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Assessment of Neuropharmacological Activities of *Terminalia Chebula* in Rats

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Abstract: Problem statement: Oxidative stress has been implicated in the pathophysiology of many neurodegenerative diseases such as Alzheimer's Disease (AD). In traditional practices of Ayurvedic medicine, numerous herbs have been used to treat cognitive disorders. *Terminalia Chebula* (Combretaceae; TC), is a well-known Ayurvedic herbal remedy which possesses an antioxidant activity. The scientific literature strongly supports its *in vitro* exerted acetylcholinesterase inhibitory and has suggested developing this herb as a potential in the treatment of AD, However, the *in vivo* neurophamacological activities of this plant have never been studied. **Approach:** Adult male Sprague-Dawley rats were orally given the different doses of aqueous extracts of TC (10, 20 and 40 mg kg^{-1}) once daily at a period of 2 weeks. The series of established neuropharmacological tests including elevated plus maze, forced swimming, open field and water maze tests were assessed after single administration, 1 and 2 weeks of treatment. **Results:** TC at a dose of 40 mg kg^{-1} treated rats exhibited a significant cognitive enhancing effect at all treatment duration. Unfortunately, this substance failed to show dose dependant manner and the other effects. **Conclusion:** With the above data, it can then be primarily concluded that aqueous extracts of TC should be further investigated about possible active ingredients and developed in line of other anti-Alzheimer herbal drugs or herbal brain booster.

Key words: *Terminalia chebula*, neurodegenerative diseases, ayurvedic medicine, cognitive enhancing effect, neuropharmacological activities, antioxidant activity, acetylcholinesterase inhibitory activity

INTRODUCTION

Accumulating lines of evidence reported that oxidative stresses such as generation of damaging reactive oxygen species are speculated to be pathophysiologically important in Alzheimer's Disease (AD) and other neurodegenerative diseases (Cross *et al*., 1987). Furthermore, oxidative stress involves redox signaling to molecular mediators of inflammation pathways, which induce further neuronal cells damage (Ritz *et al*., 2008).

External herbal supplementation through antioxidants and anti-inflammations were recommended to protect neuronal cells from the deleterious effects of such oxidative stress conditions (Ishige *et al*., 2001; Phachonpai *et al*., 2012).

Among various herb extracts, the fruit of *Terminalia Chebula* Retz (*T. chebula*; TC), a member of the family Combretaceae, is a very popular traditional Ayurvedic medicine that has been used for treatment for various diseases in Asia (Park *et al*., 2011). The dried ripe fruit TC has widely been used in the treatment of asthma, sore throat, vomiting, hiccough, bleeding, piles, diarrhea, gout, heart and bladder diseases (Reddy *et al*., 2009). The scientific literature strongly supports its *in vitro* possessed antibacteria, anticarcinogenic and antimutagenic activities (Malekzadeh *et al*., 2001; Saleem *et al*., 2002; Khan *et al*., 2007). Also, it is a strong antioxidant, which might prove useful for treating neurodegenerative disorders by inhibiting the

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production of Reactive Oxygen Species (ROS), which has been implicated in the pathophysiogenesis of age-related diseases such as AD (Cheng *et al*., 2003). Moreover, recent findings reported that TC extract exerted acetylcholinesterase inhibitory and has suggested developing this herb as a potential in the treatment of AD (Sancheti *et al*., 2010).

In view of the complexity of herbal medicines and their inherent biological variations, it is necessary to evaluate their neuropharmacological activities (Castro *et al*., 2009). Thus, it is of interest to study the neuropharmacological effects of this medicinal plant. In all respect mentioning, we hypothesized that the aqueous extract of TC might also possess the neuropharmacological activities. Therefore, this study was carried out to determine the effect of the plant extract on anxiety, depression and cognitive function.

