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Original article

Exploring the neuroprotective properties of Terminalia paniculata in alcohol induced neurotoxicity: evidence by biochemical and histological studies

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Abstract

Alcoholism is associated with numerous medical, psychiatric, social, and family problems. Although ill effects of alcohol are seen on all parts of the body, the central nervous system and the liver are most affected. The arsenal against neuronal damage in modern medicine appears to be limited. Therapeutic modalities are multidisciplinary and include antidepressants, mood stabilizers, estrogen, electro convulsive treatment, physical exercise and enriched environment. From immemorial times plants and their products are used by man as medicines. Plants as whole or their parts like branches, leaves, stem, roots, fruits, flowers etc. are known to have several pharmacological properties including antioxidant, antiviral, anticancer, antimicrobial, antifungal and antiparasitic. Terminalia paniculata is one of such medicinal plant known used for treating several ailments including diabetes, ulcer, leprosy and menstrual disorders. The aim of present study was to evaluate neuro-protective activity of Terminalia paniculata in ethanol induced neuronal damage using rat models. T. paniculata ethanolic extract was screened for its neuroprotective potential was studied by conducting various animal experiments in Male Wistar rats. These experiments included neurotransmitters and histopathological examination. Series of experiments conducted in this study, conclude that T. paniculata has promising neuroprotective activity against neuronal damage induced by ethanol. Furthermore, T. paniculata can be looked upon cost-effective, natural and safe alternative for the ailments like neuronal injury induced by alcohol.

Keywords: *Terminalia paniculata, neuronal damage, neurotransmitters, brain tissue.*

Introduction.

Among various types of addictions, alcoholism is reported to be the most common. Several national and international bodies have highlighted alcoholism as one the most common scourges to mankind. The burden of alcoholism across the world is high. According to the National Survey on Drug Use and Health (NSDUH), 85.6% of individuals above the age of 18 years reported that have consumed alcohol at some point in their lifetime.[1] On the other hand expressing the concern over alcohol consumption and its ill effects, the World Health Organization (WHO) stated that, "approximately 5.1% of global disease burden is attributed to alcohol addiction".[2]

Although negative effect of alcoholism is manifested throughout human body, nervous system and liver is most commonly affected. Alcoholism exerts severe detrimental effects on both adult and fetal central nervous system which may induce several changes that may either be localized or disseminated, temporary or permanent, acute or chronic, short term or long lasting.[3] Modern medicine offers limited hope for treating alcohol associated neurological disorders as only few therapeutic modalities are available. Alternative medicines are ray of hope for many ailments where therapeutic options are very few.[4]

India has rich heritage of alternative medicine including Ayurveda and siddha. These branches of

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alternative medicines are now gaining importance and being used by rural and urban masses for both prophylactically and therapeutically for various ailments.[5,6]

In Ayurveda, many medicines are derived from plants and their plants. Varied climatic conditions and different types forests provides natural habitat for plants with medicinal value. Although utility of many plants with medicinal properties in several acute and chronic diseases has been studied by various researchers, the scope still exists, since many properties are yet to be explored.

Terminalia *paniculata* is one among of such medicinal plant used for traditionally used for treating several ailments like diabetes, ulcer, leprosy and menstrual disorders. Considering broad and medicinal values of *T. paniculata*, in the present study neuroprotective activity of this plant was explored by conducting evaluating array of biochemical and histological parameters in alcohol induced neurotoxicity.

Material and Methods.

The present experimental research study was conducted for a period of 1 year (August 2021 to July 2022). The bark *T. paniculata* was procured from a local vendor Senngottai, Coimbatore and it was authenticated by Dr. S. Mutheeswaran, Scientist, Department of Botany, St. Xavier's college, Xavier Research Foundation, Tirunelveli, Tamil Nadu.

