari, Dept. of Toxicology and Pharmacology, Faculty of Pharmacy,
Kerman, Iran. P.O.Box: 76175-493
Tel: +98 341 3205001 Fax: +98 341 3205003
e-mail: heidarimr@yahoo.com, heidarimr@kmu.ac.ir

1. Introduction

One of the most common central nervous system disorders is epilepsy. In spite of modern drug therapy, there are some limitations in this respect including safety and efficacy of these drugs in control of seizure (Hopkins et al., 1995; Carvey et al., 1998; Katzung, 2007). Therefore there are some interest for investigation for finding new compounds with higher efficacy and minimal adverse effects. Herbal medicines are one of the valuable sources for finding these compounds (Zargari, 1997). There has been an increase in interest in Tilia species, including *T. platyphyllos*, because of their medicinal and nutritional properties. Tilia platyphyllos belongs to Tiliaceae family, which consists of several species, distributed in Europe, North America and Asia. *T. platyphyllos* is a large deciduous tree (Zargari, 1997; Aguirre-Hernandez et al., 2007; Fluke, 2000). *T. platyphyllos* grows in Baluchestan, Bandar-abbas and north parts of Iran. Flowers and flowering branches of this tree are used in Iranian traditional medicine (Zargari, 1997; Fluke, 2000; Volak, 2000; Samsam-shariat, 1994. *T. platyphyllos*, is used as diaphoretic, appetizer, diuretic, expectorant, antispasmodic and sedative in phytotherapy. It has been also used for the treatment of cough, nervous tension, migraine, insomnia, and different types of spasms in traditional medicine (Fluke, 2000; Volak, 2000; Bremness, 1994; Duke, 2002; Toker et al., 2001). Medicinal properties of Tilia species have been attributed to some active ingredients including flavonoids, volatile oil and mucilage. Quercitrin (as the main flavonoid), hyperoside, tiliroside, kaempferol derivatives, isoquercitrin, astragalin and rutin are the most important compounds in Tilia species (Zargari, 1997; Toker, 2001; Brickman, 2000; Aeinechi, 1991). There are some reports about analgesic, sedative and anxiolytic efficacy of different species of Tilia including T. americana var. mexicana (Aguirre-Hernandez et al., 2007; Martinez et al., 2009; Herrera-Ruiz et al., 2008; Perez-Ortegaa et al., 2008). Tilia is used as a non-narcotic sedative for sleep disorders or anxiety in traditional medicinePerez-Ortegaa et al., 2008). Flower tea of this species has been used for overanxious children as a mild sedative (Perez-Ortegaa et al., 2008; Arteche et al., 1998). *T. platyphyllos* have been used as sedative and tranquilizer in traditional medicine (Aguirre-Hernandez et al., 2007; Viola et al., 1994; Coleta, 2001). Sedative and antispasmodic effect of *T. platyphyllos* was reported in some traditional medicinal plant books in Iran (Zargari, 1997; Fluke, 2000; Bremness, 1994; Rojhan, 1995). *T. platyphyllos* is suggested for treatment of bronchitis, insomnia, rheumatism and epilepsy in some literatures (Zargari, 1997; Duke, 2002; Rojhan, 1995; Zeppa et al., 2000; Silva, 2000). According to these facts and popular believes in Iranian traditional medicine, it seems that this plant may be effective in different CNS disorders such as seizure. Bibliography shows that there are any reports in the literatures about study the anticonvulsant effect of *T. platyphyllos*. This study was carried out to investigative the effect of methanolic extract of flowering branches of *T. platyphyllos* on generalized seizure induced by GABA –A antagonist, picrotoxin (Heidari et al., 1996), widely used as a model for chemically-induced convulsion in mice (Swinyard, 1969; Meckenzie etal., 2002; Heidari et al., 2005), and pentylenetetrazole that induced clonic seizure (Mehrabani et al., 2007; Ojewole, 2008). This study can be used as scientific reference for the use of *T. platyphyllos* as an anti-seizure agent in Iranian traditional medicine.

