IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

"PHARMACOLOGICAL EVALUATION OF ANTI-CONVULSANT ACTIVITY OF LIPOSOMES CONTAINING VITEXNUGEUNDO"

[1] Ms. Ambika D.Nagarbhdiya, [2] Dr. Ranajit D.Tijare, [3] Ms. Varsha M. Kharat

Asst. Professor, Principal, Asst. Professor

D.B.K.S.S. ISHWAR DESHMUKH INSTITUTE OF PHARMACY, DIGRAS

Abstract

The *vitexnugeundo* or *nirgudi* is a native plant of India different part of which are used in the treatment of various diseases such as: analgesic, hepatoprotective, anxiolytic, anti-inflammatory, asthma, hormonal disorder etc. The purpose of this study was to prepare & evaluate extract &also liposome formulation consisting of methanol extract of leaves of *vitexnegundo* for the treatment of convulsion induced by PTZmodel & MES induced seizure using animals. The present study was novel as it consisted of utilizing the extract of same plant prepared by using various solvents. The extract was prepared using the combined solvents, consisting of ethanol, methanol, chloroform and water in the ratio 1:1. The prepared mixture of the solvent was used for the extraction of phytoconstituents, which revealed that, the prepared combined solvent showed the significant increment in the extractive value. Further same extract was screen for anti-convulsant activity using PTZ model, and results revealed that the significant anticonvulsant activity. Whereas the other extracts, which were prepared using solvents like ethanol, methanol, chloroform and water showed less extractive value and less anti-convulsant activity as compared to combined solvent extract. This novelty proved significantly in the present study. The significant activity of the plant was may be due to the presence of the few of the chemical constituents like, Flavonoids, Saponins and the cynogenetic glycosides also. The phytochemical investigation showed the presence of these chemicals in the plant extracts, which act as a strong base for the significant results, proved by the Tukey Kramer method.

Key Words: Antiepileptic,*Vitex-negundo*, Electroconvulsiometer, Maximum Electroshock (MES), Verbenaceae, Cynogenetic glycosides.

INTRODUCTION-

Vitexnegundo Linn. (Family: Verbenaceae) is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests and has been claimed to possess many medicinal properties. It is found throughout the greater part of India at warmer zones and ascending to an altitude of 15,00m in outer Western Himalayas. One of the ancient use of Vitex-negundo documented in Ayurveda is to provide mental peace. Vitex-negundo has been extensively studied for its anti-inflammatory3-5 and analgesic1,3,5,6activities in the past but, very few studies have been done to evaluate its anticonvulsant activity and there are conflicting reports regarding anticonvulsant activity of Vitexnegundo and no one has previously studied anticonvulsant activity of Vitexnegundo by oral route except one study7 .Therefore, the present study was undertaken to investigate anticonvulsant activity of ethanol leaf extract of Vitexnegundo in comparison with standard anticonvulsant drug in sub-protective doses per orally was studied to evaluate its potential role as an adjuvant therapy.

Medicinal uses of vitexnegundo

Herbal medicine focuses on curing the root cause of the disease, rather than its symptoms. The assortment of phytochemicals found in medicinal herbs enables them to enhance the overall well-being. Regardless of the advancement of contemporary medicine, a large segment of the population in countries like India, China, Nepal and Bangladesh still rely on folk and traditional medicine. Traditional medicine predominantly encompasses Indian Ayurveda, Arabic Unani and Chinese Pharmacopeia. CharakaSamhita and AnubhogaVaidyaBhaga, the great books of Ayurveda, elaborate on the use of Vn to treat sinusitis, syphilitic skin disease, catarrhal fever, dysmenorrhea and rheumatism. The seeds of Vn are used as an aphrodisiac and to cure swellings in Unanimedicine . Chinese medicine mentions the consumption of the Vn fruit to treat puffy eyes, arthritis and headaches . The traditional medicinal uses of various parts of Vn have been enlisted.

Material & method-

Collection &Authenticationof plant material

The fresh leaves of *vitexnegundo* were collected in the month of January 2022 from forest area near the Dehurwada Digras in Yavatmal district region, Maharashtra, India.

Preparation of crude extract-

The preparation of exact is done by using the process of maceration. The different solvents take like ethanol, methanol, chloroform & water.