For preparation of extract, *T. paniculata* bark was extracted with 99% ethanol for 48 hours using a Soxhlet extractor followed by drying in shade and finally ground into a coarse powder using appropriate grinder. This powder was then stored in an amber colored glass bottle. About, 2 litre of ethanol (98%) was added to the powder of 500 g of *T. paniculata* bark with intermettent shaking.

The resultant mixture obtained was first passed 2 layers of cotton and then re-filtered by using Whatman No.1 filter paper. The filtrate obtained was then concentrated with a rotary evaporator under reduced pressure at a temperature of 50-65° C. This concentrate was stored at 4° C for further tests. Ethanol was procured from SRL Laboratories. The resulting extract was concentrated to yield a dark crimson semisolid residue (7.60 g), which was then evaporated under a vacuum to entirely eliminate the solvent. A small quantity of extract was dissolved in 5ml of distilled water and then filtered.

For conducting animal studies, adult male albino Wistar rats (Rattus norvegicus) aged between 150-180 days and weighing between 180-200 grams were used. The animals were procured from GenTox Bioservices, Hyderabad. For care and usage of rats the guidelines for National Institutes of Health (NIH) were strictly followed through the study. The protocol of the study was approved by Institutional Ethics Committee (IAEC no: 35/A/2021, dated 18.06.2021).

As shown in table 1, for conduction of the study, the rats were categorized into 5groups (group 1to 5), each containing 6 animals.

Group	Name	Description
Group 1	Control group	Rats were treated with normal saline (5ml/kg body weight)
Group 2	Ethanol group	Rats were treated with ethanol (5g/kg body weight) daily for 28 days using intra-gastric tubes.
Group 3	Ethanol- Silymarin (E- SM) group	Rats were treated with ethanol (5g/kg body weight) using intra-gastric tubes and then orally received reference drug silymarin (25 mg/kg body weight) for daily for 28 days
Group 4	Ethanol-*Terminalia paniculata* I (E-TP)	Rats treated with ethanol (5g/kg body weight) using intra-gastric tubes and then orally received ethanolic extract of *Terminalia paniculata* (100 mg/kg body weight) daily for 28 days
Group 5	Ethanol-*Terminalia paniculata*-silymarim (E-TP -SM))	Rats treated with ethanol (5g/kg body weight) using intragastric tubes and then orally received ethanolic extract of *Terminalia paniculata* (100 mg/kg body weight) and silymarin (25 mg/kg body weight) daily for 28 days.

On 29th day, overnight fasted rats were euthanized under the isoflurane anaesthesia and the brain was obtained after performing autopsy of the animals. A portion from brain was then homogenated in tissue homogenizer to obtain 1:9 (w/v) homogenates with cold saline. This mixture was then centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was collected and utilized for measuring neurotransmitters like Dopamine, Serotonin, Acetylcholine, GABA, and Glutamate (glutamate receptor subunit zeta 1 (GRINL1A) levels were analyzed using the protocol described in the ELISA kit of E-Lab Biosciences (USA) and CUSABIO. The cresyl violet stained sections of brain tissue (collected during autopsy) were examined under 40x objective lens using Olympus binocular light microscope.

Results

Table 2. Effect of *T. paniculata* (TP)/ silymarin (SM) alone and in combination on neurotransmitter levels (ng/mg) in ethanol-induced neurotoxicity in Wistar rats.

Group	Dopamine	Serotonin	Acetylcholine	GABA	Glutamate (NMDA receptor)
Control group	144.82±3.25	79.13±2.24	54.6± 3.55	190.21 ±4.28	829±4.20
Ethanol	100.36 ±1.25*	37.56±1.67*	30.4±4.06*	150.12 ±2.19*	865±1.17*
E-Silymarin (E-SM)	115.35 ± 1.23*	49.05 ± 2.24*	32.1±5.22*	167.34± 1.70*	853±2.20*
E-TP	115.46±2.25*	49.75±1.05*	33.6±3.33*	168.12 ±2.29*	853±1.19*
E-TP-SM	117.58 ± 3.24*	53.43 ± 8.65*	35.1±5.48*	169.56 ±3.20*	850±1.05*

¥ Data are expressed as mean ± S.E.M (n = 6 animals / group).
* $P < 0.001$ compared to control group (analysed by one way ANOVA and multiple comparison tests with Tukey's post hoc test).

