ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

The effects of Ethanolic Extract in Dried Fruits of *Terminalia chebula* on learning and memory in mice

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Received:
20th May 2013
Received in revised form:
25th May 2013
Accepted:
31st May 2013
Available online:
10th June 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT:

In the traditional medicinal practices, several formulations containing *Terminalia chebula* are used for cognitive improvement. Alzheimer's disease (AD) is the most common type of dementia disorder of elderly affecting millions of people. The pathophysiology of the disease is complex and involves multiple pathways of neuronal damage. In the present study Nootropic acitivity of Ethanolic Extract Of *Terminalia chebula* was studied in mice. In order to evaluate the beneficial effect of *Terminalia chebula* on learning and memory, an experimental study was conducted in normal male mice. In this study we used a Shuttle Box device to evaluate the active avoidance learning and memory in mice. Different doses of ethanolic extract of *Terminalia chebula* (100, 200 and 400 mg/kg) were administered to animals in the test groups. The learning ability and memory recalls were assessed and compared statistically with those of control animals. Our data showed that during the learning procedure the mean numbers of free shock trials in the test groups were increased compared with the control group but the difference was significant only in the case of the 100 mg/kg *Terminalia chebula* extract. In the short- and long-term memory assessments the animals in the test groups received less shocks than the control group and the differences were significant in the case of the 100 and 200 mg/kg *Terminalia chebula* extract. Our findings indicate that acute administrations of ethanolic extracts of *Terminalia chebula* enhance the learning and memory recall ability in mice in an inverse dose-dependent manner.

Keywords: *Terminalia chebula*; learning; memory; mice.

1. INTRODUCTION

Terminalia chebula is a miracle herb of the nature and has many therapeutic properties from head to toe. *Terminalia chebula Retz*. (Fam. Combretaceae)[1], is called the "king of medicines" in Tibet and is always listed first in the Ayurvedic meteria medica because of its extraordinary powers of healing. In Ayurveda it is considered to destroy all diseases and eliminate all waste from the body. At the same time, it is known to promote tissue growth and health. But no systematic updated information on the therapeutic effectiveness of *Terminalia chebula*, a popular herbal remedy in India and South-East Asia a wide list of synonyms or meanings like, cures all diseases, eliminates fear of death, suitable for all age groups, keeps body strong, and helps stop aging process. All the synonyms denote the importance of *Terminalia chebula*. Ancient wisdom described Chebulic in various health disorders and was administered in different forms as it has a unique property of modifying therapeutic action based on the root cause of a problem. Charaka an eminent ancient physician mentioned that Haritaki is good for all body tissues as it cleanses the impurities of body tissues and it is supposed to be as good as mother's milk. In 14th century, another eminent scholar described seven varieties of *Terminalia chebula* in his book called BhavaPrakasa. It has been said in ancient books that even the smell of Chebulic will have a laxative effect and helps with constipation relief via cleansing the body and in the process it helps with weight loss. Therapeutic effects of *Terminalia chebula* are as antibacterial laxative for constipation, for ano-rectal

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disorders, for carminative tissue building skin disorders, for urinary disorders, anemia, hepato-biliary disorders, eye disorders, aging disorders, uterine disorders, soft tissue disorder, neurological disorders, anxiety[2] etc. In view of the complexity of herbal medicines and their inherent biological variations, it is necessary to evaluate their neuropharmacological activities[3]

Nootropics, a new generation of smart drugs, have been proved to increase the cognitive power of the human brain. Like steroids, nootropics have a potential for abuse. Piracetam have become widely used among workers and students as a means of increasing focus, mood and working memory (Sandberg). The field of nootropics is relatively young, and knowledge of the human brain is currently growing exponentially. First created to combat the degenerative effects of Alzheimer's disease, nootropics have existed since 1972. Dr. Corneliu E. Giurgea, a Romanian scientist did much of the early research in the field (Wilsher). He coined the word "nootropic" from the Greek nous for "mind" and trepein for "to turn." Dr. Giurgea also synthesized the first nootropic, Piracetam, to help his elderly patients maintain cognitive functions like learning, working memory, and concentration (Wilsher) (Hall). In traditional practices of Ayurvedic medicine, numerous herbs have been used to treat cognitive disorders. However, the in vivo neurophamacological activities of this plant have never been studied.

