International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 6 | Issue 1 | JAN-MAR | 2016 | 207-213
Research Article – Pharmaceutical Sciences

ANTIEPILEPTIC ACTIVITY OF *Alstonia scholaris* LINN. ON MES, PTZ AND STRYCHNINE INDUCED CONVULSIONS IN RATS

Krishna Mohan Chinnala[*1], Durraivel Shanmugam[2] and Madhan Mohan Elsani[1]

[1]*Department of Pharmacology, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad - 500088, Telangana, INDIA.*

[2]*SGT College of Pharmacy, SGT University, Chandhu bhudera, Gurgaon, New Delhi NCR.*

*Corresponding Author Email: drchinnala@gmail.com

ABSTRACT

Epilepsy continues to be a neurological disorder awaiting for safer drugs with improved anticonvulsant effectiveness as currently available drugs fail to provide adequate control of epileptic seizure in about one-third of patients and do not prevent progressive epileptogenic changes. In this regard, the medicinal plants have been an important source to the development of drugs with this biological activity. In the present study ethanolic extract of Alstonia scholaris Linn. was studied for its protective effect against maximum electroshock (MES), pentylenetetrazole (PTZ) and strychnine induced convulsions in Wistar rats. In MES method Seizures were elicited with a 60 Hz alternating current of 150 mA intensity for 0.2 sec. The Wistar rats were pretreated with ethanolic extract of Alstonia scholaris Linn (EEAS) for 14 days and standard group animals with Phenytoin (25mg/kg/i.p.). In PTZ and strychnine induced convulsions method animals were pretreated with EEAS for 14 days and standard group animals with Diazepam (4mg/kg/i.p). On 14th day seizures were elicited by the PTZ (80mg/kg/i.p.) and strychnine (4mg/kg/i.p.) administration. It is found that treatment with ethanolic extract of Alstonia scholaris Linn. (200 mg/kg and 400mg/kg) significantly protected the animals especially hind limb tonic extensor (HLTE) stage in MES induced epilepsy. It is also found that EEAS 200 mg/kg and 400 mg/kg had shown a dose dependent significant increase in onset of clonic convulsions comparable with standard treated animals in PTZ and strychnine induced convulsive rats. The results obtained from this study indicate that EEAS has promising dose dependent antiepileptic activity in Wistar rats against MES and PTZ and strychnine induced convulsions.

KEY WORDS

Antiepileptic activity, Alstonia scholaris Linn., MES, PTZ, Strychnine..

INTRODUCTION

Epilepsy is a neurological disorder denoted by the periodic occurrence of seizures and continuing severe mortal disease throughout the globe; numerous types of epilepsy have been described with variety of pathological conditions. As per a recent study, 70 million people have epilepsy worldwide and nearly 90% of them are found in developing regions[1]. From the 70 million persons with epilepsy worldwide, nearly 12 million patients are expected to reside in India, which contributes to nearly one-sixth of the global burden[2]. There are different multiple health problems that can cause epilepsy for example, brain tumors, either benign or malignant, brain trauma, autoimmune irregularities, and neurological diseases such as stroke and Alzheimer's can lead to epileptic seizures[3]. Epileptic seizures are caused by a disruption in electrical activity among neurons in the cerebral cortex, the most highly developed part of the human brain[4].

Currently available antiepileptic drugs are able to efficiently control epileptic seizures in about 50% of the patients, another 25% may show improvement, whereas the remaining 25% of antiepileptic drugs do not benefit significantly. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult so that a demand for new types of antiepileptics exists. One of the approaches to search for new antiepileptic drugs is to investigate the naturally occurring compounds, which may

belong to new structural classes. One of the approaches to search for the new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes. Herbal medicines are often considered to be gentle and safe alternative to synthetic drugs. More than half of the medicinally important pharmaceutical drugs are either natural products or derivatives of the natural products.