MATERIALS AND METHODS

Source of the plant material and preparation of the plant extract: The fruits of TC ripen from November to March and fall soon after ripening. The fully ripe fruits were collected from the ground as soon as they have fallen and shade dried. The herbarium was authenticated by Assistant Professor Dr. Nathida Weerapreeyakul, Faculty of Pharmaceutical Science, Khon Kaen University, Thailand.

The dried fruit skins were hammered in to small pieces and heated in 800 mL distilled water for 24 h in water bath at 40°C. This process was repeated twice. The final yield of the aqueous extract used for this study was 47.6%. The dried extract was stored at 4°C prior to use.

Animals: Healthy male Sprague-Dawley rats weighing 180-220 gm and aging 8 weeks were obtained from National Laboratory Animal Center, Salaya, Nakorn Pathom. They were housed in group of 5 per cage in standard metal cages at 2 ± 22°C on 12:12 h light-dark cycle. All animals were given access to food and water ad libitum. Experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC) and approved by the Ethical Committee of the Khon Kaen University.

Drugs: Diazepam (2 mg tablet^{-1}), fluoxetine (20 mg tablet^{-1}) and donepezil (10 mg tablet^{-1}) (Government Pharmaceutical Organization) were used as standard drugs in this study. All drugs and TC extract were dissolved in saline solution which used as vehicle to a desired concentration. Then, they were filtered through gauze and given to the animals via the intragastric feeding tube. All administered substances including the TC suspension were freshly prepared.

Experimental protocol: All rats were randomly assigned to 6 groups (n = 8 in each group). Group I: Naïve intact control rats. Group II: Vehicle treated group. Group III: Positive control treated group. In each test, the positive control group was treated with the standard drugs used for treating the related disorders. In order to determine anxiolytic effect, the animals were treated with diazepam (2 mg kg^{-1}). In addition, fluoxetine (20 mg kg^{-1}), was the positive control of anti-depressant effect while during the determination of cognitive function, the positive control group was treated with donepezil (1 mg kg^{-1}). Group IV-VI: TC treated group, the animals were treated with different doses of the aqueous extracts of TC (10, 20 and 40 mg kg^{-1}) via oral route for 2 weeks once daily throughout the experimental period.

The animals in all groups were assessed all behavioral tasks except that in the assessment of spontaneous locomotor behaviors, there was no positive control treated group.

Behaviors evaluation: The rats were divided into various groups as mentioned earlier. The behavioral profiles were assessed both after the single dose and after the repetitive administrations of TC extract (1 and 2 weeks). Each animal was subjected to the following behavior task forces: (a) Spontaneous locomotor activities (b) Elevated plus maze test (c) Forced swimming test and (d) Morris water maze test.

Spontaneous locomotors activities: In order to assure that anxiolytic, anti-depression like behavior and cognitive enhancing effect which determined by various tests just mentioned earlier were not false positive due to the effect of TC on motor behaviors, we also determined the effect of TC on the spontaneous locomotor activities by open field test (Hawiset *et al*., 2011). The open field apparatus was an arena 80 cm in diameter with a white, opaque wall 30 cm high. Rats were individually placed in the center of the arena and locomotor activities including the number of grooming, licking and rearing were scored within 5 min.

Elevated plus maze test: The elevated plus maze for rat consisted of open arms (50×10 cm) and two enclosed arms (50×10 cm) with 40 cm high walls, extending from a central platform (10×10 cm). The arms were connected with a central square, 10×10 cm, to give the apparatus a plus sign appearance. The maze was raised to a height of 50 cm above floor. The maze

floor and walls were constructed from dark opaque wood. Each rat was placed on the center of the platform facing an enclosed arm. Animals were tested individually and only once for 5 min according to the following parameters: Number of entries in the open and closed arms and time of performance in each of them (Wattanathorn *et al*., 2007). The time of performance measures the time spent by the animal in the opened and closed arms. The maze was cleaned following each trial to remove any residue or odors. Each rat was assessed individually 30 min after the treatment.

