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Synergistic Nootropic Activity of Ethanolic Leaf Extracts of *Ziziphus Jujuba* and *Tinospora Cordifolia* against Stress Induced Rats

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ABSTRACT

Aim: The present investigation deals with the preliminary phytochemical analysis, synergistic nootropic activity of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* against stress induced rats.

Methods: Nootropic activity was observed in Albino Wistar rats of either sex using behavior paradigms such as elevated plus maze, staircase and morris water maze.

Results: In elevated plus maze and morris water maze, the rats were treated with extracts at doses (100 and 200 mg/kg-po) for 9 days and were observed on 9th day of treatment. Piracetam (100 mg/kg) was used as standard drug for comparison. In the elevated plus maze, the combination of leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* at 200mg/kg significantly decreased the transfer latency when compared to 100 mg/kg. In the staircase, the combination of leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* at 200mg/kg significantly decreased the number of climbings and the number of rearings when compared to 100 mg/kg. In the morris water maze, the combination of leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* at 200mg/kg significantly decreased the escape latency when compared to 100 mg/kg and control.

Conclusion: The combination of these two medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia* possessed significant synergistic nootropic activity and further research attempts can be made so as to isolate the novel moiety molecules which can be elucidated and can be evaluated for their therapeutic potential.

Introduction

Creating efficient and secure therapeutic treatment plans for cognitive diseases including forgetfulness, attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease (AD) continues to be difficult in the medical sector [1]. Well-planned, representative epidemiological surveys published report estimate that over 55 million people worldwide living with dementia in 2020 and by the year 2030, there will be 63 million people. Hence diagnosis and treatment of these conditions is a big challenge in front of medical fraternity.

Modern system of medicine provides medicaments to treat cognitive deficits still limitations are seen in these treatment modalities, thus it is worthwhile to search for different medicines which helps to maintain memory loss of patients with neuropsychiatric disorders or improve memory functions [2]. The word "nootropic" which may be shortened to "smart drug," "brain booster," or "memory enhancing drug" is frequently used to refer to the substance that improves mental ability. Nootropics are substances that improve mental abilities including memory, motivation, focus and attention, according to their description [3]. There are two types of nootropics: they are natural and herbal nootropics like *Ginkgo biloba* and *Panax quinquefolius* (*American Ginseng*) and synthetic nootropics made in a lab, like Piracetam. Natural nootropics have been shown to improve brain health while also enhancing cognitive performance. Nootropics have a vasodilator effect on the brain's tiny arteries and veins [4]. When natural nootropics are introduced into the system, the blood flow to the brain will be increased [5]. The brain receives 15% of the body's entire blood flow and oxygen while only making up 3% of total body weight. In reality, the brain can only produce energy by burning glucose, demonstrating how much neuronal function depends on a steady stream of oxygen and nutrients [6].

Ziziphus jujuba belonging to the family Rhamnaceae and is a well- known cognitive enhancer in the Indian system of traditional medicine [7]. It is also referred to as Indian jujube and commonly called "Regu" in Telugu. *Ziziphus jujuba* was traditionally used as a brain (nerve) tonic to enhance memory, learning and concentration. It has been scientifically proven to possess anti-inflammatory, anti-diabetic,

anti-cancer, liver protection, anti-oxidant properties, anti-allergic, anti-aging, anti-fatigue and coagulant properties [8-10]. *Tinospora cordifolia* commonly named as "Guduchi" or "Giloy" in Sanskrit belonging to family Menispermaceae [11].

Certain medications, known as *rasayana*, are advised by Ayurveda. They are claimed to have memory-enhancing and anti-aging properties. *Phyllanthusemblica* (*Pe*, Amala), *Tinospora cordifolia* (*Tc*, Gulavela) and *Ocimum sanctum* (*Os*,Tulsi) are the examples of *rasayana* drugs [12]. It has been scientifically proven to possess anti-diabetic, anti-arthritic, anti-osteoporotic effects, anti-HIV effects, anti-cancer effects, anti-microbial, anti-oxidant, anti-toxic effects and wound healing properties [13-18].

Materials and Methods

Plant material collection and preparation of extractions:

Leaves of *Ziziphus jujuba* and *Tinospora cordifolia* were procured from the surrounding areas of Yeleswaram, Kakinada district, Andhra Pradesh, India and were validated at Maharani College, Peddapuram. The recently harvested leaves of *Ziziphus jujuba* and *Tinospora cordifolia* were cleared of dirt and then the leaves were dried under shade for 60 days and then coarsely powdered in a mechanical grinder separately.

The fine powdered materials were weighed and macerated with the required quantity of ethanol for 3 days, followed by hot percolation for around 180 minutes. The extracts were then concentrated through distillation. The products obtained were dried and stored in desiccator for one week. After one week the phytochemical tests have been started [19].

Animals:

Wistar albino rats were procured from the animal house of Aditya College of Pharmacy, Surampalem. Wistar rats weighing around 150 gm to 200 gm were used for the study. Housing of animals was maintained with provision of 12 hours, day and night cycles.