Alstonia scholaris Linn. belonging to family Apocynaceae is an evergreen or briefly deciduous tree up to 40 m. tall, branches horizontally to the main trunk. Large tree to 40 m high, stem to more than 100 cm diameter, often fluted; outer bark light brown or creamish, lenticellate; inner bark with copious white latex; the crown often tiered or interrupted. The plant is distributed many countries, includes India, Sri Lanka, southern China, throughout Malaysia, Philippines, northern Australia, Bismarck's and the Solomon Islands[5]. It is known to be a rich source of alkaloids, flavonoids and terpenes which turns the interest among the scientist to use this plant for therapeutic purposes. Amongst the chemical classes present in medicinal plant species, alkaloids stand as a class of major importance in the development of newer drugs because alkaloids possess a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal plants. However, of the large variety of the alkaloids (about 180 alkaloids) isolated, so far only few have been assessed for biological activities[6]. The principal terpene constituents were also reported in *Alstonia scholaris* like linalool (35.7 %), cis and trans linalool oxides, alpha-terpineol and terpinen-4- ol[7] which are used to treat several central nervous system disorders. The bark is used in Ayurvedic medicine to treat fever, malaria, troubles in digestion, tumors, ulcers, asthma, and so forth. The leaves and the latex are applied externally to treat tumors. The bark and roots are boiled with rice and eaten by girls daily for several weeks to treat excessive vaginal discharge. In Traditional Chinese Medicine, the dried leaves of *Alstonia scholaris* used as an expectorant. Members of the *Alstonia* genus are used around the world to treat malaria[8]. The plant was evaluated for various pharmacological studies like Antimicrobial activity[9], Hepatoprotective activity[10], Anticancer activity[11], Antimutagenic activitiy[12], Immunomodulatory activity[13], Antiasthmatic activity[14], Anti-fertility activity[15], Wound healing activity[16], Analgesic and anti-inflammatory activities[17], Anti-ulcer activity[18], hypoglycaemic activity[19], Antioxidant activity[20]. However there are no reports on the antiepileptic activity of the plant leaves. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS & METHODS

Collection and authentication of plant

The aerial parts of *Alstonia scholaris* Linn. were collected surroundings of Warangal and authenticated by P**rof. Vatsavaya S Raju,** Senior Professor, Department of Botany, Kakatiya University, Warangal, Telangana. A voucher specimen was submitted at Department of Botany, Kakatiya University, Warangal.

Preparation of extract

The leaves were shade-dried and pulverized to coarse powder then passed through the 40 mesh sieve. Weighed quantity of the powder was subjected to continuous hot extraction by using Soxhlet Apparatus at 77 to 80ºC. The extract was then evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. A greenish waxy residue of *Alstonia scholaris* L. was obtained. The dried ethanolic extract *Alstonia scholaris* (EEAS) was stored in desiccators until use.

Preliminary Phytochemical screening Phytochemical Screening

The phytochemical examination of the ethanolic extract of *Alstonia scholaris* was performed by the standard methods[21,22]**.** Further investigation was carried out using the ethanolic extract suspended in1% w/v Sodium carboxy methylcellulose (SCMC).

Experimental animals

Wister rats of either sex weighing 150-200g were obtained from CPCSEA approved (Reg no: 1278/ac/09/CPCSEA) animal house of St.John College of Pharmacy, Yellapur, Warangal, Telangana. The

animals were maintained in a standard laboratory condition with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of institute (Reference No: 03/IAEC/StJCOP/2013) and experiments were conducted strictly according to the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Acute Toxicity Study

The acute toxicity of 90% ethanolic extract of *Alstonia scholaris* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study[23].

Anti epileptic activity

Method I

Maximal electroshock (MES) induced convulsions

Group-I: Served as control (received 1% w/v SCMC, 1mL/100 g).