Forced swimming test: In order to assess the anti-depression like behavior of the plant extract, the modified (Tong-Un *et al*., 2010) test was conducted. The apparatus used in this study is the cylinder glass aquarium (22 cm diameter X 40 cm high) filled to the depth of 20 cm with fresh water at 25°C. After 30 min of drug administration, each animal was placed individually into the cylinder for 5 min-test and observed for immobility (by keeping the head of the animals above water in the way that animal made no further attempts to escape and neither hind leg was moving; the rats were slightly hunched forward) by blind observer who has been trained for the observation. Upon removal from the water, rats were towel-dried and finally returned to their home cage.

Morris water maze test: Learning and memory of animals were tested in Morris water maze (Morris, 1984; Phachonpai *et al*., 2012). It consisted of a circular water tank (170 cm diameter X 60 cm height) that was partially filled with water (25±2°C, 40 cm deep). A non-toxic paint divided virtually into four equal quadrants, labeled North-South-East-West by two imaginary lines crossing the center of the pool. An escape platform (10 cm in diameter) was hidden 2 cm below the surface of the water on a fixed location in one of the four quadrants of the pool. The platform remained in the same quadrant during the entire experiment.

Before the training started, rats were allowed to swim freely into the pool for 60 s without platform. They were given four trials (once from each starting position) per session for 5 days, each trial having a ceiling time of 60 s and a trial interval of approximately 30 s. After climbing on to the platform, the animal remained there for 30 s before the commencement of the next trial. If rat failed to reach the escape platform within the maximum allowed time of 60 s, it was gently placed on the platform and allowed to remain there for the same interval of time. The time to reach the hidden platform was recorded as escape latency. In addition to the acquisition test, the determination of retention memory was carried out on the next day. According to this test, the platform was removed and the rats were placed into the water maze for 60 s. The time spent in the target quadrant, which had previously contained the hidden platform was recorded. The time spent in the target quadrant indicated the degree of memory consolidation taken place after learning. Any enhancement of cognition would be reflected by a decrease in escape latency and increase in retention time.

Statistical analysis: Data were presented as mean ± Standard Error of Mean (SEM). One-way Analysis Of Variance (ANOVA), followed by Duncan's test. A probability level less than 0.05 were accepted as significance.

RESULTS

Effect of TC on cognitive enhancing effect: In order to determine the cognitive enhancing effect of TC using Morris water maze test, a valid test which is sensitive to the spatial learning and memory abilities or hippocampus-dependent memory (Mehrdad *et al*., 2008). The present results showed no significant changes in escape latency and retention times in control and vehicle treated group. However, rats which received either donepezil (a cholinesterase inhibitor) or the aqueous extract of TC at a dose of 40 mg kg^{-1} significantly decreased the escape latency while increased the retention time ($p<0.05$ all; compared with control and vehicle treated groups) as shown in Fig. 1 and 2 respectively. This indicates that the aqueous extract of TC could produce cognitive enhancing effect.

Effect of TC on anxiolytic effect: To determine the effect of TC via oral administration on anxiolytic activity, the results depicted in Fig. 3 and 4 showed that oral administration of saline or vehicle produced no significant changes in both the number of opened arms entries and time spent in the opened arms after single and repetitive doses. Diazepam, a standard drug used for the treatment of anxiety, which used as positive control in this study significantly increased the number of opened arms entries and time spent in the opened arms at all duration of treatment (p-value<0.05 all; compared to that of control and vehicle treated groups). On the other hand, all dosage ranges of TC extract did not produce the significant changes on this parameter.

Effect of TC on anti-depression like behavior: The effect of TC on anti-depression activity was determined using the forced swimming test, a valid tool using for the screening the effect of substances possessing anti-depressant activity and the neurobilogical mechanism related to depression.