Ethics approval:

All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration number 1269/PO/E/S/08/CPCSEA.

Experimental Protocol:

Wistar albino rats were sub-grouped into two categories based on stress. They are Normal rats and Stress induced rats. The Normal rats were randomized into 8 groups, each with 4 animals. The animals in Group 1 were treated with vehicle and served as control. Animals in Group 2 were treated with 100 mg/kg Piracetam p.o. serves as standard, Group 3 were treated with 100 mg/kg *Ziziphus jujuba* extract, Group 4 were treated with 200 mg/kg *Ziziphus jujuba extract*, Group 5 were treated with 100 mg/kg *Tinospora cordifolia* extract, Group 6 were treated with 200 mg/kg *Tinospora cordifolia* extract, Group 7 were treated with mixture of 100 mg/kg of *Ziziphus jujuba* and *Tinospora cordifolia* extracts, Group 8 were treated with mixture of 200 mg/kg of *Ziziphus jujuba* and *Tinospora cordifolia* extracts respectively. The stress induced rats were also randomized into 8 groups, each with 4 animals same as normal rats, but for these groups stress was induced.

Stress procedure:

With the aid of a basin that was roughly 45 cm in diameter and 60 cm height, modest chronic stress was inflicted onto the rats. The basin was kept at a temperature of roughly 22˚C to 28˚C and filled with water to a depth of about 40 cm. A rostrum or raised platform is kept in the center of the basin during the 5 to 7 days stress induction period. The rats were placed on top of the rostrum and submerged in water 2 cm deep for the whole of the day without access to food or water. On alternate days for around 7 days, the rats were restricted to food for 3 hours and water for 2 hour [20].

Drug administration:

The standard drug Piracetam p.o (100 mg/kg) and extracts were administered to animals as per standard protocol. In Morris water maze and elevated plus maze tests all animals were treated for 9 days. For Staircase test all animals were treated for 7 days.

Staircase:

Animals of different groups received their assigned drug treatments (as mentioned previously) for 7 days. On Day 1, one hour after the assigned drug treatments, all animals were tested on the staircase and the total number of climbings and rearings were recorded. The rats were placed on the staircase floor with its back to the box on the first day, and a timer is

provided and set for 180 seconds. During this time, the total number of steps climbings and rearings were recorded. After one hour of drug administration on Day 2, the total number of steps climbed and rearings were once again counted. Each specific group of rats was splitted into two sub-groups as stress and normal groups on Day 2 after learning. Each stress sub group was subjected to stress from Day 2 to Day 7 for 4 days without training. On Day 7, the total number of climbings and rearings were checked again for stress and normal groups [21].

Elevated plus maze:

Animals of different groups received their assigned drug treatments (as mentioned previously) for 9 days. On Day 1, one hour after the assigned drug treatments, all animals were tested on the elevated plus maze (EPM) for transfer latency (TL) and recorded. After measuring the transfer latency, the rats were given five minutes to explore the maze. The animals were each positioned at the end of an open arm that faced away from the maze's centre and the length of time it took for them to enter the closed arm was recorded and termed the TL. On Day 1, all animals were allowed to explore the EPM for 300 seconds. The animals remaining in the open arm without entering into the closed arm within 300 seconds were gently pushed into one of the enclosed arms and TL was recorded as 300 seconds (first trial). These animals were once again allowed to explore the maze for another 60 seconds and returned to their home cages after completion of the first trial. Similarly, retention of memory was assessed as TL on Day 2 (24 hours after the first trial) and Day 9.

Each specific group of rats was splitted into two sub-groups as stress and normal groups on Day 2 after learning. Each stress sub group was subjected to stress from Day 2 to Day 9 for 7 days. On Day 9, the transfer latency was checked again for stress and normal groups [22, 23].

Morris water maze:

This approach has been used for many years to evaluate memory activity in learning and retention. A pool or swimming pool is created by the basin and its contents. The water is colored with powdered milk or non-toxic paint. The rat uses the platform as a means of escape. By exposing the platform, which is in the center of the pool, one inch above the water's surface, the platform's location is made known to the rats. A constant 26°C is maintained for the water temperature. Three consecutive trials will be performed on each animal. Place the animal on the platform first and then remove it after 20 seconds. The four entry points into the water maze are north, south, east and west. The rats were individually placed in the water at any of the beginning locations, comfortably and without stressing the animal. A maximum of 60 seconds may be spent by the animals looking for the platform. On day 1, the animals could first have trouble finding the platform, but eventually they will pick it up without any trouble. After one hour of drug administration on Day 2, the escape latency was recorded. Each specific group of rats was splitted into two sub-groups as stress and normal groups on Day 2 after learning. Each stress sub group was subjected to stress from Day 2 to Day 9 for 7 days. On Day 9, the escape latency was checked again for stress and normal groups. Care should be taken after completion of experiment [24].

Statistical analysis

Mean and SEM were used to express the results. Dunnett's test was used to compare the treatment groups to the standard groups after one-way analysis of variance (ANOVA). The minimum level of significance was fixed at $p < 0.05$.