Dopamine levels were significantly ($p<0.001$) decreased in Ethanol group (100.36 ±1.25) compared to control group (144.82±3.25). The drug treatment groups E-SM (115.35 ± 1.23), E-TP (115.46±2.25) and combinations (117.58 ± 3.24) showed significant protection ($p<0.001$) by changing the levels of dopamine as compared with control group.

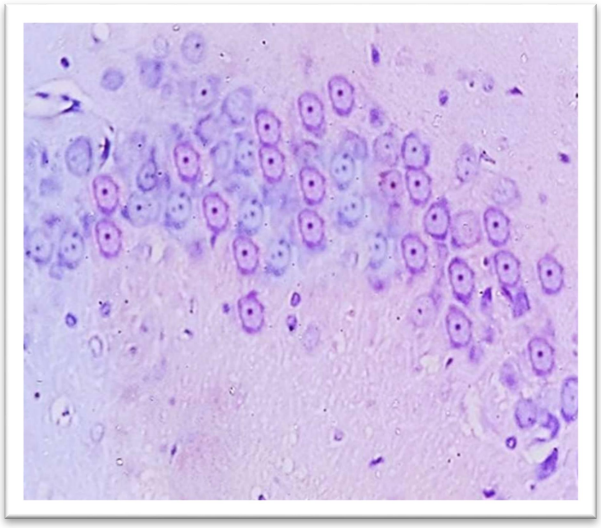
The Ethanol group (37.56±1.67) showed significant ($p<0.001$) decrease in serotonin levels when compared with control (79.13±2.24). The treatment groups E-SM, E-TP and E-TP-SM combination in both doses revealed significant ($p<0.001$) change in the serotonin levels with the values of 49.05 ± 2.24, 49.75±1.05, and 53.43 ± 8.65respectively when compared with control group.

Acetylcholine levels in the Ethanol group (30.4±4.06) were also significantly ($P<0.001$) decreased compared to control (54.6± 3.55). After treatment with E-SM (32.1±5.22), both of E-TP (33.6±3.33) and E-TP-SM combination (35.1±5.48), the values were significantly ($p<0.001$) altered when compared with control group.

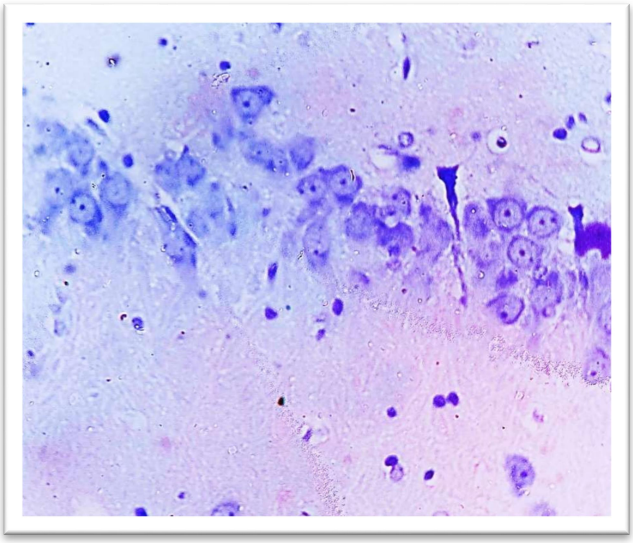
The GABA levels were significantly ($p<0.001$) decreased in Ethanol group (150.12 ±2.19) compared to control group (190.21 ±4.28). Significant protection ($P<0.001$) was seen with the levels of GABA in drug treatment groups E-SM (167.34± 1.70), E-TP (168.12 ±2.29) and combination E-TP-SM (169.56 ± 3.20) when compared with control group.