Group-II:received Standard drug Phenytoin (25mg/kg/i.p)

Group-III: received ethanolic extract of the *Alstonia scholaris* Linn. (200mg/kg/p.o)

Group-IV: received ethanolic extract of the *Alstonia scholaris* Linn. (400mg/kg/p.o)

The Wister rats of 150-200 g of either sex animals (n=6 in each group) were used for the study. All the animals were administrated with respective treatment for 14 days before inducing seizures. On 14th day, seizures are induced to all the groups by using an electroconvulsiometer. Maximal electroshock seizures were elicited by a 60Hz current of 150mA for 0.2sec[24]. A drop of electrolyte solution (0.9% NaCl) was applied to the corneal electrodes prior to application to the rats; this increases the contact and reduces the incidence of fatalities. The duration of various phases (like flexion, extensor, clonus, stupor, and recovery or death) of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition and duration of Hind Limb Tonic Extension (HLTE).

Method II

Pentylenetetrazole (PTZ) Induced convulsions

Group-I: Served as control (1% w/v SCMC, 1ml/100 g)

Group-II:received Standard drug Diazepam (25mg/kg/i.p)

Group-III: received ethanolic extract of the *Alstonia scholaris* Linn. (200mg/kg/p.o)

Group-IV: received ethanolic extract of the *Alstonia scholaris* Linn. (400mg/kg/p.o)

The Wister rats of 150-200 g of either sex animals (n=6 in each group) were used for the study. Group III, IV animals received 200mg/kg, 400mg/kg of EEAS respectively for 14 days and test conducted for antiepileptic activity 1hr after the last doses of extract. PTZ (60mg/kg/i.p) is used as the inducing agent[25]. After the administration of PTZ each animal was placed in an individual plastic cage for observation lasting for 1hr. Seizures and tonic clonic convulsions were recorded. The control group animals were received 1% w/v SCMC regularly while standard group animals were received diazepam (4.0mg/kg/i,p.) on 14th day 1hr prior to PTZ administration[26].

Method III

Strychnine Induced convulsions

Group-I: Served as control (1% w/v SCMC, 1ml/100 g)

Group-II: received Standard drug Diazepam (5mg/kg/i.p)

Group-III: received ethanolic extract of the *Alstonia scholaris* Linn. (200mg/kg/p.o)

Group-IV: received ethanolic extract of the *Alstonia scholaris* Linn. (400mg/kg/p.o)

The Wister rats of 150-200 g of either sex animals (n=6 in each group) were used for the study. The test groups (III and IV) received 200mg/kg, 400mg/kg of EEAS orally for 14 days respectively and test conducted for antiepileptic activity 1 hr after the last dose of the extract. Strychnine (2.5mg/kg) is used as the inducing agent[27]. After the administration of strychnine, the animals were placed in an individual plastic cage for observation convulsions. The control group animals were received 1% w/v SCMC while standard group animals were received Diazepam

(4.0mg/kg/i.p) on 14th day 1hr prior to Strychnine administration[28].

Statistical analysis:

Graph pad prism software 6.0 was used in the statistical analysis of experimental data. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests. $p< 0.001$, $p< 0.01$ and $p< 0.05$ was considered as significant.

RESULTS

The preliminary phytochemical analysis of EEAS showed that the plant has alkaloids, flavonoids, glycosides, terpenes, phenols, proteins, essential oils, Gums and mucilage which are potent anti oxidants.

Effect of EEAS on MES induced convulsions

Phenytoin (PHT) treated animals have shown 100% protection against MES induced convulsions where as EEAS 200 mg/kg and 400 mg/kg have shown 64.5% and 86.33% protection respectively against MES induced convulsions. The EEAS at both doses 200 and 400 mg/kg exhibited significant ($p<0.05$ and $p<0.01$) antiepileptic activity when compared with control (Table 1).