Results

Table 1: Results of phytochemical analysis of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia*

Compounds	Chemical tests	*Ziziphus jujuba* and *Tinospora cordifolia*
Carbohydrates	Molisch'stest	Positive
	Benedict's test	Positive
	Fehling's test	Positive
Flavonoids	Lead acetate test	Positive
	Zinc chloride test	Positive
	NaOH test	Positive
Glycosides	General test	Positive
	Legal's test	Positive
	Modified-Borntrager's test	Positive
Proteins	Xanthoproteic test	Positive
	Biuret test	Positive
Saponins	Froth formation test	Positive
Tannins	Ferric chloride test	Positive
Triterpenoids	Salkowski's test	Positive
	Liebermann-Burchard test	Positive
Alkaloids	Dragendorff's test	Positive
	Mayer's test	Positive
	Hager's test	Positive

Elevated plus maze:

Table 2: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg) on transfer latency of stress induced rats using elevated plus maze

Groups (With stress)	Transfer latency (secs)		
	Day 1	Day 2	Day 9
1	74 (± 0.57)	52 (± 0.46)	60 (± 0.28)
2	32 (± 0.24)	28 (± 0.32)	36 (± 0.18)
3	50 (± 0.40)	40 (± 0.11)	44 (± 0.22)
5	52 (± 0.94)	38 (± 0.33)	42 (± 0.28)
7	37 (± 0.48)	34 (± 0.20)	38 (± 0.26)

Values are presented as the mean SEM (n = 4)

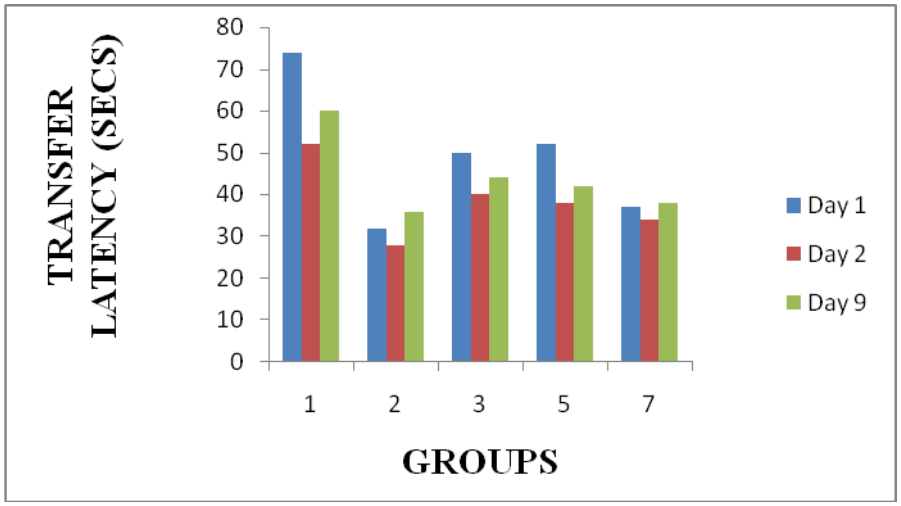

Figure 1: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg) on transfer latency of stress induced rats using elevated plus maze

Table 3: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (200 mg/kg) on transfer latency of stress induced rats using elevated plus maze

Groups (With stress)	Transfer latency (secs)		
	Day 1	Day 2	Day 9
1	74 (± 0.57)	52 (± 0.46)	60 (± 0.28)
2	32 (± 0.24)	28 (± 0.32)	36 (± 0.18)
4	46 (± 0.48)	36 (± 0.11)	38 (± 0.22)
6	50 (± 0.54)	46 (± 0.40)	40 (± 0.25)
8	34 (± 0.48)	30 (± 0.20)	28 (± 0.19)

Values are presented as the mean SEM (n = 4)

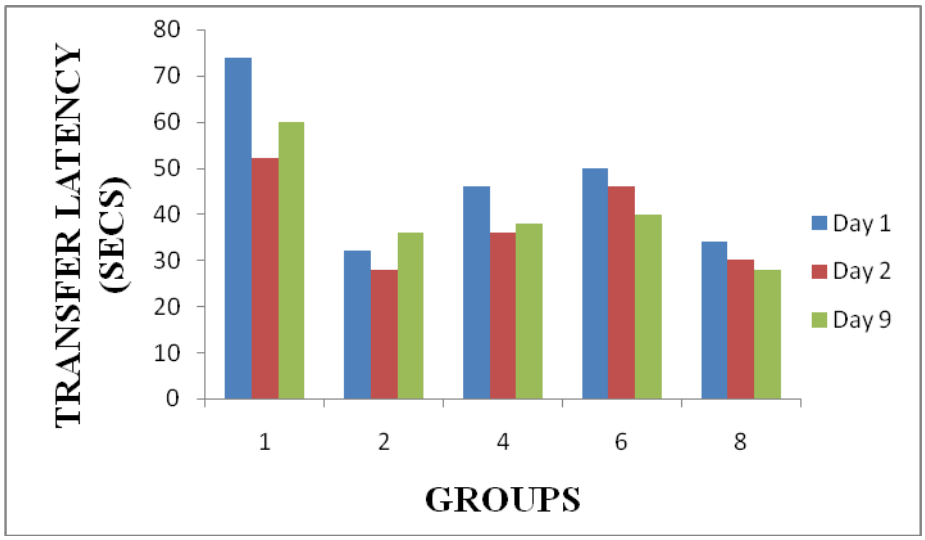

Figure 2: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (200 mg/kg) on transfer latency of stress induced rats using elevated plus maze