2. Methodology

2.2. Animals

Male albino mice weighing 23-27g were used in this experiment. The animals were obtained from The Neuroscience Research Center of Kerman University of Medical Sciences. They were housed in a room temperature 21 ± 2 at 12/12h light/dark cycle. They had free access to food and water except during the time of experiments. Animals were acclimatized to the laboratory for at least one hour before testing and were used for once experiment only. The experiments were carried out between 9.00 and 14.00 hours. For each experiment, animal groups of five mice were used. This study was down in laboratory of Toxicology and Pharmacology of Faculty of Pharmacy in Kerman University of Medical Sciences in Iran. According to international rules of considering animal experiments (Zimmermann, 1983), all efforts were made to minimize animal suffering and to reduce the number of test animals.

2.2. Plant Material

Flowering branches of *T. platyphyllos* were purchased from an official medicinal plant store in Kerman, Iran. The samples were authenticated by Dr. M. Mehrabani, Department of pharmacognosy, faculty of pharmacy, kerman, Iran and voucher specimens (No. 1006) were deposited in Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

2.3. Extract Preparation

The dried flowering branches of *T. platyphyllos*, (50 g) were powdered and extracted with 80% methanol by percolation method during 72 hours. The extract was concentrated by rotary evaporator apparatus and dried at room temperature. The weight of crude dried extract was 10 g, (20%) yield.

2.4. Preparation of solutions for administration to animals

The dried total extract was dissolved in normal saline for final suitable concentrations to give the desired experimental concentration (100, 200 and 300 mg/10ml). Experimental convulsant drug, picrotoxin, with concentration of 1 mg/ml and Pentylenetetrazole (PTZ) 8 ml/ml were prepared in normal saline. Phenobarbital 4 mg/ml was also dissolved in normal saline. All extracts and drugs were prepared freshly in the first day of the experiments and administered in a volume of 10 ml/kg body weight intraperitoneally (Heidari et al., 2006a). Negative and positive control groups received Normal saline (10ml/kg, i.p.) and Phenobarbital; reference anti-seizure drug, (40 mg/kg, i.p.) instead of the extracts, respectively (Meckenzie et al., 2002; Avallone et al., 2000; Ngo-Bum et al., 2004). The extract, saline and Phenobarbital were injected i.p. 20 min before picrotoxin or pentylentetrazole (Ojewole, 2008; Heidari et al., 2006b; Heidari et al., 2004).

2.5. Seizure evaluation and data recording

The animals received different experimental doses of *T.Platyhyllos* extract, 100, 200 and 300 mg/kg, 20 min before convulsive and fatal dose of picrotoxin; 10mg/kg or PTZ 80 mg/kg (Avallone et al., 2000; Ngo-Bum et al., 2004). Latency time of first convulsion symptoms, duration of seizure, death latency and percentage of death were measured in the test and control groups (Meckenzie et al., 2002; Avallone et al., 2000; Ngo-Bum et al., 2004; Gower et al., 1995). Mice that did not show tonic or clonic convulsion within 30 min. after picrotoxin or PTZ administration were considered protected. Mice were observed for the convulsive behavior signs including hind-limb tonic clonic seizure, convulsive waves through the body, ear and facial twitching, myoclonic jerks and rearing, clonic convulsions with falling on its side, repeated severe tonic-clonic convulsions. The criterion for seizure was defined as the exhibition of at least one of the above symptoms in mice. Mice were observed 90 min after picrotoxin or PTZ injection in individual places. The ability of the extract to prevent the seizure or delay the latency or onset of tonic or clonic seizure was considered as anticonvulsant activity of the extract (White, 1997).

2.6. Statistic analysis

Results are presented as Mean ± S.E.M and statistical significance between groups were analyzed by ANOVA followed by Newman - Keuls test. Fisher exact test was used for comparison of percentage of mortality. *P values* less than 0.05 were considered significant (Ojewole, 2008; Ngo-Bum et al., 2004; Heidari et al., 2009a,).

3. Results

3.1. Seizure induction

Single injection of picrotoxin 10 mg/kg/ip, or PTZ 90 mg/kg/ip, induced tonic–clonic seizure in mice.