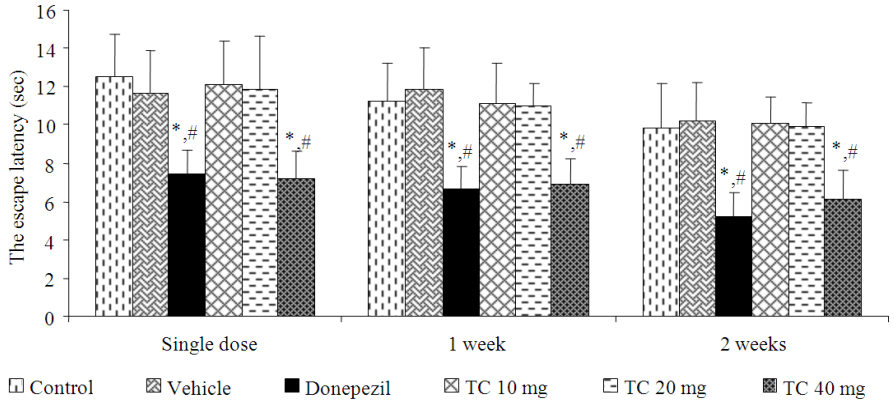

Fig. 1: Effect of donepezil and TC (10, 20 and 40 mg kg^{-1}) on the escape latency in Morris water maze test. Values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to naïve control group, # p<0.05 as compared to vehicle treated group

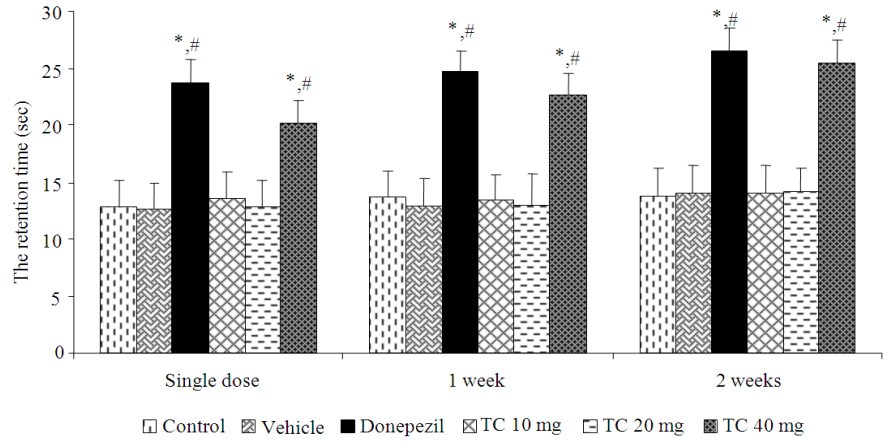

Fig. 2: Effect of donepezil and TC (10, 20 and 40 mg kg^{-1}) on the retention time in Morris water maze test. Values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to naïve control group, # p<0.05 as compared to vehicle treated group

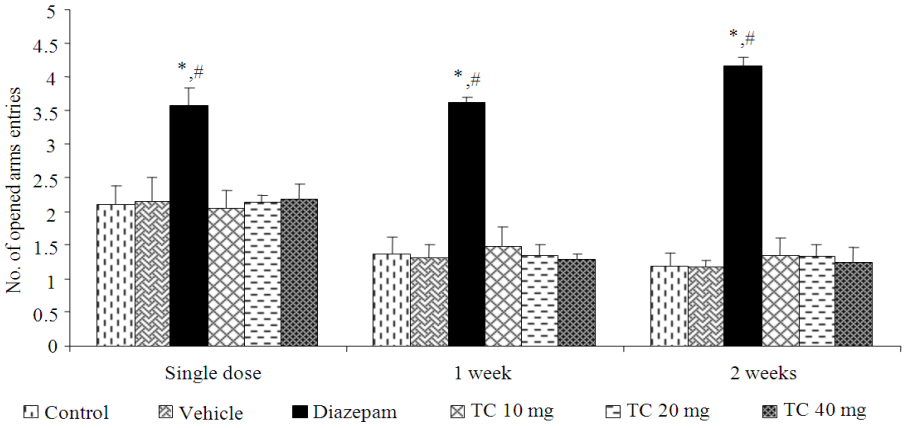

Fig. 3: Effect of diazepam and TC (10, 20 and 40 mg kg^{-1}) on the opened arms entries in Elevated plus maze test. Values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to naïve control group, # p<0.05 as compared to vehicle treated group