The plant drug is used to dry under the shade and ready for coarse grinding by using the grinder. Once the powder is set in the maceration assembly it is allow for the 24 hrs under the pressured temperature for the extraction of

crude drug. The different solvents allow to the exatrtion on crude drug. After 24 hrs. the extract is allow to concentrate & dry for the utilisation.

Finally, the extract was transferred to air tight container and stored at cool place for further pharmacological activity.

The crude drug extraction is vary with the solvent which is used.i.e it may be methanol, ethanol, chloroform& water by using the soxhlet apparatus.(Successive solvent determination)

Percentage yield

Following formula was used for calculation of percentage yield of crude extract

$$\% \text{ yield} = \frac{\text{Weights of extractives}}{\text{Weight of the crude drug}} \times 100$$

Method for preparation of liposome

Fusion method

First, components of lipid phase (Table 1) and propylene glycol were kept at 60°C water bath to form uniform lipid phase. Then, herbal extract dissolved in suitable amount of acetone was added to lipid phase. To evaporate acetone, the mixture was kept at 60°C. Subsequently, the aqueous phase (phosphate buffer saline) was warmed up to 60oC and added to lipid phase. Liposomes were formed after 15 min overtaxing. (Jaafari et.al. 2005)

Sr. no	Ingredients	Percent
1	Egg phosphatidylcholine	15 %
2	Cholesterol	2%
3	α tocopherol	0.3%
4	Methyl parabens	0.1%
5	Propyl parabens	0.02%
6	Propylene glycol	7%
7	Ethyl acetate extract	2%
8	Aqueous phase	Up to 100

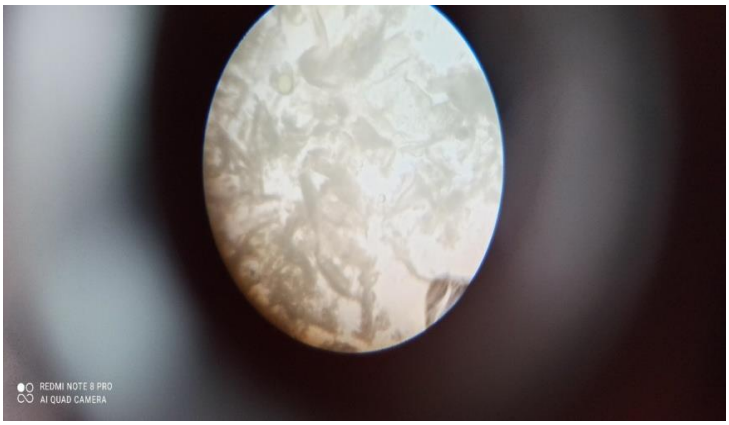


Diagram showing prepared liposomes of vitex by using fusion method.

Pharmacological screening design

It contains two types: chemical & physical method.

Chemical method

- **PTZ- induced convulsion in mice**
    1. Divide the animal into four groups (control, STD, extract, liposomes) consisting of the 3 mice.
    2. Weigh and number them.
    3. Inject pentylenetetrazole to control animal.
    4. Note the onset of action of the jerk movement of the body, duration of the jerk, duration of clonus, extensor.
    5. Inject diazepam to the test group. Inject extract to the third group and liposomes to the fourth group.
    6. After 30 min inject PTZ to these animals which have received STS, extract, & liposomes.
    7. Note the onset and the severity of the convulsion.
    8. Note eighter delay or complete abolition of convulsion in the mice treated with STD, extract, liposome.

Physical method

- **MES-induced convulsion in mice**
    1. Divide animal into four group (control, STD, extract, liposomes) each consisting of 3 rats.
    2. Weigh and number them.
    3. Hold the animal(control) properly place electrodes on the ear pinna.
    4. Apply prescribed current (150 mA for 0.2 sec).
    5. Note the time sec spent by the animal in each phase of the convulsion (a) tonic flexion, (b) tonic extensor phase, (c) clonic convulsion, (d) stupor, (e) recovery or death.
    6. Inject phenytoin intraperitonially to the test group. Extract and liposomes to the third and fourth group.
    7. After 30 min subject the animal to electro convulse meter and hold the animal properly place the electrodes on the ear pinna. Apply prescribed current (150 mA for 0.2 sec).
    8. Note down the reduction in time or ablolism of tonic extensor phase of MES.