3.2. Picrotoxin

3.2.1. Effect of T. platyphyllos extract on the onset time of seizure induced by picrotoxin:

Pretreatment of animals with doses of 100, 200 and 300 mg/kg of the plant extract delayed the onset of seizure. The most effective dose was 200 mg/kg and this dose delayed the onset of seizure from 208 Sec to 570 Sec ($p<0.01$). (Fig. 1A). The percentage of animals showing convulsions was 100% in all groups of mice

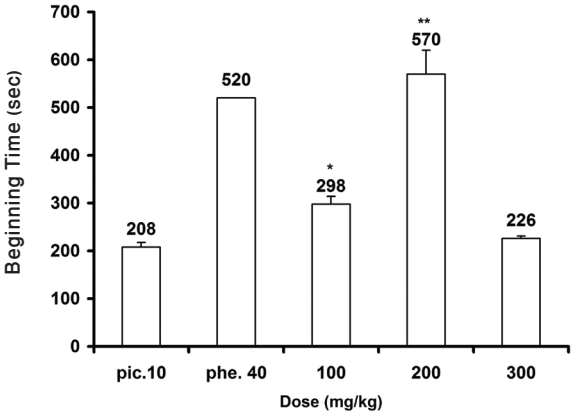

Figure 1A. *The effect of the T. platyphyllos extract on the onset time of seizure.Normal Saline10 ml/kg, Phenobarbital 40mg/kg or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Picrotoxin 10mg/kg. Each column indicates the Mean ± SEM in 5 mice.*
**P<0.05 and ** P<0.01; significant difference from saline control group.*

3.2.2. Effect on death time

Pretreatment of animals with different doses of *T. platyphyllos* extract decreased the severity of seizure and delayed the death time that was significant with doses of 200 ($p<0.01$). (Fig. 1B)

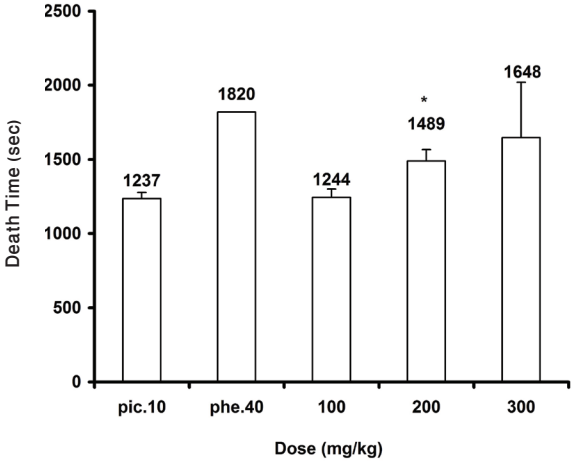

Figure 1B. *The effect of the T. platyphyllos extract on the Death time Normal Saline 10 ml/kg, Phenobarbital 40mg/kg or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Picrotoxin 10mg/kg. Each column indicates the Mean ± SEM in 5 mice.*
**P<0.05; significant difference from saline control group.*

3.2.3. Effect on duration of seizure:

The extract *of T. platyphyllos* decreased the severity of seizure. However the duration of seizure was not significantly changed by the extract (Fig. 1C).

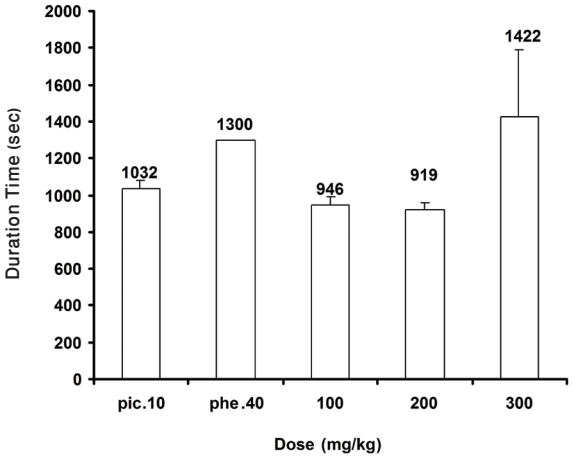

Figure 1C. *The effect of the T. platyphyllos extract on the Duration of seizure Normal Saline 10 ml/kg, Phenobarbital 40mg/kg or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Picrotoxin 10mg/kg. Each column indicates the Mean ± SEM in 5 mice.*

3.2.4. Effect on percentage of death

Figure 1D shows that only the dose of 300 mg/kg of the extract decreased mortality from 100 to 80% ($p<0.01$).