The mean value of control group was 829±4.20 in glutamate. The Ethanol group (865±1.17) had a significant decrease ($P<0.001$) when compared to control group whereas the treatment groups of E-SM, E-TP and E-TP-SM combinations in both doses also had significant change ($p<0.001$) when compared to the control group and their values were 853±2.20, 853±1.19 and 850±1.05 respectively.

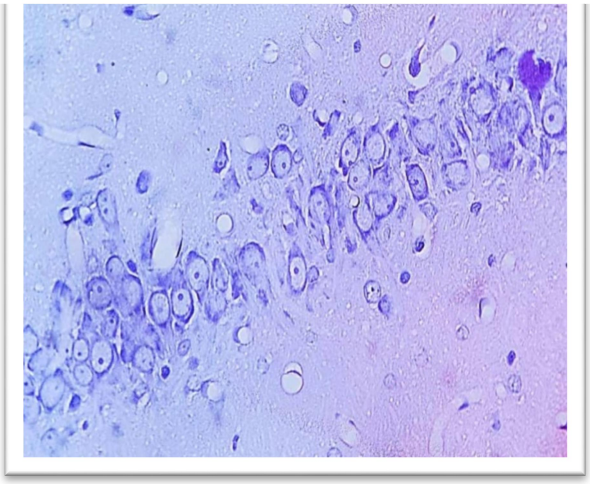
Effect of TP/SM alone and TP with SM combination on ethanol-induced histological changes in brain in Wistar rats is shown in figure 1. Upon histological examination, it was noted that the effect of standard drug silymarin and plant extracts on hepatocytes and neuronal cells clearly showed the protective effects of ethanol extracts of *T. paniculata* bark against alcohol-induced liver and neuronal damage.



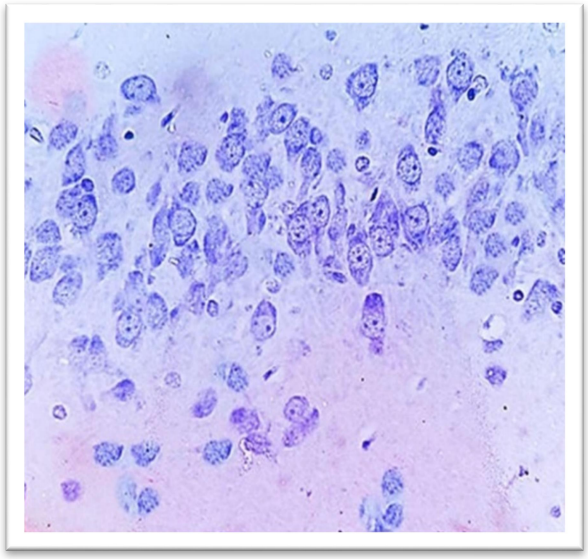
Control group: intact neuronal cell bodies with normal morphological characterization.



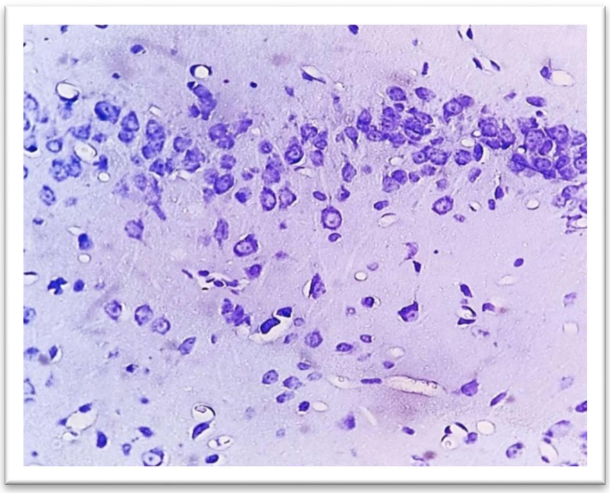
Ethanol Group: degenerated pyramidal cellular morphology with cell death and moreover pyknotic nuclei and necrotic cells.