2. MATERIALS AND METHODS

2.1. Preparation of extract

Dried Fruits of *Terminalia chebula* were purchased from a local market (Hyderabad, Andra pradesh) and identified by botanists of Herbal Medicine Research Center Hyderabad. The plant extract was prepared by maceration of 50 g of the chopped, dried fruits in a mixture of 200 ml ethanol and 200 ml distilled water by shaking them for 48 h and pressing the solution out of the material using a filter press. The extraction solvent was then removed under reduced pressure until the extract was obtained as a dried gum. The extract was then diluted with normal saline to provide a 400 mg/ml stock solution.

2.2. Animals

In this study 28 male Balb/c strain mice aged 60-70 days and weighing 25-30 g were raised in the institutional animal house of the Department. These animals were maintained under controlled temperature (23 ± 2°C) and a 12:12-h light-dark cycle (lights on at 7:00 a.m.). Chow and tap water were provided ad libitum prior to starting the investigation. Animals were randomly divided into 4 groups (7 animals in each group). The animals in the control group received normal saline while the animals in test groups were given 100, 200 or 400 mg/kg ethanolic extract of *Terminalia chebula* diluted in 1 ml of normal saline. All injections were administered intraperitoneally (IP) before each test. Animals in both control and test groups were exposed to three stages of cognitive behavioral tests (learning, short-term memory and long-term memory). For behavioral testing, permission was obtained from the animal ethics committee.

2.3. Apparatus[4-5]

In this study we used a Shuttle Box to evaluate the active avoidance learning and memory

Briefly, the shuttle box designed for the active avoidance test consists of two compartments, each measuring 22 × 21 × 22 cm, connected by a sliding door. The walls of the safe compartment were white, whereas the other compartment, where the animals received foot shock, had black walls. The top of the safe compartment was covered with a transparent acrylic sheet while the top of the other compartment had an opaque pattern. The floor consisted of a metal grid (0.4 cm-diameter) connected to a shock generator and a control module (Ugo Basile model 7551), via which foot shocks of 1 mA and 1 second duration could be delivered.

2.4. Shuttle Box Test

To investigate the effect of *Terminalia chebula* extract on learning and memory in mice, 20 minutes after intra-peritoneal injection of normal saline or *Terminalia chebula* extracts the active avoidance test was performed on each animal, basically as described by Datta S. The mice were placed in a shuttle box divided into two compartments. Two initial conditioned stimuli (CS), a sound beep (3600 Hz; 45 dB) and a light flash (adjustable intensity, 6 W at 220 V AC), were applied simultaneously, followed after 10 sec by an unconditioned stimulus (US), an electric footshock (1 mA), whenever animals avoided leaving the dark compartment during this period. Foot shocks were delivered until the animal escaped to the safe compartment. Animals in both control and case groups performed this test in three different stages i.e. learning ,short-term memory and long-term memory, respectively, each for three successive days, and three times each day at an interval of 30 minutes and 10 successive trails each time. The data were recorded as the number of successful (shock free) trials in which the animal did not receive a foot shock in each seriesof 10 trials. The mean±SEM of these results were calculated for both control and case groups in each stage. The mice with auditory and visual abilities can learn to associate sound and light, respectively, with the unconditional stimulus, and cross into the other compartment within 10 sec of the conditional stimulus without receiving a foot-shock. The test was performed for 3 successive days in the first week of the testing period to evaluate the learning ability. Then, after all the animals had been trained to reach at least 70 % of their full learning capacity (i.e. for each 10 trials at

least in 7 trials the animal would leave the dark compartment without receiving an electrical shock), they were kept in solitary confinement for another week. The test to evaluate short-term memory was then performed for 3 successive days and again after 3 weeks, and the same experimental procedures were performed for long-term memory.

2.5. Statistical analysis

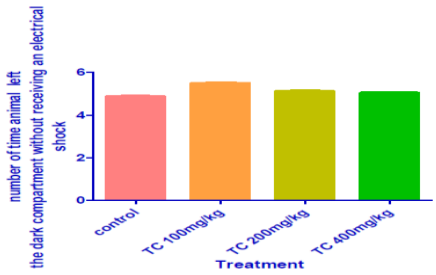
The data are presented as means±SEM of the number of successful (shock free) trials. The results were analyzed statistically using one way ANOVA followed by Tukey's post test to estimate differences among means. A value of $P<0.05$ has chosen as the level of statistical significance.