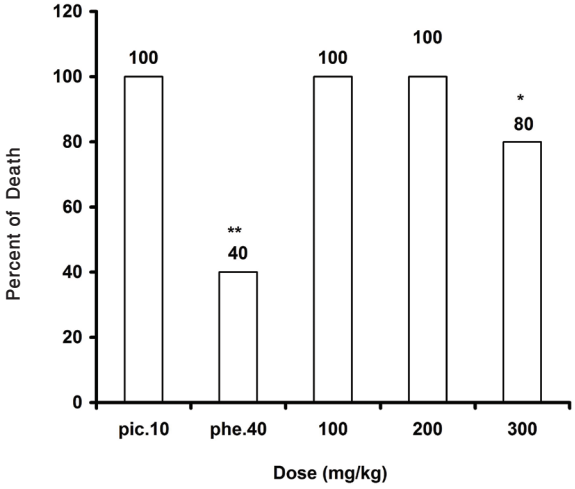

Figure 1D. *The effect of the T. platyphyllos extract on the percent of death Normal Saline 10 ml/kg, Phenobarbital 40mg/kg or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Picrotoxin 10mg/kg. Each column indicates the Mean ± SEM in 5 mice.*
**p<0.05 and ** p<0.01; significant difference from saline control group.*

3.3. Pentylenetetrazole (PTZ)

3.3.1. Effect of T. Platyphyllos extract on the onset time of seizure induced by PTZ:

Pretreatment of animals with doses of 100 and 200 mg/kg of the extract delayed the onset of seizure. The most effective dose was 200 mg/kg and this dose delayed the onset of seizure from 233 Sec to 351 ($p<0.01$). (Fig. 2A). The percentage of animals showing con

vulsions was 100% for all groups of animal pretreated with the extract. Pretreatment of animal with phenobarital inhibited convulsion in 100% of them in this group.

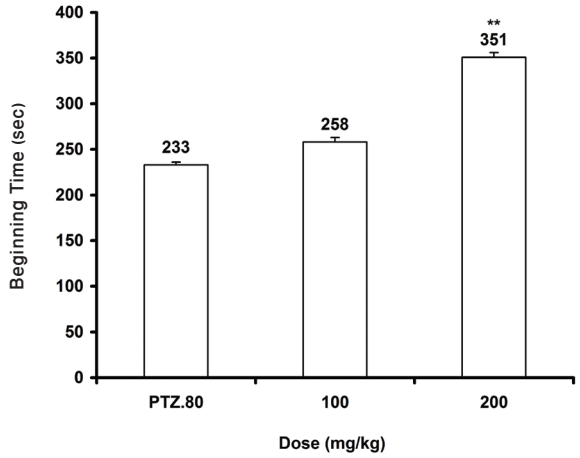

Figure 2A. *The effect of the T. platyphyllos extract on the Onset time of seizureNormal Saline10 ml/kg, or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes beforePentylentetrazole 80mg/kg. Each column indicates the Mean ± SEM in 5 mice.*
*** p<0.01; significant difference from saline control group.*

3.3.2. Effect on death time

Pretreatment of animals with different doses of *T. platyphyllos* extract decreased the severity of seizure and delayed the death time that was significant with doses of 200 mg/kg ($p<0.01$). (Fig. 2B)

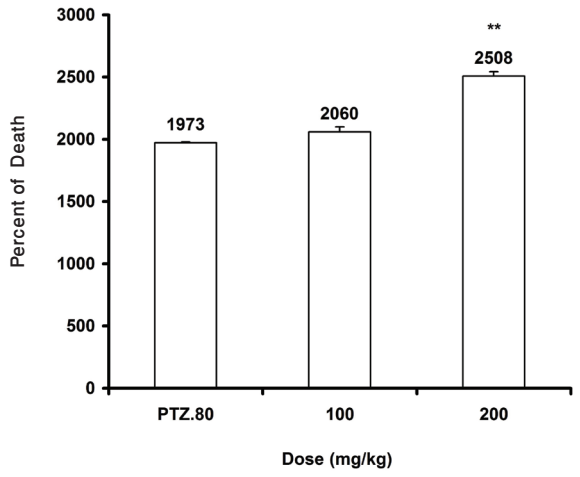

Figure 2B. *The effect of the T. platyphyllos extract on death time.*

Normal Saline10 ml/kg, or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Pentylentetrazole 80mg/kg. Each column indicates the Mean ± SEM in 5 mice.
*** p<0.01; significant difference from saline control group.*

3.3.3. Effect on duration of seizure

The extract of *T. platyphyllos* decreased the severity of seizure. The duration of seizure was prolonged by the dose of 200 mg/kg (Fig. 2C , $p < 0.01$).