Table 1: Effect of EEAS on MES Induced Convulsions

Groups	Flexion (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	Recovery (sec)
I. Control	9.333±0.3333	12.17±0.4014	18.50±0.2236	38.50±0.2236	175.5
II.PHT (25mg/kg.ip)	4.667±0.3333**	0***	8.333±0.3333**	15.50±0.3416**	64.2
III.EEAS (200mg/kg.p.o)	8.500±0.2236**	7.12±0.2582*	17.17±0.6009**	32.67±0.7601*	105.3
IV.EEAS (400mg/kg.p.o)	5.500±0.2236***	3.167±0.3073***	12.830±0.3073**	17.667±0.4944**	94.2

The values are expressed as mean ± SEM of 6 animals
Comparisons were made between: Group I with Group II, III and IV
Statistical significant test for comparison was done by ANOVA, followed by
Dun net's-'t' test. ***p<0.001, **p< 0.01 and *p<0.05

Effect of EEAS on PTZ induced convulsions

Diazepam treated animals have shown 100% protection against PTZ induced convulsions where as EEAS 200 mg/kg and 400 mg/kg have shown 79.16% and 92.30% protection respectively against PTZ induced convulsions. The EEAS at both doses 200 and 400 mg/kg exhibited significant ($p<0.05$ and $p<0.01$) antiepileptic activity when compared with control (Table 2).

Effect of EEAS on Strychnine induced convulsions

Diazepam treated animals have shown 100% protection against strychnine induced convulsions where as EEAS 200 mg/kg and 400 mg/kg have shown 86.33% and 100% protection respectively against strychnine induced convulsions. The EEAS at both doses 200 and 400 mg/kg exhibited significant ($p<0.05$ and $p<0.01$) antiepileptic activity when compared with control (Table 3).

Table 2: Effect of EEAS on PTZ Induced Convulsions

Groups	Treatment	Latency(onset of epileptic seizure in sec)	Duration of seizures
I	Vehicle control	160.3±3.480	73.17±0.3073
II	Diazepam	648.2±3.092***	11.50±0.2236***
III	EEAS 200mg/kg.p.o	314.5.±4.703**	70.17±0.5426*
IV	EEAS 400mg/kg.p.o	545.5±4.031***	26.17±0.792**

The values are expressed as mean ± SEM of 6 animals
Comparisons were made between: Group I with Group II, III and IV
Statistical significant test for comparison was done by ANOVA, followed by
Dun net's-'t' test. ***p<0.001, **p< 0.01 and *p<0.05

Table 3: Effect of EEAS on Strychnine Induced Convulsions

Group	Treatment	Latency (sec)
I	Vehicle control	128.7±1.202
II	Diazepam	545±2.971***
III	EEAS 200mg/kg.p.o	213.5±1.803*
IV	EEAS 400mg/kg.p.o	470.7±5.925**

The values are expressed as mean ± SEM of 6 animals
Comparisons were made between: Group I with Group II, III and IV
Statistical significant test for comparison was done by ANOVA, followed by
Dun net's-'t' test. ***p<0.001, **p< 0.01 and *p<0.05

DISCUSSION

Maximal electro shock (MES) induced seizures model of epileptic seizure have made possible discovery of anticonvulsant properties of the antiepileptic drugs[29]. The possible mechanism involved in MES stimulation leads to high frequency repetitive potentials, thus opening of Na^{+} channels and thereby increasing the intracellular Ca^{++} levels leads to depolarization of the cell. It is found that treatment with EEAS on rats significantly reduces in the tonic hind limb extensor stage in MES induced epilepsy. Protection against HLTE in MES predicts the ability of EEAS to prevent the spread of seizure discharge from the epileptic focus in the brain. γ- Amino butyric acid (GABA) is known to be a major inhibitory neurotransmitter in the central nervous system and is thought to play important roles in various neurological disorders[30]. One generally accepted mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the picrotoxin–sensitive site at $GABA_A$ receptor complex[31]. Impairment of GABA mediated inhibitory has been implicated in different forms of epilepsy in experimental animal models. Treatment with EEAS on PTZ induced rats significantly reduces the duration of convulsions and increase in the time taken for the onset of convulsions. It is known that strychnine-induced convulsions involve blockage of the inhibitory effect of glycine[32]. Glycine is an inhibitory neurotransmitter. Impairment of glycine mediated inhibitory has been implicated in different forms of epilepsy in experimental animal models. Treatment with EEAS on strychnine induced rats significantly reduces the duration of convulsions and increase in the time taken for the onset of convulsions.

