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Original article

Protective effects of ethanolic extract of Terminalia paniculata bark on ethanol-induced behavioral alterations

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Abstract

Introduction: Incidence of alcohol consumption has significantly increased in last few decades. Alcoholism is associated with serious medical and economic consequences. Behavioral disorders like mood swings, inappropriate behaviour, poor judgment and concentration, slurred speech, memory loss, and lack of coordination are common in alcoholics. Apart from psychotherapy, the allopathic armamentarium against alcohol induced behavioural alterations is too limited. The rich heritage of Ayurveda in India is looked upon as a new hope for treating behavioural alterations in alcoholics

Aim: To investigate the protective effects of ethanolic extract of *Terminalia paniculata* bark on ethanol-induced behavioral alterations.

Material and methods: Behavioural aspects such as anxious behaviour, exploratory behavior, and locomotor activity by using a photoactometer (PAM), Elevated Plus Maze (EPM), Rotarod, and Open Field Activity (OFA) were performed in male albino Wistar rats treated with ethanol (E), ethanol and *Terminalia paniculata* (E-TP), ethanol and silymarin (E-SM). Additionally, Rats treated with normal saline were included as controls.

Results: In the case of animal administered Terminalia paniculata in dose of 100mg/kg, locomotor activity increased significantly compared with animals receiving only ethanol. *Terminalia paniculata* and Silymarin have shown an increase in activity compared to depressed rats. In comparison with ethanol and other groups, time spent in open arm were significantly decreased with both doses of *Terminalia paniculata (*100mg/kg, 200mg/kg) and Silymarin. In case of entry of rats in close arm, after 4 weeks, time spent in close arm was significantly increased in control rats compared to all other groups.

Conclusion: Behavioral affects in alcoholics is linked with serious personal and family problems. Since apart from psychotherapy, the allopathic armamentarium against alcohol induced behavioural alterations is too limited, alternative/conventional medical streams are gaining importance in recent years. Based on the results of the present study, *Terminalia paniculata* alone or in combination with other drugs can be looked upon as a future alternative prophylactic and therapeutic ayurvedic formulation for treating behavioral alteration in alcoholics.

Key words: Behavioural alteration, ethanol, *Terminalia paniculata,* Wistar rats

Introduction.

Across the globe, addiction is a major threat to the well-being of an individual. It is a chronic condition that negatively affects almost all aspects of life including physical and psychological health, personal and social relationships, career, and financial status. As per definition, addiction is a chronic and relapsing disorder characterized by compulsive use of drug/substances despite adverse consequences.[1]

As per the systemic study done to assess global disease burden, addiction like alcoholism, tobacco chewing/smoking habits and use of illicit drugs are major risks for both disability and premature death.[2] Among various types of addiction, alcohol consumption is the most common.[3]

According to a survey, 85.6% of individuals of age more than 18 years have consumed alcohol at some point of their life. [4] Highlighting the worldwide

menace of alcohol addiction, the World Health Organization (WHO) reported that alcoholism is linked with approximately 3.3 million deaths per annum and nearly 5.1% of the total global burden of disease. [5]

Alcoholism is associated with serious medical and economic consequences. Deleterious effect of alcohol abuse on a health of an individual include cirrhosis, cardiomyopathy, various types of malignancies, infectious diseases, fetal abnormalities, different types of neurological complications including dementia whereas mood swings and anxiety are common psychiatric disorders experienced by alcoholics. Diminished productivity, frequent hospitalization, premature death and legal fees add to incremental financial burden. [6]

Chronic alcoholism is known to induce changes in neural circuits that have direct control behavioural processes like arousal, reward and stress.[7] Apart from psychotherapy, the allopathic armamentarium against alcohol induced behavioural alterations is too limited. On the other hand, in recent years alternative/conventional medical streams are gaining importance especially for chronic ailments.