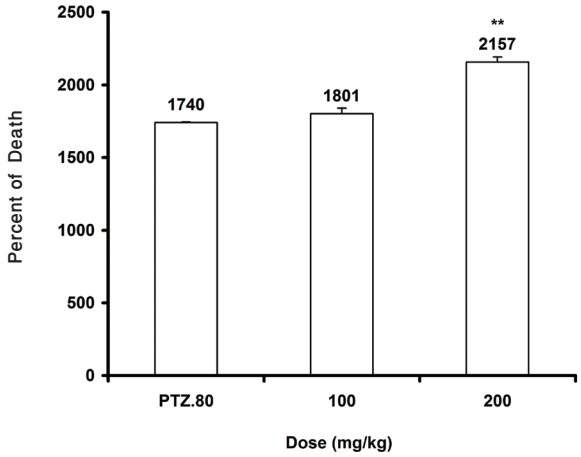

Figure 2C. *The effect of the T. platyphyllos extract on Duration time of seizureNormal Saline10 ml/kg, or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Pentylentetrazole 80mg/kg. Each column indicates the Mean ± SEM in 5 mice.*
*** p<0.01; significant difference from saline control group.*

3.3.4. Effect on percentage of death

Figure 8 shows that the dose of 200 mg/kg of the extract decreased percentage of death from 100 to 40% ($p<0.01$). (Fig. 2D)

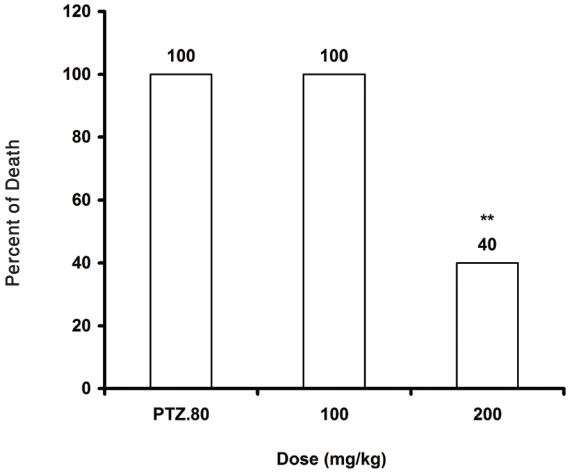

Figure 2D. *The effect of the T. platyphyllos extract on Percent of deathNormal Saline10 ml/kg, or different doses of T. platyphyllos extract were injected intraperitoneally 20 minutes before Pentylentetrazole 80mg/kg. Each column indicates the Mean ± SEM in 5 mice. ** p<0.01; significant difference from saline control group.*

4. Discussion

The benefits of *T. platyphyllos* for many diseases such as CNS disorders were reported in some literatures in Iranian traditional medicine (Zargari, 1997; Aeinechi, 1991; Rojhan, 1995; Mozaffarian, 1996). In this research the effect of methanol extract of *T. platyphyllos* on the chemically induced generalized tonic-clonic seizure by picrotoxin (Swinyard, 1969; Meckenzie et al., 2002; Avallone et al., 2000; Ngo-Bum et al., 2004), as a GABA-A antagonist (Heidari et al., 1996), and clonic seizure induced by pentylene tetrazole (Mehrabani et al., 2007; Ojwole, 2008) were studied. Our results showed that the methanol extract of flowering branches of *T. platyphyllos* have some anticonvulsant effect in mice. Pretreatment of animals with doses of 100, 200 and 300 mg/kg of *T. platyphyllos* extract, 20 min. before administration of picrotoxin 10 mg/kg, delayed the onset of seizure. The maximum effect was seen with dose of 200 mg/kg of the extract. It seems that this dose of extract produced enough blood concentration for this effect (Shargel, 2001). Higher doses not only did not increase the effect, but also decreased it. It may be speculated that higher doses of extract may be induced non pharmacologic effect (Shargel, 2001). These non-therapeutic effects may be due to the presence of some unknown component or metabolites in *T. platyphyllos*. This variation of response is relatively common with crude herbal extracts and also reported by others Heidari et al., 2005; Heidari et al., 2006; Lian et al., 2005). The explanation for this observation may be due to existence of other components in the crude extract that may have opposite or toxic effects. Thus the effect of crude extract is a sum of the relative doses and maximal effects of the individual components of extract (Lian et al., 2005).