E-SM Group: regenerated neurons less evidence of damage in the neuronal cells. The apoptotic and necrotic cells are absent. The number of neurons also increased.



E-TP Group: Less number of pyramidal neuronal cells and presence of pyknotic nuclei.



E-TP-SM Group: normal pyramidal neurons glial cells and intact arrangements with no signs of damage.

Figure 1: Effect of TP/SM alone and TP with SM combination on ethanol-induced histological changes in brain in Wistar rats.

Discussion.

Since ancient times, plants are considered as the important source of medicines used for treating vast clinical manifestations ranging from mild seasonal illness to dreadful diseases. Even the era, where modern medicine has progressed beyond leaps and bounds, 80% of global population still believes that therapeutic agents derived from natural moieties like plants are effective and free from side-effects**.** [7] In general, many modern drugs mimic completely or partially, the structure and functions of medicines derived from natural moieties.

As per available scientific reports, the plants belonging to the family of *Combretaceae* are very rich in phytochemicals that have various vital physiological and pharmacological effects. Owing to potential medicinal properties, these plants have attracted the attention of many researchers for their application in various non-treatable/difficult to treat and chronic diseases like cancer, hepatitis, diabetes mellitus, cardiac ailments and HIV/AIDS.[7] *T. paniculata* is one among the plants from the family *Combretaceae* which are known for its medicinal potential that are attributed to phytochemicals.

In this study, the neuroprotective role of *T. paniculata* was evaluated in neurotoxicity induced by ethanol. Although various mechanisms are suggested for alcohol induced brain pathology, recent studies have underscored the involvement of various pathways that are directly or indirectly altered by heavy consumptions of ethanol.[8]Alcohol consumption (acute/chronic) affects most if not all currently known neurotransmitters of CNS.[9]

Neurotransmitters are chemical messengers, endogenous in nature, and perform various functions including neurotransmission, differentiation, the growth of neurons, and the development of neural circuitry.[10] Studies have clearly indicated that various neurotransmitters are directly or indirectly involved in the overall pathophysiological mechanism of alcoholism.[11]

Alcohol consumption leads to imbalance of neurotransmitters be either due to their excess activity or inhibition. Examples of the neurological pathways that are affected by alcohol consumption are the dopaminergic, serotoninergic, γ-amino butyric acid (GABA) and glutamate pathways. [11]

Dopamine, serotonin, acetylcholine (ACH) and GABA were the serum neurotransmitter studied. Dopamine is a neuromodulatory agent.[12] Within the CNS, dopamine binds to specific receptors that are present on the cell membrane that are presented by neurons. Dopamine plays an important role in controlling certain activities like locomotion, learning, working memory, cognition, and emotion.[13] In this study, dopamine levels were significantly decreased in Ethanol group compared to control group. The drug treatment groups showed significant protection by changing the levels of dopamine as compared with control group.

Serotonin is a well-known neurotransmitter of the brain that plays a vital role in many peripheral tissues, including the immune system and cognitive functions.[14] In this study, there significant decrease noted in the Ethanol group when compared with control (79.13±2.24) whereas serotonin level was improved in groups that were treated with *T. paniculata* alone or in combination with silymarin.

In this study similar protective potential of *T. paniculata* was also noted for another neurotransmitter like ACH and GABA. The most of actions of alcohol that occurs in an individual is due to ACH.[15] In alcoholic intoxication, elevated level of ACH causes certain manifestations that includes alcohol sensitivity involving vasodilation leading to increased dermal- temperature, facial flushing, hypotension, increased heart and respiration rate, dryness of mouth or throat that is associated with bronchoconstriction and allergy reactions, nausea and headache, and euphoria.[15]

Alcohol intoxication also alters the equilibrium between γ-aminobutyric acid (GABA) and glutamate. GABA is the primary inhibitory neurotransmitter whereas the glumate is the major excitatory neurotransmitter. In this study, the GABA level was significantly decreased in ethanol group compared to control group. Decreased GABA levels are known to be associated common characteristics of alcohol addiction like disinhibition, poor impulse control, and decreased executive function. [16]When the GABA levels were studied in groups treated with *T. paniculata* alone or in combination with silymarin, there was improvement noted in ACH levels compared to ethanol group.