Since historical times, man is versed with medicinal properties of plants and has been using various plants and their derivatives for treating diseases. The phytochemicals have similar effects to the chemical compounds in conventional medicines. The present study was undertaken with an aim to investigate protective effects of ethanolic extract of *Terminalia paniculata* on ethanol induced behavioral alterations.

Material and methods

The present study was carried in the Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Hyderabad, Telangana. The chemical reagents required for the study were procured from SRL laboratories and Sigma-Aldrich. The protocol of the study was approved Institutional Ethics Committee.

1. Preparation of plant extract.

The bark of Terminalia paniculata (1 Kg) was dried in the shade, and pulverized to coarse powder with the help of a suitable grinder. The powder obtained was stored in an airtight and clean glass container and used in the process of extraction. The plant was authenticated by Dr.Sharadha, Professor, Department of Pharmacognosy, Challa Malla Reddy College of Pharmacy, Hyderabad.

For the process of extraction, the bark of T. paniculata was dried under shade, pulverized to coarse powder and extracted with 99% ethanol for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under a vacuum to remove the solvent completely and concentrated to obtain a dark reddish semisolid residue (7.60 g).

2. *In Vivo* experiments.

Adult male albino Wistar rats (180-220 g) were purchased from GenTox Bioservices, Hyderabad. The animals were timely fed with standard laboratory food and water. Through the study, for the care and usage of rats the guidelines for National Institutes of Health (NIH) were strictly followed.

For the purpose of experimental study, the rats were divided in 5 groups. Each group contained 6 rats.

Group I (control): Rats treated with normal saline (5g/kg body weight).

Group II: (E) Rats treated with ethanol (5g/kg body weight) daily for 28 days using intragastric tubes.

Group III: (E-TP_1) Rats treated with ethanol (5g/kg body weight) along with extract of *Terminalia paniculata* (100 mg/kg body weight) daily for 28 days.

Group IV: (E-TP_2) Rats treated with ethanol (5 g/kg body weight) along with extract of Terminalia paniculata (200 mg/kg body weight) for 28 days.

Group V: (E-SM) Rats treated with ethanol (5g/kg body weight) along with drug silymarin (25 mg/kg body weight) orally for 28 days.

Behavioural aspects such as anxious behaviour, exploratory behaviour, and locomotor activity by using a photoactometer (PAM), Elevated Plus Maze (EPM), Rotarod, and Open Field Activity (OFA) – (MKM, India) were performed in these groups.

2.1 Photoactometer (PAM)

The spontaneous locomotor (horizontal) activity of the experimental animal was studied on 2nd and 4th week using the actophotometer. The ambulatory activity was recorded by placing the animal in the center square arena of the actophotometer for 5 minutes. When the animal moved inside the apparatus, the beam of light falling on the photocell was cut and it was displayed on the front panel meter as a movement count.[8]

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2.2 Elevated Plus Maze (EPM)

EPM used to assess anxiety-like behavior.[9,10] The Digital EPM is constructed in the way of + symbol and comprises two open arms (25 x 5 x 0.5 cm) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm) with a center platform (5 x 5 x 0.5 cm). The open arms have a very small (0.5 cm) wall to decrease the number of falls, whereas the closed arms have a high (16 cm) wall to enclose the arm. The entire apparatus is 50 cm above the floor. Entire setup is connected to the digital meter which will record the entries and time based on censors present on starting and ending of each arm. Individual animal anxiety will be assessed by placing it in the center platform, facing open arm to explore the EPM for 5 minutes and the behavioral parameters will be noted. Total number of entries into open arm and closed arm, Transfer latency (time taken for the entry into a closed arm with all the four limbs), time spent in closed arm and open arm.