Phytopharmacological investigations-

Compound	Chloroform Extract	Methanol Extract	Ethanol extract	Water extract
Carbohydrate	–	–	–	–
Alkaloid	–	+	–	–
Tannis	+	–	–	–
Glycoside	–	+	+	+
Steroids	+	–	–	–
Terpenoids	–	–	–	–
Saponins	+	+	+	+
Flavonoids	–	+	+	+
Soluble materials	–	+	–	+
Cynogenetic glycosides	+	+	+	–

Result

Statistical analysis

Groups	Treatment	Onset of seizure	Recovery / Death
Group I	**PTZ-treated**	**30.66±1.22**	**Death**
Group II	**STD- 80mg/kg**	**33.66± 1.20*** **	**Recover**
Group III	**Ethanol Extract- 400 mg/kg**	**52.00±1.00*** **	**Recover**
Group IV	**Methanol Extract- 400 mg/kg**	**52.00±1.00*** **	**Recover**

Group V	**Chloroform Extract -400 mg/kg**	**51.00±1.00*** **	**Recover**
Group VI	**Water Extract - 400 mg/kg**	**39.00±1.00*** **	**Slightly recover**
Group VII	**Combined extract Liposomes -400 mg/kg**	**56.33±1.00 **** **	**Recover**

Effect of extract on the frequencies of onset of seizure and the duration of clonus in mice.

Value expressed in mean ± SEM [n =3]

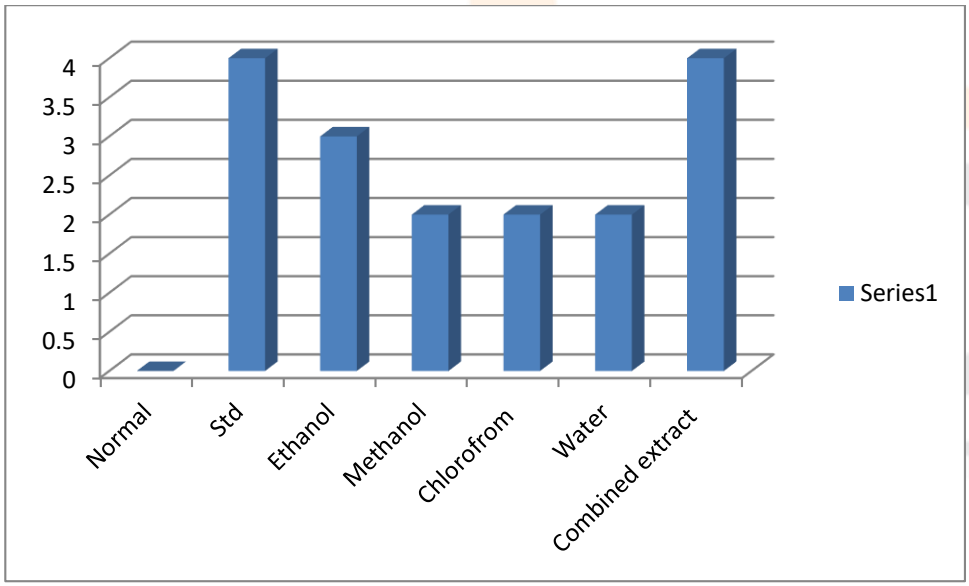
* Statistically significant at (p< 0.012)

** Statistically significant at (p<0.0011)

*** Statistically significant at (p<0.0001)

**** Statistically significant at (p<0.000)

When control group is compared with STD, extracts, & liposomes the STD increase the time of onset of jerk & shows the significant value at (p<0.0011).treated with extract, it prolongs the onset of jerk than the STD so it shows more significance at significant value at (p<0.0001). When treated with liposomes it shows more significant action than the extract at significant value at (p<0.000).



Result of PTZ induced seizure in animal model

The PTZ induced epilepsy in animal model the STD shows the significant value, the extract shows the moderately significant value,& the liposomes shows the highly significant value.