From the above study it concluded that in Preliminary phytochemical analysis shows that alkaloids, terpens and flavonoids are the major components of the EEAS. Hence, these properties could be mediated by several compounds present in the extract and could explain the use of this plant in traditional medicine in the treatment of epilepsy. The study concluded with significant antiepileptic activity of ethanolic extract of *Alastonia scholaris* Linn. against various models of epilepsy. This therefore, supports the traditional use of the plant in the treatment of epilepsy. Further studies are required to isolate the compounds

responsible for the extract's activities and as well as establish its mode of action.

ACKNOWLEDGEMENTS

Authors are sincerely thankful to Management St.John College of Pharmacy, Yellapur, Warangal, for their contribution and facilities provided regarding our Research work.

REFERENCES:

1. Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: A metaanalytic approach. Epilepsia 2010;51:883-90.
2. Senthil Amudhan, Gopalkrishna Gururaj, Parthasarathy Satishchandra. Epilepsy in India I: Epidemiology and public health. *Annals of Indian Academy of Neurology* 2015; 18(3): 263-277.
3. Vincent A, Irani S et al. Potentially Pathogenic Autoantibodies Associated with Epilepsy and Encephalitis in Children and Adults. *Epilepsia* 2011; 52(8): 8-11.
4. Fukao K. Psychopathology of epileptic psychosis. In: Matsuura M, Inoue Y. (Eds.) Neuropsychiatric Issues in Epilepsy 2010:35-44.
5. Gardner S, Sidisunthron P and Anusarnsunthron V. A field guide to forest trees of northern Thailand. Kobfai publishing project Bangkok 2000: pp 545.
6. Versha P., Ghosh B., Anroop B., Ramanjit M. Antimicrobial activity of *Alstonia scholaris* leaf extracts. Indian Drugs 2003; 40(7): 412- 413.
7. Dung N.X., Ngoc R.H., Rang D.D., Nhan N.T., Klinkby N., Leclercq P.A. Chemical composition of the volatile concentrate from the flowers of Vietnamese *Alstonia scholaris* (L.) R.Br., Apocynaceae. J. Essent. Oil Res. 2001; 13(6): 424-426.
8. Gandhi M., Vinayak V.K. Preliminary evaluation of extracts of *Alstonia scholaris* bark for *in vivo* antimalarial activity in mice. J. Ethnopharmacol. 1990; 29(1): 51-57.
9. Varshney A., Goyal M.M. Phytochemical study on the leaves of *Alstonia scholaris* and their effects on pathogenic organisms. Ancient Science of Life 1995; 15(1): 30-34.
10. Lin SC, Lin CC, Lin YH, S Supriyatna S and Pan SL. The protective effect of Alstonia scholaris R.Br. on hepatotoxin-induced acute liver damage. Am. J. Clin. Med. 1996; 24(2): 153-64.
11. Keawpradub N., Houghton P.J., Eno-Amooquaye E., Burke P.J. Activity of extracts and alkaloids of Thai *Alstonia scholaris* against human lung cancer cell lines. Planta Med. 1997; 63(2): 97-101.
12. Lim-Sylianco CY, Jocano AP and Linn CM. Antimutagenicity of twenty Philippine plants using the micronucleus test in mice. Philippine Journal of Science 1990; 117(3): 231-235.
13. Iwo M.I., Soemardji A.A., Retnoningrum D.S., Sukrasno U.U.M. Immunostimulating effect of pule (*Alstonia scholaris* L. R.Br., Apocynaceae) bark extracts. Clin. Hemorheol. Microcirc. 2000; 23(2-4): 177-183.
14. Channa S., Dar A., Ahmed S., Rahman A.U. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. J. Ethnopharmacol. 2005; 97(3): 469-476.
15. Gupta R.S., Sharma R., Sharma A., Bhatnager A.K., Dobhal M.P., Joshi Y.C., Sharma M.C. Effect of *Alstonia scholaris* bark extract on testicular function of Wistar rats. Asian J. Androl. 2002; 4(3): 175-178.
16. Arulmozhi S.L., Rasal V.P., Sathiyanarayanan L., Ashok P. Screening of *Alstonia scholaris* Linn. R. Br., for wound healing activity. Oriental Pharmacy and Experimental Medicine 2007a; 7(3): 254-260. S. Arulmozhi *et al.,*2007.
17. Arulmozhi S., Mitra Mazumder P., Ashok P., Hulkoti B., Narayanan L.S. Antinociceptive and anti-inflammatory activities of *Alstonia scholaris* Linn. R.br. Phcog. Mag. 2007b; 3(10): 106-111.
18. Arulmozhi S., Mazumder P.M., Sathiyanarayanan L., Thakurdesai P.A. Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris*. Pharmacologia 2012; 3(5):132-137.
19. Akhtar S.M., Bano H. Hypoglycemic effect of powdered *Alstonia scholaris*(Satona). Prof. Med. J. 2002; 9: 208-271.
20. Arulmozhi S., Mitra Mazumder P., Ashok P., Sathiya Narayanan L. *In vitro* antioxidant and free radical scavenging activity of *Alstonia scholaris* Linn. R.Br. Iranian Journal of Pharmacology and Therapeutics2007ca; 6(2): 191-196.
21. Kokate CK. *Practical Pharmacognosy*. Vallabh Prakasham Delhi, 5th Edition 1991: 107-121.
22. Jayaraman J. *Laboratory Manual in Biochemistry*. New age International (P) Ltd, 1st Edition 1981: 51.
23. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.