2.3 Rotarod test

The motor performances in rats were measured by the rotarod test on 2nd and 4th week. The animals were placed on the Rotarod during the testing trials at a speed of 4 initially and slowly the speed was raised to 15 rpm for 3 minutes and the falls of the animals from the rotating rod were calculated. This test was performed before euthanasia.[11]

2.4 Open Field Activity (OFA)

Locomotor activity will be assessed by using the open field test on the 2nd and 4th week, a glass chamber measures 40cm in length, 40 cm in width, and 30 cm in height serving as open field apparatus. Animals were placed into the center of the field and allowed to explore the chamber for 5 minutes. The movement of animals was analyzed based on the number of line crossings, time spent in the center, and time spent in the peripheral area.[12]

Results

When locomotor activity was studied at 2 weeks, it was significantly increased in the control group whereas in an ethanol-treated group, it was significantly reduced to 111.5±6.189 per 5 min at 2 weeks ($p<0.001$) **(Figure 1)**

In the case of animals administered with *Terminalia paniculata* in the dose of 100mg/kg, locomotor activity was increased significantly i.e., 160.3±6.653 ($p<0.001$) compared with ethanol alone receiving animal. However, locomotor activity was significantly increased to 169.5 ± 9.397 ($p<0.001$) in 200mg/kg with *Terminalia paniculata* as compared to ethanol alone.

There was no significant difference between locomotor activity in *Terminalia paniculata* at a dosage of 100mg/kg and 200mg/kg. Animals treated with Silymarin in a dose of 25mg/kg showed significantly increased locomotor activity to 182.5 ± 8.118 ($p<0.001$), compared with ethanol alone. However, in the case of Terminalia paniculata at both doses 100mg/kg and 200mg did not show a significant difference with Silymarin (25mg/kg) alone also.

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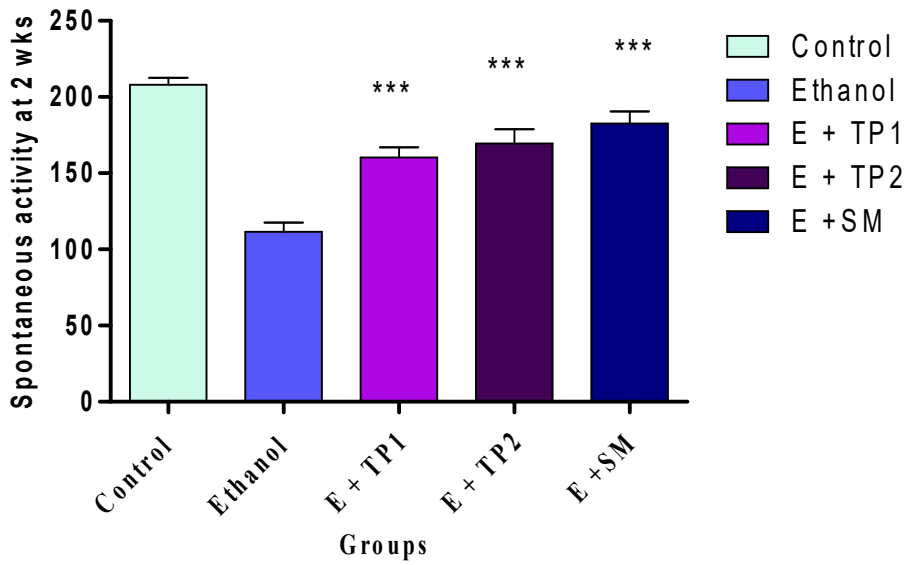

Figure 1. Comparison between effects of Ethanol-induced depression models (locomotor activity) in rats at 2 weeks.

Figure 2, shows locomotor activity in rats after 4 weeks. Ethanol significantly reduced spontaneous locomotor activity, compared to the control treated group. In the case with *Terminalia paniculata* in both doses, locomotor activity was significantly increased compared to ethanol treated group. Moreover, silymarin showed significantly increased locomotor activity (183.17± 7.387) compared with the ethanol-treated group.