The *T. platyphyllos* extract also delayed the death time and significantly decreased the death percentage from picrotoxin induced seizure.The potency of *T. platyphyllos* extract is similar with the effects of rosmarinus officinals on seizure induced by picrotoxin in previous study (Heidari et al., 2005) but the potency of *T. platyphyllos* extract, is lower than echium amoenum in previous study (Heidari et al., 2006). There is not any reports about anti-seizure effect of *T. platyphyllos* in the literature to compare of our findings with others.

Tilia species contain different components including hydrocarbons, esters, aliphatic acids, polyphenols and terpenoids (Duke, 2002; Viola et al., 1994; pietta et al., 1993; Buchbauer et al., 1995; Matsuda et al., 2002). Flavonoids such as quercitrin, isoquercitrin, kaempferol, astragalin, rutin, hyperoside, tiliroside, quercetin-3,7-Odirhamnoside and kaempferol-3,7-O-dirhamnoside have been reported as major components in Tilia species (Zargari, 1997; Toker et al., 2001; Aeinechi, 1991; Herrera-Ruiz et al., 2008; Arteche et al., 1998; Pietta et al., 1993). However one or most of these chemical compounds are suspected to responsible for the anticonvulsant effect of the *T. platyphyllos* extract.

Since picrotoxin is a GABA-A antagonist (Heidari et al., 1996), and inhibit or attenuate the GABA system, therefore it may be supposed that the effect of *T. platyphyllos* extract on seizure induced by picrotoxin may be mediated through GABAergic system. In the other hand, *T. platyphyllos* extract may have some compounds that increase the release of GABA or potentiate the GABA system by other mechanisms.

There are some reports showing anxiolytic effects of some natural and synthetic flavonoids in rats and found that these compounds exerted their effects through the central benzodiazepine receptors (Salgueiro et al., 1997; Medina et al., 1997; Du et al., 2002). There are also some reports about existence of pharmacologically active benzodiazepine receptor ligands in Tilia species (Viola et al., 1994). Therefore, it seems that the anti seizure effect of *T. platyphyllos* may be related in part to flavonoid compounds or other ligands present in the extract that interact with benzodiazepine receptors that coupled to GABA-A receptors. Similar effect was observed in our previous experiments with Rosmarinus officinalis L. extract (Heidari et al., 2005).

Pentylentetrazole with dose of 80 mg/kg induced clonic seizure in animals (Mehrabani et al., 2007; Ojewole, 2008:

Heidari et al., 2009b). *T. platyphyllos* extract delayed the onset of seizure from pentylentetrazole and death time and decrease the death percentage. It is concluded that *T. platyphyllos* extract may be effective in control of clonic seizures. This extract increased the duration of seizure. Observation of animals during experiments indicates that the extract attenuated the severity of seizure, therefore it is maybe speculated that the animals have survived for longer period with milder seizure (Ojewole, 2008). This can be an explanation for increased duration of seizure induced with pentylentetrazole, with this extract. Seizure from Pentylentetrazole and picrotoxin induced from inhibition or attenuation of GABAergic neurotransmission (Heidari et al., 1996; Ojewole, 2008: Heidari et al., 2009a), therefore it is concluded that the anti-seizure effect of *T. platyphyllos* extract may probably mediated by potentiating the GABAergic neurotransmission. This article is the first report about the anti-seizure effect of *T. platyphyllos* in medical literatures. However there is a necessity to research more and find effective and safe anticonvulsant agents from infinitive sources of medicinal plants.

In conclusion, the findings reported in this article indicate that *T. platyphyllos* may contain bioactive components with anticonvulsant properties that are in favor with use of this plant in Iranian folk medicine. Determination of the role of each compounds in the anti-seizure effect of *T. platyphyllos* extract remains to be determined in other investigations. However completed pharmacological and toxicological investigations are needed for more supporting of using this plant as an official herbal drug in clinical applications.