Staircase:

Table 4: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg) on number of steps climbed and number of rearings of normal rats using staircase

Groups	Day 1		Day 2		Day 7	
	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings
1	60 (± 0.57)	46 (± 0.44)	37 (± 0.33)	33 (± 0.88)	35 (± 0.3)	32 (± 0.16)
2	36 (± 0.12)	34 (± 0.2)	28 (± 0.04)	22 (± 0.08)	25 (± 0.06)	21 (± 0.1)
3	48 (± 0.4)	42 (± 0.8)	38 (± 0.06)	34 (± 0.07)	32 (± 0.8)	30 (± 0.06)
5	52 (± 0.8)	46 (± 0.7)	40 (± 0.6)	38 (± 0.3)	36 (± 0.4)	32 (± 0.5)
7	42 (± 0.06)	40 (± 0.04)	36 (± 0.02)	32 (± 0.01)	30 (± 0.04)	29 (± 0.06)

Values are presented as the mean SEM (n = 4)

Table 5: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg) on number of steps climbed and number of rearings of stress induced rats using staircase

Groups (With stress)	Day 1		Day 2		Day 7	
	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings
1	60 (± 0.57)	46 (± 0.44)	37 (± 0.33)	33 (± 0.88)	44 (± 0.32)	38 (± 0.21)
2	36 (± 0.12)	34 (± 0.2)	28 (± 0.04)	22 (± 0.08)	34 (± 0.38)	25 (± 0.13)
3	48 (± 0.4)	42 (± 0.8)	38 (± 0.06)	34 (± 0.07)	46 (± 0.26)	38 (± 0.39)
5	52 (± 0.8)	46 (± 0.7)	40 (± 0.6)	38 (± 0.3)	50 (± 0.06)	42 (± 0.02)
7	42 (± 0.06)	40 (± 0.04)	36 (± 0.02)	32 (± 0.01)	38 (± 0.22)	32 (± 0.26)

Values are presented as the mean SEM (n = 4)

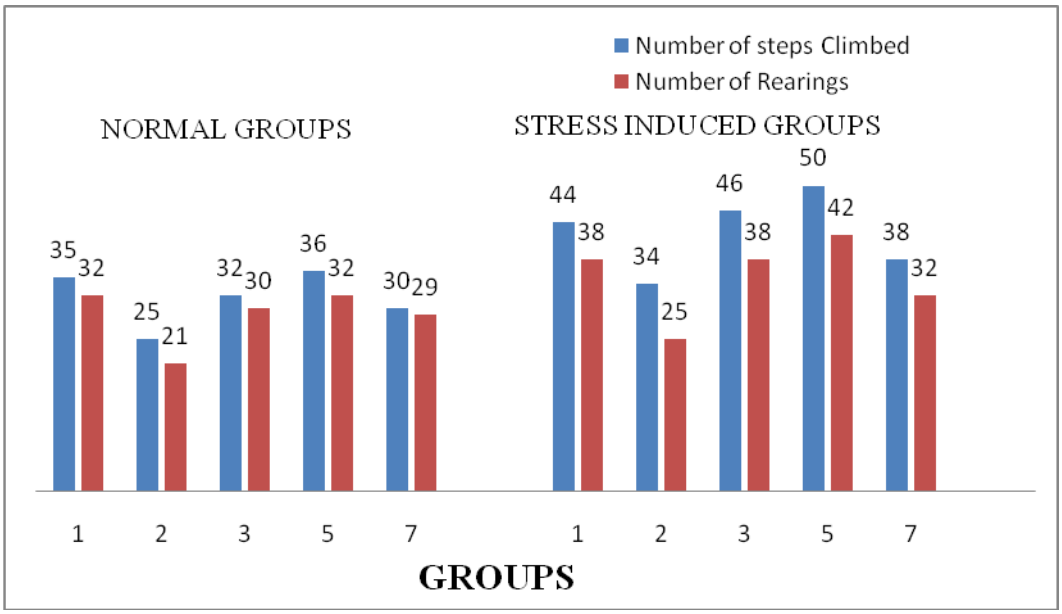

Figure 3: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg) on number of steps climbed and number of rearings of normal rats and stress induced rats using staircase

Table 6: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (200 mg/kg) on the number of steps climbed and the number of rearings of normal rats using staircase