Finally, upon histopathological examination of brain tissues, it was noted that the effect of standard drug silymarin and ethanol extracts of *T. paniculata* on neuronal cells was protective against alcohol-induced liver and neuronal damage. Administration of ethanol extract of *T. paniculata* bark reduced inflammation and necrosis of neuronal cells that seen in ethanol treated group.

Conclusion.

Based on the findings of this study, the administration of *Terminalia paniculata* alone and in combination with silymarin to male Wistar rats subjected to chronic ethanol intake resulted in appreciable improvements in neuronal functions via comprehensive findings of histopathological parameters and neurotransmitter levels. Therefore *T. paniculata* can be considered as a novel therapeutic candidate in treating ethanol-induced neurotoxicity either alone or in combination with silymarin.

References.

1. SAMHSA, Center for Behavioral Health Statistics and Quality. 2019 National Survey on Drug Use and Health. Table 2.17B – Alcohol Use in Lifetime among Persons Aged 12 or Older, by Age Group and Demographic Characteristics: Percentages, 2018 and 2019.
2. World Health Organization (WHO) *Global Status Report on Alcohol and Health.* 2014.
3. Alderazi Y, Brett F. Alcohol and the nervous system. Current Diagnostic Pathology. 2007 Jun 1;13(3):203-209.
4. Jaiswal YS, Williams LL. A glimpse of Ayurveda - The forgotten history and principles of Indian traditional medicine. J Tradit Complement Med. 2016 Feb 28; 7(1):50-53.
5. Pandey M, Rastogi S, Rawat A. Indian traditional system of medicine and nutritional supplementation. Evidence Based Complementary and Alternative Medicine 2013; 376327; 2013: 12 pages.
6. Shakya AK. Medicinal plants: Future source of new drugs. International journal of herbal medicine. 2016; 4(4):59-64.
7. Dwevedi A, Dwivedi R, Sharma YK. Exploration of Phytochemicals Found in *Terminalia* sp. and their Antiretroviral Activities. Pharmacogn Rev. 2016;10(20):73-83.
8. Tsermpini EE, Plemenitaš Ilješ A, Dolžan V. Alcohol-Induced Oxidative Stress and the Role of Antioxidants in Alcohol Use Disorder: A Systematic Review. *Antioxidants*. 2022; 11(7):1374.
9. Nevo I, Hamon M. Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. Neurochemistry international. 1995;26(4):305-36.
10. Rizo J. Mechanism of neurotransmitter release coming into focus. Protein Sci. 2018;27(8):1364-1391.
11. Banerjee N. Neurotransmitters in alcoholism: A review of neurobiological and genetic studies. Indian J Hum Genet. 2014;20(1):20-31.
12. Di Chiara G. Alcohol and dopamine. Alcohol health and research world. 1997;21(2):108.
13. Drożak J, Bryła J. Dopamine: not just a neurotransmitter. Advances in Hygiene and Experimental Medicine. 2005 Aug 12;59.
14. Wu H, Denna TH, Storkersen JN, Gerriets VA. Beyond a neurotransmitter: The role of serotonin in inflammation and immunity. Pharmacological research. 2019 ;140:100-14.
15. Eriksson CJ. The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res. 2001 May;25(5 Suppl ISBRA):15S-32S.
16. Shyu C, Chavez S, Boileau I, Le Foll B. Quantifying GABA in Addiction: A Review of Proton Magnetic Resonance Spectroscopy Studies. Brain Sci. 2022 13;12(7):918.