Treatment	Duration of tonic extension	Recovery / death	% protection
Group I (NS-10 ml/kg)	18.8± 1.98	Recover	0 %
Group II (Phenytoin 25mg/kg)	1.4±1.4	Death	100%
Group III (Ethanol Extract 500mg/kg)	5±5**	Recover	80%
Group IV (Methanol Extract 500 mg/kg)	5 ± 3.52***	Recover	85%
Group V (Chloroform Extract 500 mg/kg)	4 ± 3.52***	Recover	70 %
Group VI (Water Extract 500 mg/kg)	3 ± 3.52***	Recover	65%
Group VI (Combined extract liposome's 500 mg/kg)	6± 3.52***	Recover	85%

Conclusion-

The present study concludes that the, The preliminary phytochemical studies were carried out in ethanol, ethanol chloroform & water extract of vitex nugundo &observed the significant presence of alkaloids,carbohydrates, phenol, sterol, tannis, saponis& cynogentic glycosides. However on the basis of our experimental result the higher response of combined extracts shows the significant anti convulsant activity.

Reference

- Epilepsy founder & epilepsy foundation by Joseph I. SirvenMDPattyObsorne Shafer RN, MNon. Tuesday, January 21, 2014.
- SC Ahuja1, Siddharth Ahuja2, and Uma Ahuja3Nirgundi (Vitexnegundo) – Nature's Gift to Mankind Asian Agri-History Vol. 19, No. 1, 2015 (5–32).
- Gollapalle L. Viswanatha1, Marikunte V. Venkataranganna, NunnaBheemaLingeswara Prasad3, Godavarthi Ashok1Evaluation of anti-epileptic activity of leaf extracts of Punicagranatum on experimental models of epilepsy in mice. Journal of Intercultural Ethnopharmacology. DOI: 10.5455/jice.20160904102857.
- V. R. Tandon* and r. K. Guptaan experimental evaluation of anticonvulsant activity of vitex-negundoIndian J PhysiolPharmacol 2005; 49 (2):199–205.
- LucianaCristinaBorgesFernandes,CarlosCamposCamara,andBenitoSoto-BlancoAnticonvulsantActivityofExtractsofPlectranthusbarbatusLeavesinMiceHindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 860153, 4 pages doi:10.1155/2012/860153.
- Karunakar Hegde1*, Shalin P Thakker2, Arun B Joshi3, CS Shastry1, KS Chandrashekhar3Anticonvulsant Activity of Carissa carandas Linn. Root Extract in Experimental Mice Tropical Journal of Pharmaceutical Research, April 2009; 8 (2): 117-125.
- Uzma Bano1 Azhar Jabeen2*, Asrar Ahmed3, M. Akhtar Siddiqui4 World Journal of Pharmaceutical Research.SJIF Impact Factor 5.990 Volume 4, Issue 12, 589-606. Review Article ISSN 2277– 7105.
- OguzSoguta, UmranAydemirSezer, PhDa, b, c, SerdarSezer, PhDa, journal of Drug Delivery Science and Technology journalLiposomal delivery systems for herbal extracts.
- Herciliamarialinsrolim, nereidestelasantos-magaalhaes, iabellamarialinsrolim, short communication of acute toxicity & anticonvulsant activity of liposomes containing nimodipine on pilocarpine induced seizure in mice.
- MangatSingha, Shanti Devib, Virendra S. Ranac, Bhuwan B. Mishraa, JitendraKumarc and VivekAhluwaliaa.Delivery of phytochemicals by liposome cargos: recent progress, challenges and opportunitiesMicro and Nano Carriers.ISSN: 0265-2048.
- http://www.ayurvedaconsultants.com/herb_consult.aspx?commonName=NIRGUNDI accessed on Nov 2010.
- Bansod MS, Harle UN, Vitexnegundo L: Phytochemical constituents, traditional uses and pharmacological properties: Comprehensive review. Pharmacologyonline Newsletter. 2009; 1: 286-302.
- Achari, B., Chowdhuri, U.S., Dutta, P.K. and Pakrashi, S.C. 'Two isomeric flavones from Vitexnegundo', Phytochemistry, 1984; 23: 703-704.