Groups	Day 1		Day 2		Day 7	
	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings
1	60 (± 0.57)	46 (± 0.44)	37 (± 0.33)	33 (± 0.88)	35 (± 0.3)	32 (± 0.16)
2	36 (± 0.12)	34 (± 0.2)	28 (± 0.04)	22 (± 0.08)	25 (± 0.06)	21 (± 0.1)
4	46 (± 0.4)	40 (± 0.8)	36 (± 0.06)	32 (± 0.07)	30 (± 0.8)	28 (± 0.06)
6	50 (± 0.8)	44 (± 0.7)	42 (± 0.6)	31 (± 0.3)	40 (± 0.4)	29 (± 0.5)
8	38 (± 0.06)	36 (± 0.04)	29 (± 0.02)	24 (± 0.01)	27 (± 0.04)	26 (± 0.06)

Values are presented as the mean SEM (n = 4)

Table 7: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (200 mg/kg) on number of steps climbed and number of rearings of stress induced rats using staircase

Groups (With stress)	Day 1		Day 2		Day 7	
	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings	Number of steps climbed	Number of rearings
1	60 (± 0.57)	46 (± 0.44)	37 (± 0.33)	33 (± 0.88)	44 (± 0.32)	38 (± 0.21)
2	36 (± 0.12)	34 (± 0.2)	28 (± 0.04)	22 (± 0.08)	34 (± 0.38)	25 (± 0.13)
4	46 (± 0.4)	40 (± 0.8)	36 (± 0.06)	32 (± 0.07)	40 (± 0.26)	34 (± 0.39)
6	48 (± 0.8)	44 (± 0.7)	42 (± 0.6)	31 (± 0.3)	46 (± 0.06)	35 (± 0.02)
8	38 (± 0.06)	36 (± 0.04)	29 (± 0.02)	24 (± 0.01)	36 (± 0.22)	28 (± 0.26)

Values are presented as the mean SEM (n = 4)

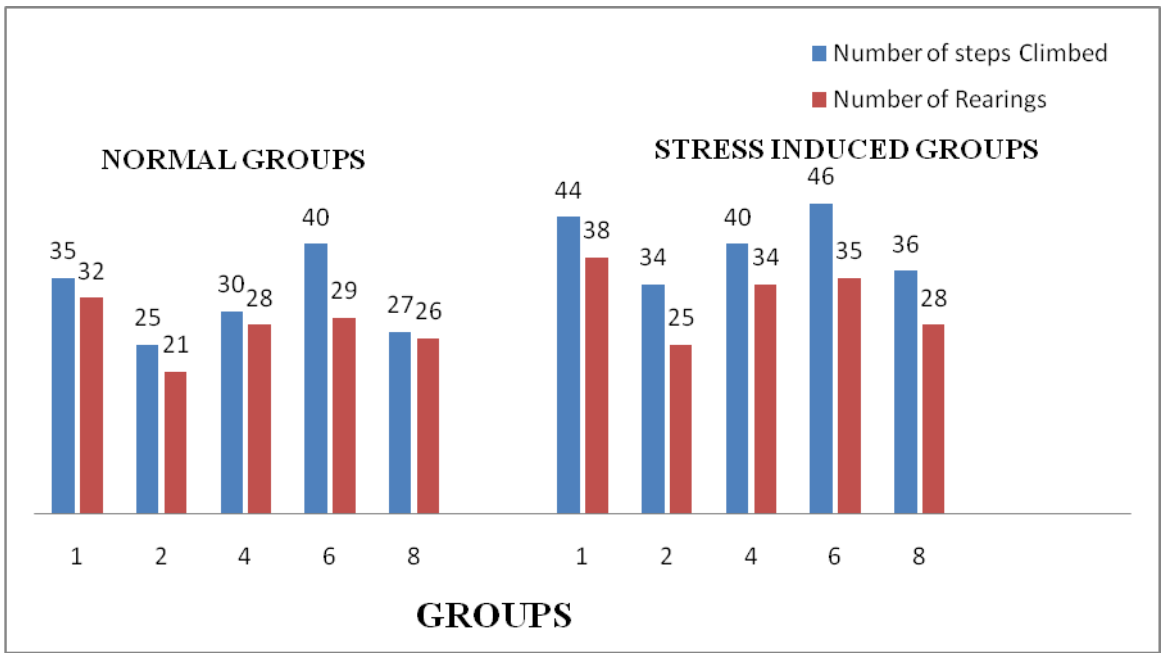

Figure 4: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (200 mg/kg) on number of steps climbed and number of rearings of normal and stress induced rats using staircase

Morris water maze:

Table 8: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100, 200 mg/kg) on escape latency of normal rats using Morris water maze