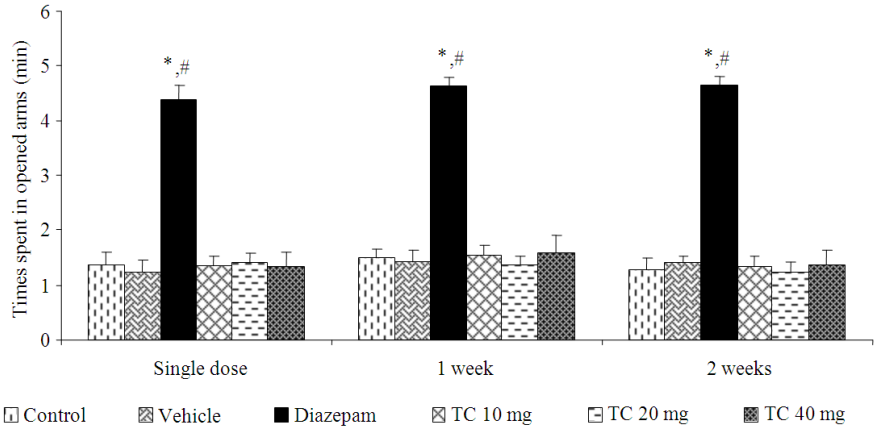

Fig. 4: Effect of diazepam and TC (10, 20 and 40 mg kg^{-1}) on the time spent in opened arms in Elevated plus maze test. Values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to naïve control group, # p<0.05 as compared to vehicle treated group

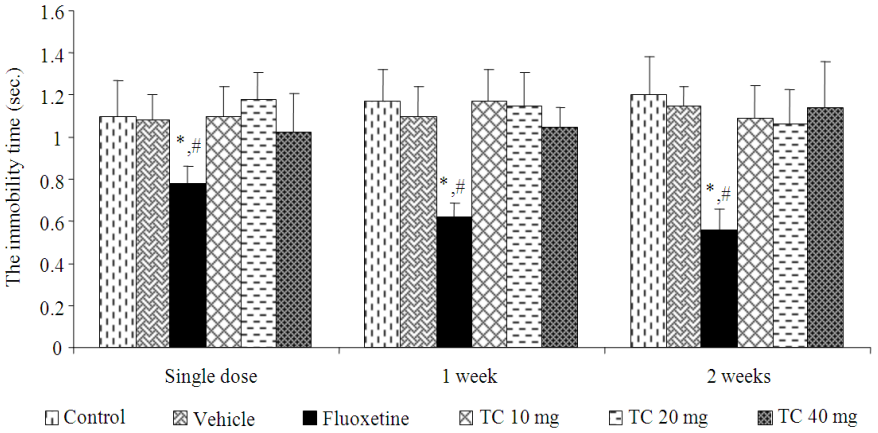

Fig. 5: Effect of fluoxetine and TC (10, 20 and 40 mg kg^{-1}) on the immobility time in Force swimming test. Values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to naïve control group, # p<0.05 as compared to vehicle treated group

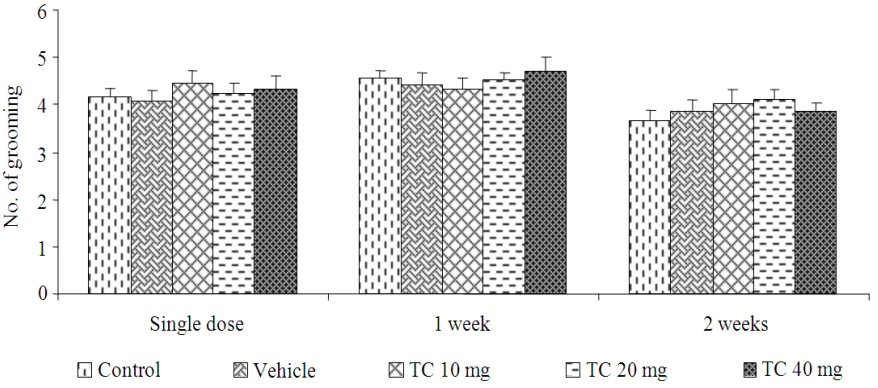

Fig. 6: Effect of TC (10, 20 and 40 mg kg^{-1}) on grooming behavior. Values given are the mean ± S.E.M. (n = 8)