3. RESULTS

Our findings involving the mean±SEM of the number of trials on three successive days in which the animals in the test and control groups successfully left the dark compartment are summarized in Tables 1 to 3 for learning, short-term memory and long-term memory, respectively. These data showed that in the learning test there was an increase in the total number of trials in which the animals in the test groups were able to leave the dark compartment "without receiving a foot shock", compared with those in the control group, but only in the case of the 100 mg/kg *Terminalia chebula* extract was the difference significant. In the case of short-term memory there was a significant increase in the total number of successful (shock free) trials in response to 100 and 200 mg/kg *Terminalia chebula* extract, compared with the control group. Pre-treating the animals with 100 and 200 mg/kg *Terminalia chebula* extract in the long term memory also led to a significant increase in shock-free trials. However, 400 mg/kg *Terminalia chebula* extract could not significantly increase the shock- free trials neither for short- nor long-term memory.

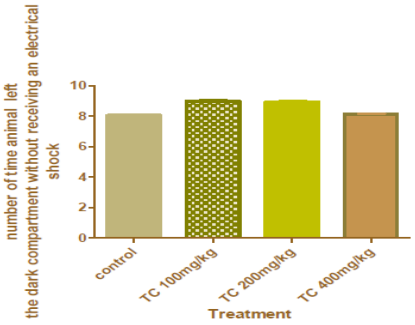
Sl no	groups	Number of successful trail
01	control	4.8±0.0166
02	*Terminalia chebula* extract (100 mg/kg)	5.5±0.0365
03	*Terminalia chebula* extract (200 mg/kg)	5.1±0.0210
04	*Terminalia chebula* extract (400 mg/kg)	5.0±0.0223

Table 1. comparison between the number of successful trials in control (normal saline) and case (100, 200 and 400 mg/kg *Terminalia chebula* extract) groups, during 3 successive days of the learning test (*n*=6).



Sl no	groups	Number of successful trail
01	control	8.6±0.0333
02	*Terminalia chebula* extract (100 mg/kg)	8.9±0.0872
03	*Terminalia chebula* extract (200 mg/kg)	8.8±0.0557
04	*Terminalia chebula* extract (400 mg/kg)	8.1±0.0223

Table 2. comparison between the number of successful trials in control (normal saline) and case (100, 200 and 400 mg/kg *Terminalia chebula* extract) groups, during 3 successive days of the short- term memory test (*n*=6).



Sl no	groups	Number of successful trail
01	control	8.6±0.0333
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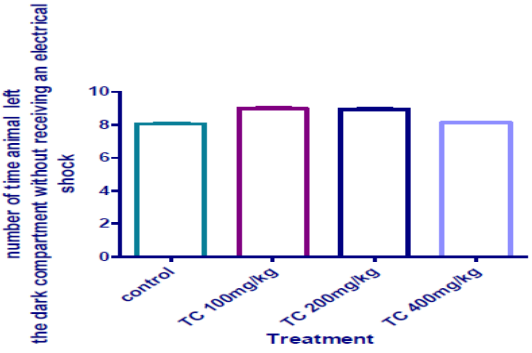
04	*Terminalia chebula* extract (400 mg/kg)	8.1±0.0223

Table 3. comparison between the number of successful trials in control (normal saline) and case (100, 200 and 400 mg/kg *Terminalia chebula* extract) groups, during 3 successive days of the longterm memory test (*n*=6).



Among the various approaches attempted to increase cholinergic activity, the inhibition of Acetylcholiesterase (AChE) is the most successful one[8]. 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[9]. 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[10]. 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[11]. 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[12].

Since the pattern of changes of learning & memory 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. 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. 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.

5. CONCLUSION

With the above data, it can then be primarily concluded that ethanolic 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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Conflict of Interest: None Declared

Cite this article as:
Nageswara rao S, Palaksha MN, Satish S, Ravishankar. The effects of Ethanolic Extract in Dried Fruits of *Terminalia chebula* on learning and memory in mice. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (20), 59-62.