Day	Group 8				Group 7			
	Trials (seconds)				Trials (seconds)			
	Trial -1	Trial -2	Trial -3	Trial -4	Trial -1	Trial -2	Trial -3	Trial -4
1	3.8 (± 0.3)	5.6 (± 0.5)	2.9 (±0.4)	3.6 (± 0.1)	5.1 (± 0.6)	4.8 (± 0.5)	6.2 (± 0.1)	6 (± 0.04)
2	2.8 (± 0.6)	3.8 (± 0.3)	3.9 (± 0.3)	4.0 (± 0.2)	4.0 (± 0.3)	4.0 (± 0.2)	4.1 (± 0.6)	4.9 (± 0.5)
9	2.0 (± 0.5)	2.5 (± 0.4)	2.4 (± 0.8)	2.3 (± 0.3)	4.2 (± 0.1)	3.0 (± 0.3)	3.2 (±0.5)	3.4 (± 0.7)

Values are presented as the mean SEM (n = 4)

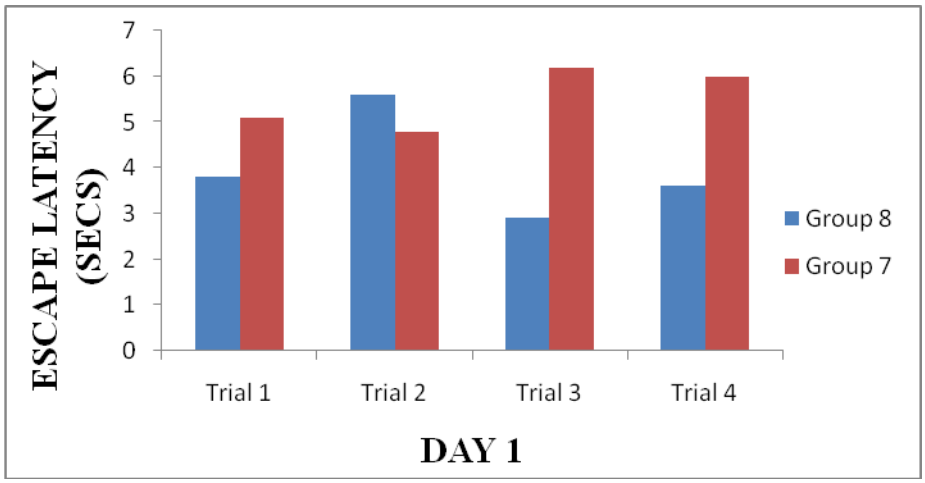

Figure 5a: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of normal rats using Morris water maze (Day-1)

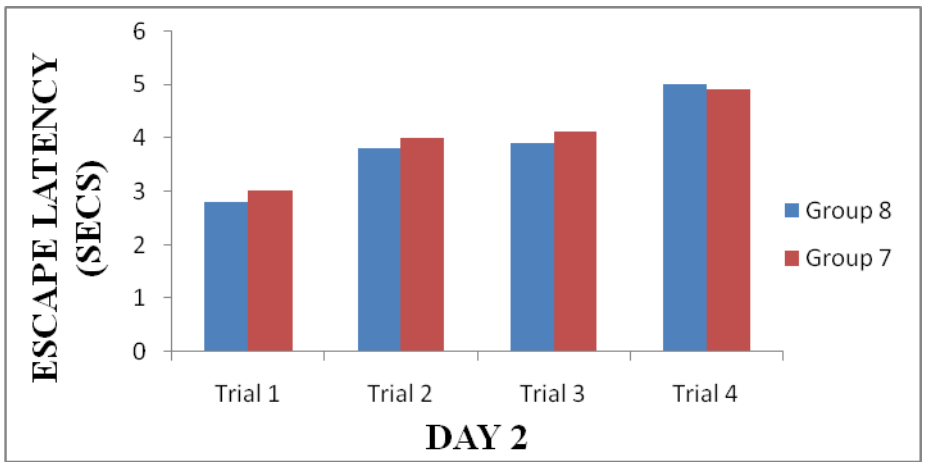

Figure 5b: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of normal rats using Morris water maze (Day-2)

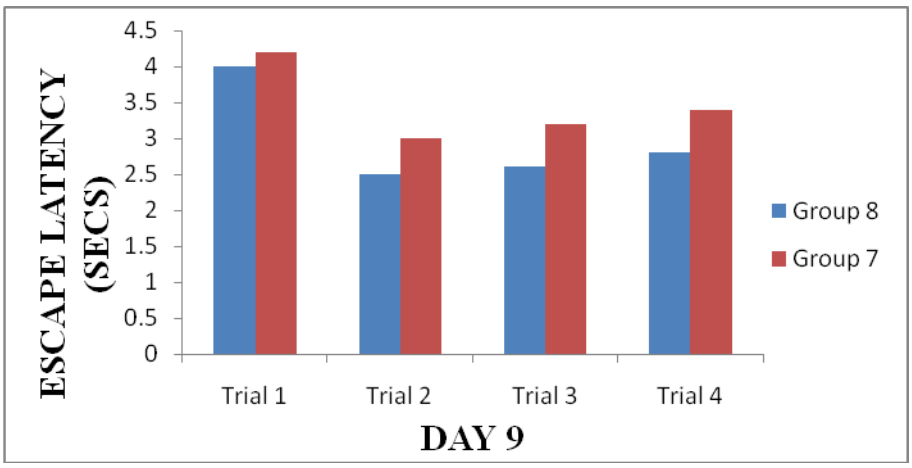

Figure 5c: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of normal rats using Morris water maze (Day-9)