Rreferences:

Aeinechi, Y. Medicinal plants. Tehran, Tehran University Publications; 1991.

Aguirre-Hern´andez E, Martinez AL, Gonzalez-Trujano ME, et al. Pharmacological evaluation of the anxiolytic and sedative effects of Tilia americana L. var. mexicana in mice. J Ethnopharma 2007;109:140–145.

Arteche A, Vanaclocha B. Fitoterapia, Vadem´ecum de Prescripci´on, 3a edici´on. Masson, Barcelona, 1998; pp: 434–437.

Avallone R, Zanoli P, Puia G, et al. Pharmacological profile of apigenin, a flavonoid isolated from Matricaria chamomilla. Biochem Pharmacol 2000; 59(11):1387-94.

Bremness, L. Herbs, Dorling Kindersly Book, Kyodo Printing Co. Singapore 1994; p: 88.

Brickman G. Herbal medicine, 1th ed; Integrative medicine communication, 2000; pp: 240-242.

Buchbauer G, Remberg B, Jirovetz L, et al. Comparative headspace analysis of living and fresh cut lime tree flowers (Tiliae flores). Flav Frag J 1995;10:221–224.

Carvey PM. Drug action in the central nervous system, New York: Oxford University Press, 1998; pp: 201.

Coleta M, Campos MG, Cotrim MD, et al. Comparative evaluation of Melissa officinalis L., Tilia europea L., Passiflora edulis Sims. and Hypericum perforatum L. in the elevated plus maze anxiety test. Pharmacopsychiatry 2001;34:520–521.

Du XM, Sun NY, Takizawa N, et al. Sedative and anticonvulsant activities of goodyerin, a flavonol glycoside from Goodyera schlechtendaliana. Phytother Res 2002;16:261-3.

Duke JA. Handbook of Medicinal herbs. 2th ed. CRC Press, 2002; pp: 467-468.

Fluke H. Medicinal plants, Tehran, Roozbahan publication, Edition 5, 2000; p:97.

Gower AJ, Noyer M, Varl CS, et al. Anti-convulsant drug: Pharmacological profile in animals, Eur J Pharmacol 1995;222(2-3):109-203.

Heidari MR, Asadipour A, Rashid-Farokhi P, et al. Effect of Rosmarinus officinalis L. Extract on the seizure induced by picrotoxin in mice. Pak J Biol Sci 2005;8(12):1807-1811.

Heidari MR, Dadollahi Z, Mehrabani M, et. al. Study of anti seizure effects of Matricaria recutita L. extract in mice. ANNALS of the New York Academy of Sciences, 2009a: 1171: 300–304.

Heidari MR, Heidari M, Eimani G and Sepehri Gh. Effect of methanlic extract of Hyoscyamus niger L. on seizure induced by picrotoxin in mice. Pakistan Journal of Pharmaceutical Sciences, 2009b: 22(3): 308-312.

Heidari MR, Khalili F, Ghazi Khansari M, et al. Effects of picrotoxin on antinocieption in the formalin test, Pharmacol Toxicol 1996;78(5):313-316.

Heidari MR, Mandegary A, Hosseini A, et al. Anticonvulsant effect of methanolic extract of Echium ameoneom Fisch and C.A Mey. against seizure induced by picrotoxin in mice. Pak J Biol Sci 2006;9(4):772-776.

Heidari MR, Moein Azad E, Mehrabani M. Evaluation of the analgesic effect of Echium amoenum Fisch & Mey. Extract in mice : possible mechanism involved. J Ethnoharmacol 2006b;103:345-349.

Heidari MR, Razban F. Effect of Valeriana officinalis extract on the seizure induced by picrotoxin in mice. J Kerman University of Medical Sciences 2004;11(2):100-108.

Herrera-Ruiz M, Román-Ramos R, Zamilpa A, et al. Flavonoids from Tilia americana with anxiolytic activity in plus-maze test. J Ethnopharmacol 2008;118(2):312-7.

Hopkins A, Shorvon S, Cascino G. Epilepsy, Chaman & Hall, 1995;38-42, 82.

Katzung BG. Basic & Clinical Pharmacology. 10th ed; United States: Appleten and Lange; 2007;374-394.