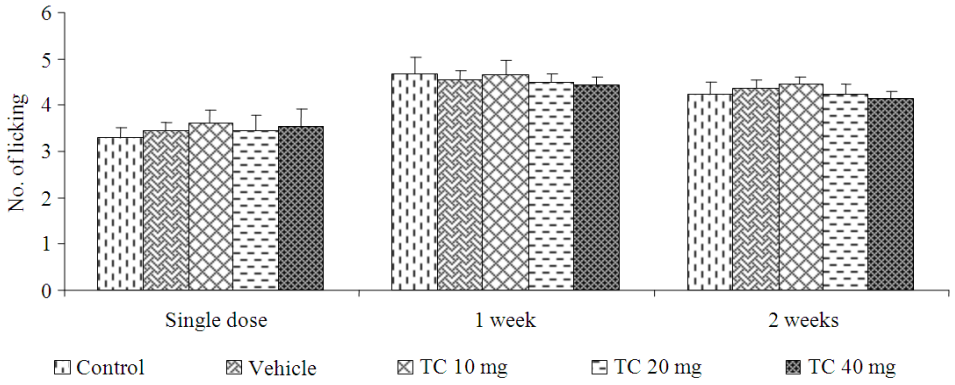

Fig. 7: Effect of TC (10, 20 and 40 mg kg^{-1}) on licking behavior. Values given are the mean ± S.E.M. (n = 8)

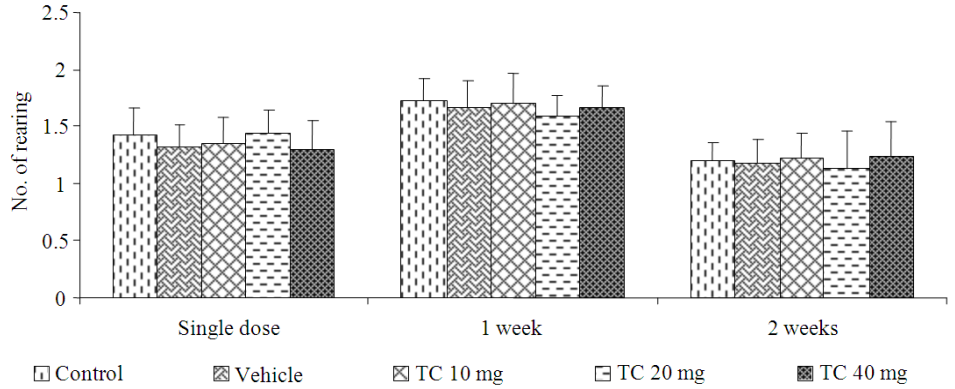

Fig. 8: Effect of TC (10, 20 and 40 mg kg^{-1}) on rearing behavior. Values given are the mean ± S.E.M. (n=8)

Our results demonstrated that all rats subjected to vehicle and TC supplementation did not produce significant changes in the immobility times in forced swimming test throughout the observation time while the rats treated with fluoxetine, a standard drug used for the treatment of depression, which used as the positive control in this study significantly decreased the immobility times at all treatment duration ($p<0.05$ all; compared with control and vehicle treated groups) as shown in Fig. 5.

Effect of TC on spontaneous locomotor activities: In order to assure that the anxiolytic, anti-depression like behaviors and cognitive enhancing effect which determined by various tests just mentioned earlier were not false positive induced by the effect of TC on motor behaviors, we also determined the effect of TC on the spontaneous locomotor activities including grooming, licking and rearing. Our results showed that the spontaneous behaviors as mention earlier did not differ significantly between the control, vehicle and TC treated groups throughout the experimental period as shown in Fig. 6-8 respectively.