Table 9: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of stress induced rats using Morris water maze

Day	Group 8				Group 7			
	Trials (seconds)				Trials (seconds)			
	Trial -1	Trial -2	Trial -3	Trial -4	Trial -1	Trial -2	Trial -3	Trial -4
1	4.5 (± 0.3)	6.0 (± 0.5)	5.6 (± 0.6)	4.2 (± 0.1)	5.9 (± 0.6)	6.2 (± 0.5)	5.5 (± 0.1)	5.0 (± 0.4)
2	3.9 (± 0.6)	4.5 (± 0.3)	4.0 (± 0.3)	5.0 (± 0.2)	3.2 (± 0.3)	4.8 (± 0.2)	4.5 (± 0.6)	5.6 (± 0.5)
9	5.6 (± 0.5)	4.8 (± 0.4)	3.9 (± 0.8)	5.1 (± 0.3)	7.0 (± 0.1)	5.0 (± 0.3)	6.0 (± 0.1)	5.2 (± 0.7)

Values are presented as the mean SEM (n = 4)

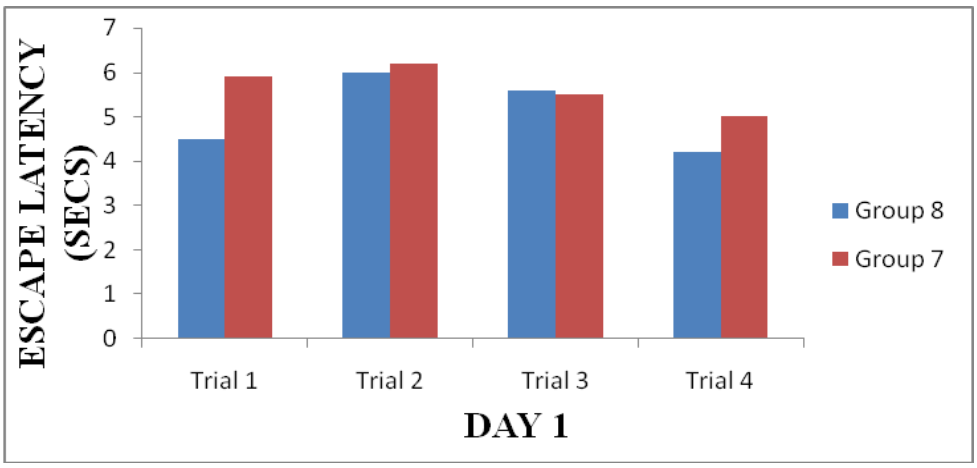

Figure 6a: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of stress induced rats using Morris water maze (Day-1)

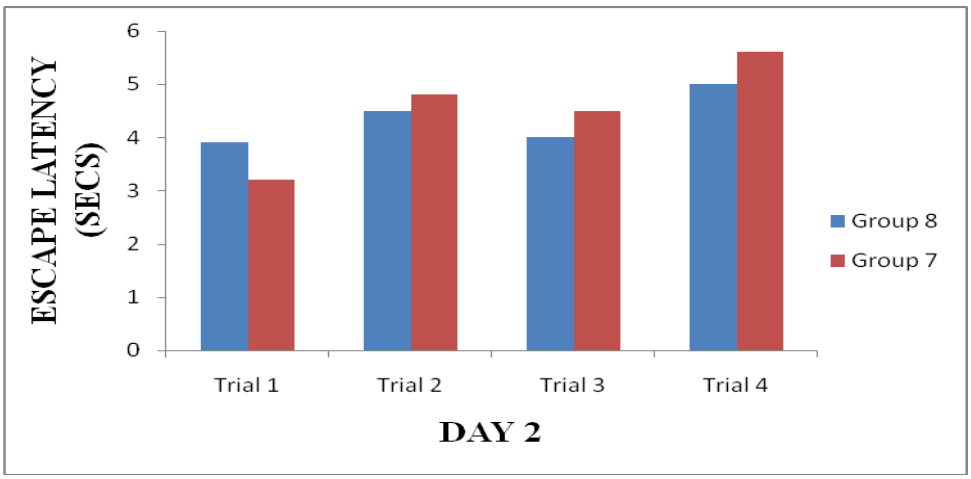

Figure 6b: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of stress induced rats using Morris water maze (Day-2)