Lian XY, Zhang ZZ, Striger JL. Anticonvulsant activity of ginseng on seizures induced by chemical convulsant. Epilepsia 2005;46(1):15-22.

Martinez AL, Gonzalez-Trujano ME, Aguirre-Hernández E, et al. Antinociceptive activity of Tilia americana var. mexicana inflorescences and quercetin in the formalin test and in an arthritic pain model in rats. Neuropharmacol 2009;56(2):564-71.

Matsuda H, Ninomiya K, Shimoda H, et al. Hepatoprotective principles from the flowers of Tilia argentea (Linden): structure requirement of tiliroside and mechanism of action. Bioorg Med Chem 2002;10:707–712.

Meckenzie L, Medvedev A, Hiscock JJ, et al. Picrotoxin-induced generalized convulsion seizure in rats changes in regional distribution and frequency of the power of electroencephalogram rhythmus. Clin Neurophysiol 2002;113(4):586-596.

Medina JH, Viola H, Wolfman C, et al. Overview-Flavonoids a new family of benzodiazepine receptor ligands. Neurochem Res 1997;22(4):419-25.

Mehrabani M, Modirian E, Ebrahimabadi AR, et al. Study of the Effect of Hydro-methanol Extract of Lavandula vera DC. and Cuscuta epithymum Murr. on the Seizure Induced by Pentylentetrazol in Mice. Journal of Kerman University of Medical Sciences 2007;14(1):25-32.

Mozaffarian VA. Dictionary of Iranian plant names. Tehran, Farhang Moaser, 1996;548-49.

Ngo Bum E, Dawack DL, Schmutz M, et al. Anticonvulsant activity of Mimosa pudica decoction. Fitoterapia 2004;75(3-4):309-14.

Ojewole JA. Anticonvulsant effect of Rhus chirindensis (Baker F.) (Anacardiaceae) stem-bark aqueous extract in mice. J of Ethnopharmacol 2008;117(1):130-5.

Pérez-Ortegaa G, Guevara-Feferb P, Chávezd M, et al. Sedative and anxiolytic efficacy of Tilia americana var. mexicana inflorescences used traditionally by communities of State of Michoacan, Mexico. J Ethnopharmacol 2008;116(3):461-468.

Pietta P, Mauri P, Bruno A, et al. High-performance liquid chromatography and micellar electrokinetic chromatography of flavonol glycosides from Tilia. J Chrom 1993;638:357–361.

Rojhan MS. Drug and herbal treatment, Tehran, Tehran University publication, 1995; p: 86.

Salgueiro JB, Ardenghi P, Dias M, et al. Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. Pharmacol Biochem Behav 1997;58:887-891.

Samsam-shariat H. Herbal treatment advantages: principle of prescription. 6th ed. Tehran, Roozbahan publication, 1994; p: 134.

Shargel L, Mutnick AH., Souney PF, et al. Comprehensive Pharmacy Review. 4th ed; Lippincott Williams & Wilkins, 2001; pp: 822-844.
Silver DB. Healing Plants 2000; pp: 160-161.

Swinyard EA. Laboratory evaluation of antiepileptic drugs. Review of laboratory methods. Epilepsia 10, 1969;107 – 119.

Toker G, Aslan M, Yesilada E, et al. Comparative evaluation of the flavonoid content in officinal Tilia Flos and Turkish lime species for quality assessment. J Pharma Biomed Ana 2001;26(1):111-21.

Viola H, Wolfman C, Levi de Stein M, et al. Isolation of pharmacologically active benzodiazepine receptor ligands from Tilia tomentosa (Tiliaceae). J Ethnopharmacol 1994;44(1):47-53.

Volak Jan et Stodola J. Text de plantes medicinales, 4th ed. Editie Grund, Traduit Par Saed Zaman, 2000; pp: 322-323.

White HS. Clinical Significant of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia 1997;38(1):9-17.

Zargari A. Medicinal plants, Tehran, Tehran University Publications., 7th edition, Vol. 1. 1997; pp: 399-402.

Zeppa S, vallorani L, Potenza L, et al. Estimation of fungal biomass and transcript levels in Tilia plathyphyllos Tuberberchii ectomycorrhizae. FEMS Microbial Lett 2000;188(2):119-24.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals.pain 1983;6:109-110.