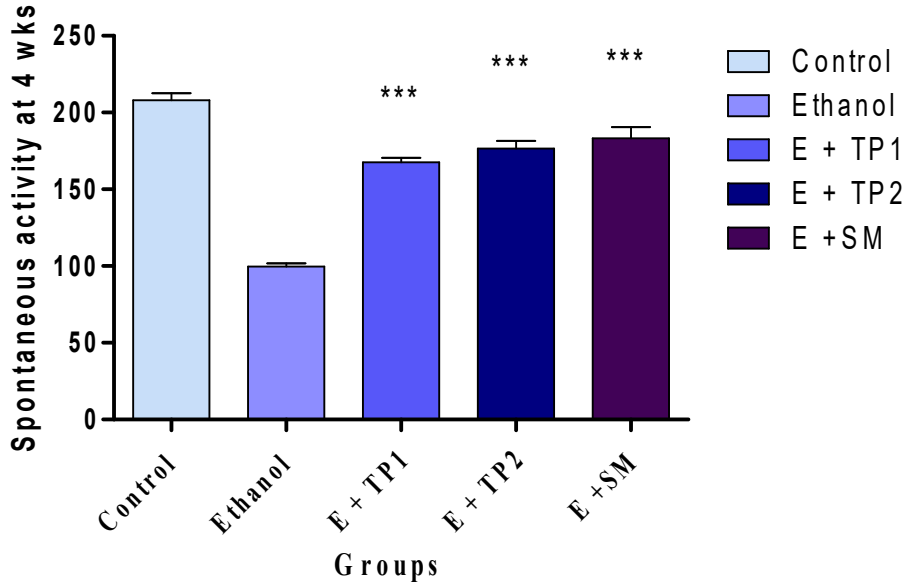

Figure 2. Comparison between effects of Ethanol induced depression models (locomotor activity) in rats at 4 weeks.

When a graph was plotted to summarize the results of the photoactometer at the end of 2nd week and 4th week, locomotor activity was significantly increased in the control group whereas it was decreased in the ethanol-treated group. However, *Terminalia paniculata* and Silymarin have shown an increase in activity compared to depressed rats (**Figure 3**).

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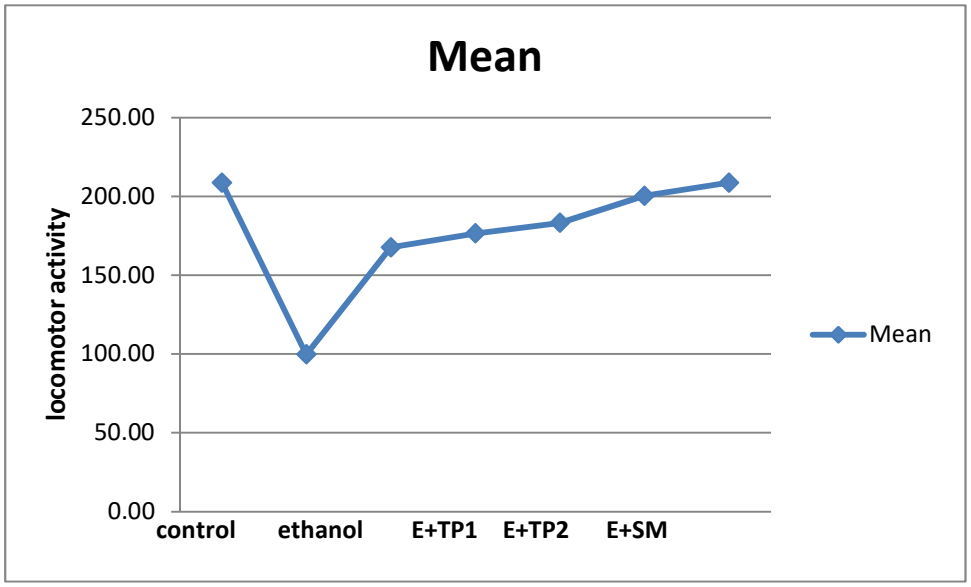

Figure 3. Locomotor activity measured by photoactometer.

The comparison between time spent in open and closed arm entries at 2 and 4 weeks is shown table 1

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Table 1: Comparison between time spent (Seconds) in open and closed arm entries at 2 and 4 weeks.