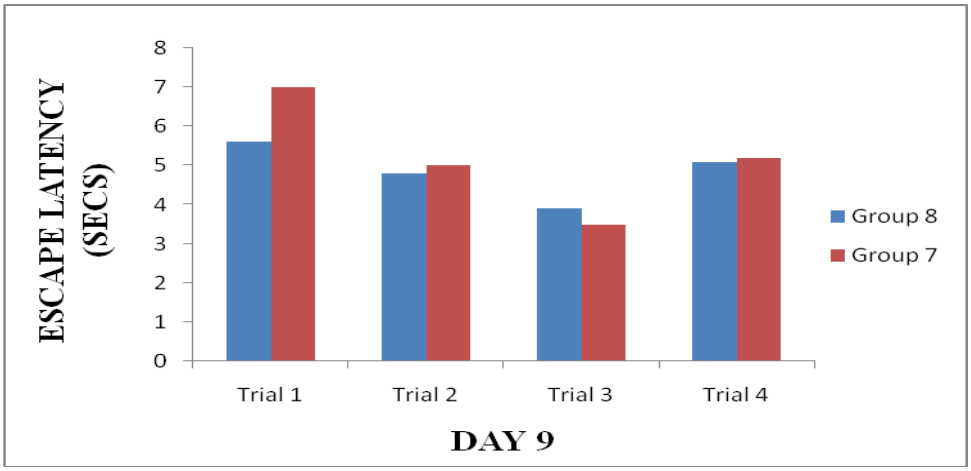

Figure 6c: Effect of ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100,200 mg/kg) on escape latency of stress induced rats using Morris water maze (Day-9)

Discussion

The preliminary phytochemical analysis of ethanolic leaf extract of *Ziziphus jujuba* possessed important constituents like carbohydrates, tannins, amino acids, proteins, sterols, triterpenoids, flavonoids, glycosides and alkaloids. Similarly *Tinospora cordifolia* also possessed important chemical constituents like carbohydrates, tannins, amino acids, proteins, sterols, triterpenoids, flavonoids, glycosides and alkaloids. It is a very well-known fact that few scientific reports indicate the presence of nootropic activity for these *Ziziphus jujuba* and *Tinospora cordifolia* plants. Though the nootropic activity is not very highly significant when used individually.

In our research study, we made a sincere attempt to evaluate the combined effect of these two medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia*. The presence of the phytochemical constituents like flavonoids and phenolic compounds present in these two medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia* may act as protector for brain against oxidative stress induced damage. Further, the presence of sterols such as stigmasterol was reported to inhibit acetyl CoA esterase activity that can be considered as an important mechanism through which the medicinal plant extracts could able to produce memory enhancing activity [25]. This activity was scientifically studied in our laboratory using different models like elevated plus maze, stair case and morris water maze methods. The ethanolic leaf extracts of *Ziziphus jujuba* and *Tinospora cordifolia* (100 mg/kg – 50 mg/kg of *Ziziphus jujuba* + 50 mg/kg of *Tinospora cordifolia* & 200 mg/kg – 100 mg/kg of *Ziziphus jujuba* + 100 mg/kg of *Tinospora cordifolia*) were administered to the experimental albino rats. These two doses (100 mg/kg &200 mg/kg) exhibited higher therapeutic improvements when compared to individual usage of single medicinal plant extracts.

Based on the experimental results obtained it is suggested that the concomitant usage of the medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia* exhibited synergistic action, clearly indicating the improvement in learning and retention of memory in the rats. The transfer latency and the inflexion ratio parameters in elevated plus maze and the total number of climbing and rearing parameters in staircase for both the standard piracetam (100 mg/kg) and the mixture of the extracts at 100 mg/kg and 200 mg/kg showed comparable results.

In the Morris water maze test, reference memory was tested in probe trials while working memory was tested by determining the escape latency or the time (seconds) it takes the animal to locate a hidden platform inside the colorized pool of water. The escape latency was significantly reduced when compared to control group (vehicle treated animal). In probe trails, the extracts treated animals were recorded using a video recorder and analyzed, the groups treated with extracts were found moving to and fro within the initial sphere of the platform which has already been removed before the start of the probe trail.

Conclusion

A sincere attempt was initiated by researchers to evaluate the synergistic nootropic activity by the combined usage of two medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia*. Both these medicinal plant extracts when concomitantly used exhibited significant synergistic activity at doses of 100 mg/kg (50 mg/kg of *Ziziphus jujuba* + 50 mg/kg of *Tinospora cordifolia*) and 200 mg/kg (100 mg/kg of *Ziziphus jujuba* + 100 mg/kg of *Tinospora cordifolia*). The standard scientific methodologies were implemented in evaluating the memory enhancing activity using elevated plus maze, staircase and morris water maze methods. The decrease of transfer latency in elevated plus maze, escape latency in morris water maze and the total number of climbings and rearings were clearly observed.

The combination of these two medicinal plants *Ziziphus jujuba* and *Tinospora cordifolia* possessed significant synergistic nootropic activity and this biological activity may be due to the presence of important phytochemical constituents like sterols, triterpenoids, flavonoids and phenolic compounds. Further research attempts can be made so as to isolate the novel moiety molecules which can be elucidated and can be evaluated for their therapeutic potential.

Abbreviations

EP: Elevated Plus Maze; **TL:** Transfer Latency; **ANOVA:** Analysis of Variance

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Conflict of interest

The authors declare no conflict of interest.

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