- Azharjabeen, therapeutics uses of vitexnegundo in the world journal of pharmaceutical research. Volume 4, Issue 12, 589-606.
- Azhar-Ul-Haq, Malik, A., Khan, M.T.H., Khan, S.B., Anwar-Ul-Haq, Ahmad, A. and Choudhary, M.I. 'Tyrosinase inhibitory lignans from the methanol extract of the roots of VitexnegundoLinn. and their structure–activity relationship', Phytomedicine, 2006; 13: 255-260.

  Epilepsy foundation. Authored By: Steven C. Schachter, MDPattyObsorne Shafer RN, MN Joseph I. SirvenMDon 07/2013 Reviewed By: Joseph I. SirvenMDPattyObsorne Shafer RN, MNon Wednesday, March 19, 2014
- Sayyah,1, * A. Khodaparast,1,2 A. Yazdi,3 and S. Sardari, Screening of the anticonvulsant activity of some plants from Fabaceae family in experimental seizure models in mice. In the review article.
- Carlos Campos Câmara,1 and Benito Soto-Blanco in the Anticonvulsant Activity of Extracts of Plectranthusbarbatus Leaves in Mice Luciana Cristina Borges Fernandes.
- Chawla, A.S., Sharma, A.K. and Handa, S.S. 'Chemical investigation and anti-inflammatory activity of Vitexnegundoseeds', Journal of Natural Products, 1992; 55: 163-167.
- Blasi, P., Giovagnoli, S., Schoubben, A., Ricci, M. & Rossi, C. Solid lipid nanoparticles for targeted brain drug delivery. Adv. Drug. Delivery Rev. 59, 454–477, https://doi.org/10.1016/j.addr.2007.04.011 (2007).
- Tam, V. H., Sosa, C., Liu, R., Yao, N. & Priestley, R. D. Nanomedicine as a non-invasive strategy for drug delivery across the blood brain barrier. Int. J. Pharm. 515, 331–342, https://doi.org/10.1016/j.ijpharm.2016.10.031 (2016).
- Jiang, Z., Jacob, J. A., Loganathachetti, D. S., Nainangu, P. & Chen, B. β-Elemene: Mechanistic studies on cancer cell interaction and its chemosensitization effect. Front. pharmacology 8, 105 (2017).
- Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.
- Liu C, Tseng A, and Yang S. 2005. Chinese herbal medicine: Modern Orwa C, Mutua A, Kindt R, Jamnadass R, and Simons A. 2009. Agroforestree Database: a tree reference and selection guide. Version 4.0. (http://www.worldagroforestry.org/af/treedb/applications of traditional formulas. CRC Press, Boca Raton, Florida, USA.
- Merlin Rose and Cathrine L. 2011. Preliminary phytochemical screening and antibacterial activity on Vitexnegundo. International Journal of Current Pharmaceutical Research 3(2):99–101.
- Pandey MM, Rastogi S, and Rawat AK. 2008. Indian Herbal Drug for General Healthcare: An Overview. The Internet Journal of Alternative Medicine 6(1). (DOI: 10.5580/1c51 ISSN: 1540-2584.)
- Wong, H.L.; Wu, X.Y.; Bendayan, R., Nanotechnological advances for the delivery of CNS therapeutics. Adv. Drug Deliv. Rev., 2012, 64, (7), 686-700.
- Gandhi, R.; Laroni, A.; Weiner, H.L. Role of the innate immune system in the pathogenesis of multiple sclerosis. J. Neuroimmunol., 2010, 221, (1-2), 7-14.

- Kohane, D.S.; Holmes, G.L.; Chau, Y.; Zurakowski, D.; Langer, R.; Cha, B.H. Effectiveness of muscimol-containing microparticles against pilocarpine-induced focal seizures. Epilepsia, 2002, 43, (12), 1462-1468.
- Mangat Singh, Delivery of phytochemicals by liposome cargos: recent progress, challenges and opportunities, Journal of Microencapsulation Micro and Nano Carriers ISSN: 0265-2048 (Print) 1464-5246 (Online) Journal homepage: https://www.tandfonline.com/loi/imnc.

- Singh, R., et al., 2016. Antifungal and phytotoxic activity of essential oil from root of SenecioamplexicaulisKunth.(Asteraceae) growing wild in high altitude-Himalayan region. Natural product research, 30 (16), 1875–1879.