International Journal of Pharma Research

Vol.9 • Issue 1 ISSN 0975-3532 January - June 2018

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

I J P R

The Research Publication from
PSG COLLEGE OF PHARMACY
Coimbatore 641 004, Tamil Nadu, INDIA

No Processing Charge & Publication Fee

www.psgpharma.ac.in

INTERNATIONAL JOURNAL OF PHARMA RESEARCH (IJPR)
THE OFFICIAL PUBLICATION OF PSG COLLEGE OF PHARMACY

International Journal of Pharma Research (IJPR) is the official publication of PSG College of Pharmacy, Coimbatore, Tamilnadu, India. It mainly covers the articles from different areas of pharma research from Academia, Industry and Community & Hospital Pharmacy.

EDITORIAL BOARD MEMBERS

EDITORIAL ADVISORY BOARD - INTERNATIONAL

Sl. No.	Name	Institution	Place
1.	Dr. Imran Amad JINA,	Pharmaceutical Inc Illinois	USA .
2.	Dr. Paul Heng	National University of Singapore	Singapore
3.	Dr. Ibrahim A Alsarra	King Saudi University	Saudi Arabia
4.	Dr. Sanjay Sehgal	Aexelar Regulatory Experts	USA
5.	Prof. Oluwatoyin. A Adeku	University of Ibadan	Nigeria.
6.	Dr. AJM Christeena	Taylors University	Malaysia
7.	Dr. Jay Ramapuram	AUBURN University	Auburn
8.	Dr. Sathis V Kamath	New Product development	USA.
9.	Dr. B. Raj kapoor	Sebha University	Libya
10.	Dr. Syed AzharSyed Sulaiman	Penang	Malaysia.
11.	Dr. Arun Shirwaikar	Gulf Pharmacy College	Ajman, UAE

EDITORIAL ADVISORY BOARD – NATIONAL

Sl. No.	Name	Institution	Place
1.	Dr. N. Udupa	Manipal College of Pharmaceutical Sci.	Manipal
2.	Dr. R. Manavalan	Annamalai University	Chidambaram
3.	Dr. K. Kannan	Annamalai University	Chidambaram
4.	Dr. K.P. Mohankumar	IICB	Kolkata
5.	Dr. R Padma	Sahasra Institute of Pharmaceuticals	Warangal
6.	Dr. Tuhinadri sen	Jadavpur	Kolkata
7.	Dr. Narayana Charyulu	NGSM Institute of Pharmaceutical science	Manglore
8.	Dr. Molly Matthew	Malik Deenar College of Pharmacy	Kasaragod.
9.	Dr. Sreenivas Reddy	Manipal College of Pharmacy	Manipal
10.	Dr. K. Gowthamarajan	JSS College of Pharmacy	Ooty
11.	Dr. Joyamma Vargees	Trivandrum Medical college	Trivandrum.
12.	Dr. A. Abdul Hassan	Madurai medical College	Madurai
13.	Prof. A.J. Chacko	MG University	Kottayam
14.	Dr. C Vijaya	Ultra College of Pharmacy	Madurai
15.	Dr. A.Rajasekaran	KMCH College of Pharmacy	Coimbatore
16.	Dr. N Nagaraj	Sri Padmavathi Mahila visva Vidyalaya	Tirupathi
17.	Dr. K. Ruckmani	Anna University	Tiruchy
18.	Dr. S. Mohan	Karpagam College of Pharmacy	Coimbatore
19.	Prof. K.S. Lakshmi	SRM University	Chennai
20.	Dr. H.G. Shivakumar	JSS College of Pharmacy	Mysore
21.	Dr. T.K. Ravi	College of Pharmacy SRIPMS	Coimbatore

SJIF#, GIF#, Ulrich's Web#, Jour Info# - Under evaluation

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Dengue-An Overview

Vinny Therissa Mangam*, Vimala Rani Nallam, Anasuri Anitha, Pappala Rama Devi, Manchala Sanisha.

Department of Pharmaceutical Analysis & Quality Assurance,
Aditya Pharmacy College, Surampalem - 533 437, India.
*E.mail: vinnytherissa@gmail.com

Received date: 22.12.2017 Accepted date: 22.01.2018

ABSTRACT

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of India in recent years. Dengue virus is transmitted by female mosquitoes (*Aedes aegypti*). This mosquito also transmits Chikungunya, yellow fever and Zika infection. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. There is no proper medication for dengue. Only by awareness, spreading of dengue to humans can be reduced. Now, there is a need to know the dengue. In present study, we reviewed Dengue related information.