DISCUSSION

Recently, the interest in the use of herbal products has grown dramatically in the western world as well as in developed countries. Although *Terminalia Chebula* (TC) extract is extensively used in Ayurvedic herbal medicine and Asia, it lacks scientific grounds for its neuropharmacological activities and to the best of our knowledge this is the first study to report its possible effects *in vivo* on the CNS. Herein, we demonstrated that the TC extract significantly exhibited the cognitive enhancing effect in normal healthy male rats.

Learning has been defined as the process acquiring the knowledge while memory is the retention of the acquired knowledge that can be retrieved (Kandel *et al*., 2000). Our results showed that TC extract at a dose of 40 mg kg^{-1} could exert its influence on both escape latency and retention time. Therefore, it indicated that this plant extract may affect on learning and memory process including acquisition, consolidation and retrieval.

The importance of central cholinergic system in cognitive function is well established (Bartus *et al*., 1982; Blokland, 1995). Thus, many therapeutic strategies to combat miseries of cognitive disorder have been aimed to improve Acetylcholine (ACh) activity.

Among the various approaches attempted to increase cholinergic activity, the inhibition of Acetylcholiesterase (AChE) is the most successful one (Giacobini, 1996). Cholinesterase Inhibitors (ChEI) are the only class of compounds consistently proven to be efficacious in treating the cognitive and functional symptoms in patients with neurodegenerative disorders such as AD, Parkinson's disease, senile dementia, ataxia and myasthenia gravis (Sancheti *et al*., 2010). In addition, new findings show that both AChE and Butyrylcholinesterase (BChE) are involved in the breakdown of acetylcholine in the brain and, thus, dual inhibition of these enzymes may prove efficient in treating dementia (Giacobini, 2004; Lane, 2006). Previous study had reported that the important active principle constituents of TC are chebulagic acid, corilagin, beta-sitosterol, gallic acid, terchebulin, caffeic acids, 1,6-di-O-galloyl-β-D-glucose, punicalagin, 3,4,6-tri-O-galloyl-β-D-glucose, casuarinin, chebulanin, neochebulinic acid, terchebulin and ellagic acid might be responsible for its medicinal properties (Juang *et al*., 2004). Moreover, recent findings showed that the dried fruits of TC led to the isolation of 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose act as both AChE and BChE agents (Sancheti *et al*., 2010).

Since the pattern of changes of escape latency and retention time induced by TC extract was similar to those of donepezil, is a golden standard medicine for curing the AD, we suggested that the herb extract might exert cognitive enhancing effect occur via its action of 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose constituents or its antioxidant effect. However, its effect to inhibit inflammatory activity still could not be omitted. It was previously reported that gallic acid in TC to possessed anti-inflammatory (Ximenes *et al*., 2010). Unfortunately, this study did not investigate about the possible active ingredients and the precise underlying mechanisms of this extract could attenuate memory impairment; this is planned in future studies.

Notably, low and medium dose TC extract do not affect cognitive function in health male rats. One possible explanation for this phenomenon might be related to the insufficient concentrations of active ingredients of TC to reach the therapeutic level.

Taken all data together, oxidative stress has been implicated in the cognitive impairment and may be responsible for the development of neurodegenerative disease including AD. So antioxidants having AChE properties may have beneficial effects in AD. Treatment with TC exhibits a beneficial cognitive enhancing effect and thus provides a rationale for the use of TC in Ayurvedic herbal medicinal treatment.

CONCLUSION

With the above data, it can then be primarily concluded that aqueous extracts of TC should be further investigated about possible active ingredients and developed in line of other anti-Alzheimer herbal drugs or herbal brain booster. However, further researches about possible active ingredients and pharmacokinetic of the extract are still required before moving forward to clinical trial study.

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