Male wistar rats	Open arm entry		Closed arm entry	
	2 weeks	**4 weeks**	**2 weeks**	**4 weeks**
Control (Mean±SD)	112.6± 1.949	113.2± 1.095	205.8 ±5.975	208.2± 4.087
Ethanol (Mean±SD)	174.0±16.447***	212.80 ± 3.03***	121.60±6.986***	122.60±2.302***
E + TP1 (Mean±SD)	131.80±3.768	162.40±3.84***###	204.20±5.762	134.40±2.881***
E + TP2 (Mean±SD)	201.80± 15.563***	213.20±2.49***	135.60±26.857	127.40±3.362***
E +SM (Mean±SD)	122.80± 6.907	162.80± 3.03***###	167.80±31.618**	135.20 ±3.633***

*** = Compare with control

= Compare with ethanol

As shown in Table 1, time spent in open arm entry of ethanol-treated rats was significantly increased i.e. 174.0±16.447 ($p<0.001$) as compared to the control group. In Terminalia paniculata 100mg/kg dose and Silymarin were not showing any significant difference compared with both control and ethanol-treated rats. However, Terminalia paniculata at dose of 200mg/kg has shown an increase in entry in the open arm significantly 201.80± 15.563 ($p<0.001$) compared to both control and ethanol-treated rats.

At 4 weeks, time spent in the open arm significantly increased in all groups compared to the control group i.e. 212.80 ± 3.03 with ethanol, 162.40±3.84 with Terminalia paniculata 100mg/kg, 213.20±2.49 with Terminalia paniculata 200mg/kg and 162.80± 3.03 with Silymarin. ($p<0.001$). However, in comparison with ethanol and other groups, time spent in the open arm was significantly decreased with Terminalia paniculata 100mg/kg and Silymarin. ($p<0.001$).

At the end of 2 weeks in control rats, time spent in close arm entry was significantly increased i.e. 205.8 ±5.975 ($p<0.001$) compared to ethanol, Terminalia paniculata in dose of 200mg/kg, and Silymarin. In comparison between ethanol-treated rats and Terminalia paniculata in a dose of 100mg/kg, significant decrease in time spent in close arm

i.e. 121.60±6.986($p<0.001$). However, there were no significant differences seen in ethanol and Terminalia paniculata in dose of 200mg/kg.

In case with entry of rats in the close arm, after 4 weeks, time spent in closed arm was significantly increased in control rats compared to all other groups.

Table 2. Transfer latency in rats at 2 and 4 weeks.

Male wistar rats	2 weeks	4 weeks
Control (Mean±SD)	31± 1.55	26.83 ± 1.60
Ethanol (Mean±SD)	33± 2.59	31 ± 2.58
E + TP1 (Mean±SD)	30.83 ± 0.98	27.33± 1.97
E + TP2 (Mean±SD)	29 ± 1.21#	23 ± 2.59
E +SM (Mean±SD)	27.83 ± 1.94* ###	22.83± 2.79

* = Compare with control
= Compare with ethanol

In 2 weeks, transfer latency was significantly reduced in the case with *Terminalia paniculata* 200mg/kg compared to ethanol. However, in the case with Silymarin, transfer latency was significantly reduced compared to both control and ethanol-treated. After 4 weeks, transfer latency was significantly reduced in the case with Silymarin as compared to both control and ethanol ($p<0.001$).

With an open field test, it was observed that time spent in the periphery was increased significantly in the case with ethanol, *Terminalia paniculata* dosages of 100mg/kg and 200mg/kg, and Silymarin as compared to control at 2 weeks whereas time spent in the central area was significantly reduced in ethanol-treated rats as compared to control. In case with Terminalia paniculata in doses of 100mg/kg and 200mg/kg as well as Silymarin there was a significant increase in the time spent in the central area as compared to the control. The number of squares crossed in ethanol-treated was significantly reduced as compared to the control and other treatment groups.