INTRODUCTION

Dengue fever is a mosquito borne tropical disease caused by the dengue virus. It is caused by the organism *Flavivirus sps* through the Vector *Aedes sps.* Synonyms[1-3] for dengue fever was Breakbone fever, Dandy fever, Dengue hemorrhagic fever, Dengue shock syndrome. Severe dengue (also known as Dengue Haemorrhagic Fever) was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today, severe dengue, affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children and adults in these regions[4].

Causative Organism

Dengue is caused by a virus member of the genus *Flavivirus* and family Flaviviridae. This virus is 50nmin size and contains a single strand RNA. There are 4 serotypes of this virus DEN1, DEN2, DEN3 and DEN4[5]. There is a short lived cross immunity between these species. Flavivirus are spherical in shape and having diameter of 40-60mm.The genome in Flavivirus is RNA infectious enveloped virus having positive sense, single sense RNA and its size is 11Kb. The Flavivirus consists of viral genome, nucleocapsid, E&M proteins, and viral envelope. Figure 1 shows image of flavivirus.

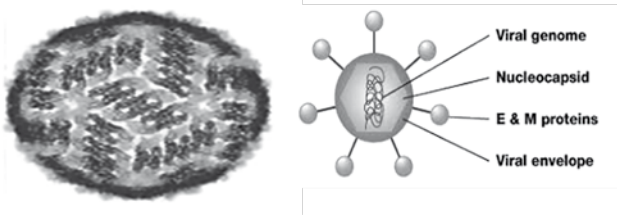


Figure 1: Flavivirus

Viral Classification[3]

The dengue viruses (species) are positive single stranded RNA viruses in the order unassigned, genus Flavivirus, family Flaviviridae.

Steps Involved in Dengue Virus Production in Body

Through mosquito (vector) transmission, dengue viruses enter in to the human skin and reach blood stream. By HSP/DC-SIGN dependent binding, it enters in to the

platelet. In platelet, the following process happens to produce dengue virus. Those are decapsidation, (+) ssRNA release, Replication and Maturation. The above steps are involved in dengue virus production in the human body. Figure 2 explains the steps involved in dengue virus production in the body.

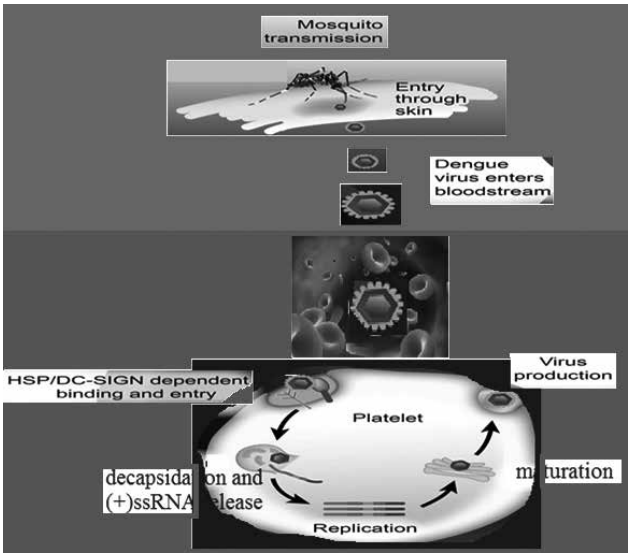


Figure 2: The steps involved in dengue virus production in body.

The Vector

Globally, *Aedes aegypti* (yellow fever mosquito) is an important vector, but it has geographical limitation. The most potent vector having epidemic potential is *A. aegypti.* In India, *A.tigris* was the most widely causes dengue. Other species are *Aedes albopictus, A.Stegomyia, A.Ploynesiensis, A.Scutellaris* and *A.Finalaya* also causes dengue. Reports[6] suggested that *Aedes aegypti* tends to be more susceptible to infection by DEN2 virus of southeast Asian genotype compared to American genotype. Local vector of Asian countries has increased tendency to transmit dengue infection particularly DEN2.Once infected, humans become the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of an infected person for 2-7 days, at approximately the same time that the person develops a fever. Patients who are already infected with the dengue virus can transmit the infection via Aedes mosquitoes after the first symptoms appear (during 4-5 days; maximum 12)[4].

Symptoms

The Primary symptoms of dengue appear within 3 to 15 days after the mosquito bite. The symptoms include high fever and severe headache, joint pain, muscle and bone pain, rash, and mild bleeding lymph nodes of the neck and groin may be swollen. Dengue infected by the young children and people infected for the first time typically has milder symptoms than older children and adults[7]. The severity of disease depends on the quantum of plasma leakage which is due to hemoconcentration, hypoproteinemia and/or serous effusion caused by dengue virus[8,9].