24. Balakrishnan S, Pandhi P, Bhargava VK. Effects of Nimodipine on the efficacy of commonly used anti-epileptic drugs in rats. *Ind J Exp Biol* .1998; 36:51-54.
25. Swinyard EA. Laboratory evaluation of antiepileptic drugs: review of laboratory methods. Epilepsia 1969; 10: 107–19.
26. Kulkarni SK. *Handbook of Experimental Pharmacology*. Vallabh Prakashan, 3rd Edition 1999: 131-134.
27. George Amabeoku, Raymond Chandomba. Strychnine-induced seizures in mice: The role of noradrenaline. Progress in Neuro-Psychopharmacology and Biological Psychiatry 1994; 18(4): 753–763.
28. Rehab F Abdel-Rahman1, Gamal A Soliman2,3*, Hasan S Yusufoglu4 , Irem Tatli- Çankaya5 , Saleh I Alqasoumi6 , Serap Arabci Anul5 and Galip Akaydin. Potential Anticonvulsant Activity of Ethanol Extracts of Cichorium intybus and Taraxacum serotinum in Rats. Tropical Journal of Pharmaceutical Research 2015; 14 (10): 1829-1835.
29. Wlaz P, Potschka H, Loscher W. Frontal versus transcorneal stimulation to maximal electroshock seizures or kindling in mice and rats. *Epilepsy Research* 1998; **30**: 219-229.
30. Kehr J and Ungerstedt U. Rapid Communication Fast HPLC Estimation of y-Aminobutyric Acid in Microdialysis Perfusates: Effect of Nipecotic and 3-Mercaptopropionic Acids. *Journal of Neurochemistry* 1988; **51(4)**: 1308-1310.
31. Obay BD, Tasdemir E, Tumer C, Bilgin HM, Sermet A. Anti epileptic effects of ghrelin on pentylenetetrazole-induced seizures in rats. *Journal of Peptides* 2007; **28**: 1214-1219.
32. Alice A. Larson and Alvin J. Beitz. Glycine Potentiates Strychnine-Induced Convulsions: Role of NMDA Receptors. The Journal of Neuroscience 1988; 8(10): 3822-3828.

***Corresponding Author:**
Krishna Mohan Chinnala
Email: **drchinnala@gmail.com**