The results of the open field test after 4 weeks showed time spent in the periphery was increased significantly in the ethanol group as compared to control and other groups whereas time spent in the central area was significantly reduced in ethanol-treated rats as compared to control and other groups. Similarly, the number of squares crossed in ethanol-treated was significantly reduced as compared to control and other treatment groups.

Table 3: Effect of the drugs on time spent (seconds) on Rota Rod apparatus.

Time spent on rotating rod in Rota rod apparatus (sec)				
Groups	0 Week	2 week	4 week	P value
Control	76.83±3.97	67±9.41	7.33±8.55	P<0.05
Ethanol	77.00±6.78	39±4.51***	±4.64***	
E + TP1	74.67±7.42	65±3.39*	±3.43**	
E +TP2	80.33±3.67	62±4.15***	9±5.57**	
E+SM	78.50±4.72	66±4.32***	69±1.83**	

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As depicted in Table 3, it was observed there was a highly significant reduction in the time spent by the animals on the revolving rod in the case with ethanol at 2 weeks and 4 weeks respectively. In the case of *Terminalia paniculata* 100mg/kg significant difference was seen in time spent on the rotating rod in prior administration at 2 weeks. After 4 weeks, *Terminalia paniculata* both at dosages 100mg/kg and 200mg/kg showed a significant difference with prior administration of the respective group.

Discussion.

Alcohol is a toxic and psychoactive substance that has the property of producing dependency. Although most individuals whether literate or illiterate are versed in the ill effects of alcoholism, it remains the most common addiction worldwide.

The term alcohol is referred to as 'ethyl alcohol' or 'ethanol'. Alcoholic beverages are consumed in various forms like beer, wine, whiskey, rum, vodka, gin, and brandy and one standard alcoholic beverage corresponds to 10 g of absolute alcohol.[4, 13]

As per a recent study published in the Lancet Journal, there is an increase in the incidence of alcohol consumption in the Indian population over the period last 30 years.[14] This could be partly attributed to the rampant proliferation of city bars and nightclubs and partly related change in the attitude of people towards alcohol and the shedding of inhibitions about alcohol consumption.[15] Studies from different states of the country indicate that currently 35% to 65% of all alcohol drinkers are within the criteria of hazardous alcohol use.[15]

Alcoholism is associated with certain behavioural disorders which negatively impact the life of an individual. Common behavioural disorders in alcoholics are inappropriate behaviour, frequent change in moods, poor judgment and concentration, slurred speech, memory loss, and lack of coordination.[16]

In modern medicine, there are limited options for treating alcohol-induced behavioural defects. On the other, India has a rich heritage of Ayurveda. In India, many patients opt for ayurvedic/conventional treatment modalities as soon they are diagnosed with certain diseases/health problems. Therefore, ayurvedic treatment is usually used with and/or after therapeutic approaches of modern medicine.[17] Nearly 90% of ayurvedic formulations are plant-based. [17]

Terminalia paniculata commonly known as Kindal is an example of a wild tree used in traditional ayurvedic medicine for treating of various ailments including parotitis, cholera, diabetics, and menstrual disorders.[18] However many other therapeutic applications of this plant are yet to be elicited.

In the present study protective effects of ethanolic extract of *Terminalia paniculata* on ethanol induced behavioral alterations. The results of various experiments carried out in the present study have highlighted the promising effect of ethanolic extract of *Terminalia paniculata* on ethanol-induced behavioral alterations in Wistar male rats. Therefore *Terminalia paniculata* alone can be looked upon as a future alternative prophylactic and therapeutic ayurvedic formulation for treating behavioral alteration in alcoholics.

Conclusion.

Alcoholism is a leading medical and social problem worldwide. Behavioral affects in alcoholics are linked with serious personal and family problems. Since apart from psychotherapy, the allopathic armamentarium against alcohol-induced behavioural alterations is too limited, alternative/conventional medical streams are gaining importance in recent years. Based on the results of the present study, *Terminalia paniculata* alone or in combination with other drugs can be looked upon as a future alternative prophylactic and therapeutic ayurvedic formulation for treating behavioral alteration in alcoholics.

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