Platelet Count Reduction

In dengue fever, platelets are destroyed and regeneration is majorly affected, leading to low count. Those with platelet counts less than 20000 units and clinical parameters such as bleeding or rashes require immediate transfusion to check hemorrhage. As per Indian red cross society data, demand for platelet units has gone up from 7-8 units/day to 100- 200 units/day[10].

Strategy in India

Till 1996, there was no reliable data to assess the exact magnitude of the dengue disease in India[11]. In 1996, more than 10000 cases and 400 deaths from dengue fever were reported in Delhi. DEN2 was a predominant serotype observed in 1996. In 2003, all dengue serotypes were detected in northern states[12]. Highest cases of dengue, which was reported till 2nd July, 2017 were found to be 9,104 cases in Kerala, 4,174 cases in Tamilnadu, 1,945 cases in Karnataka, 616 cases in Gujarat and 606 cases in Andhra Pradesh[13]. As per National vector borne disease control program (NVBDCP) by Directorate General of Health Services, Ministry of Health and family welfare, five top listed states[14,15] which have dengue cases and deaths in the country since 2011 is tabulated in table 1.

Table 1: As per NVBDCP, dengue and death cases since 2011.

State	2011		2012		2013		2014		2015		2016		2017	
	c	d	c	d	c	d	c	d	c	d	c	d	c	d
Kerala	1304	10	4172	15	7938	29	2575	11	4075	25	7439	13	19206	35
Tamil nadu	2501	9	12826	66	6122	0	2804	3	4535	12	2531	5	16086	52
Karnataka	405	5	3924	21	6408	12	3358	2	5077	9	6083	8	14690	5
Gujarat	1693	9	3067	6	6272	15	2320	3	5590	9	8028	14	2979	4
Andhra pradesh	1209	6	2299	2	910	1	1262	5	3159	2	3417	2	3177	0
Provisional till 29th oct, 2017 Report upto 4th oct, 2017 C=cases, d=deaths														

Current Diagnosis

Oral rehydration therapy, non-steroidal anti inflammatory therapy, patient counsel and platelet transfusion are diagnosis process for dengue fever. The current diagnosis of dengue by Viral isolation, Nucleic acid detection like RT-PCR (Reverse transcription polymerase chain reaction), Real time RT-PCR & Isothermal amplification methods and also by the Detection of antigens. Serological tests like igM/IgG ratio and igG ELISA and Hematological tests like Platelet count and Haematocrit values are important diagnosis test for dengue[13].

Treatment

An herbal concoction – juice of papaya leaf, common neem and hill neem been given to dengue patients in government hospitals in Tamilnadu has been found to have antiviral properties[16]. No drug and vaccine are available, vector control is the only method of choice. Attempts to identify a potential antiviral for the treatment of dengue has been faced with several challenges such as the presence of four distinct viral serotypes which frequently undergo mutations. Effective vaccination to prevent Dengue Hemorrhagic Fever (DHF) requires tetravalent vaccine because preexisting heterotypic dengue antibody is a risk factor for DHF. Treatment consists of pain medications (Analgesics, NSAIDS like aspirin, ibuprofen should be avoided) and fluids like IV fluids. Severe cases require hospital care. Supportive care like Fluid replacement i.e, Supplies or replenishes water and nutrients in the body are required. Oral rehydration therapy (Giving fluid by mouth) is used to treat dehydration caused by diarrhoea. Self-healing-Condition usually improves over time without treatment for few cases[17].

Vaccine

No current dengue vaccine is present. Vaccine development is problematic as the vaccine must provide immunity to all 4 serotypes. Even though, first vaccine is introduced by 'Sanofi Pasteur'. The vaccine has approved by The Health Sciences Authority (HSA), Singapore. The vaccine name is Dengvaxia. It was launched in late 2015. It was against Den-1 and Den-2 strains which account for three-quarters of the dengue cases in Singapore. The vaccine's efficacy was 50 per cent and 40 per cent respectively, compared with 75 and 77 per cent for the other two strains. The vaccine was approved for use in anyone aged 12 to 45 years.

As studies showed that the risk of hospitalization from dengue was reduced in those above 12 years old, but also showed that the vaccine was not very effective in those aged above 45. The disadvantage of this vaccine is that it is not providing immunity to all 4 serotypes[18].

Natural Medicine

Papaya & neem leaf extract: A herbal concotion – juice of papaya leaf, common neem and hill neem being given to dengue patients[19]. King institute director Dr.P.Gunasekaram from Chennai said the preparation of neem and papaya leaves effective in preventing and treating disease[20].

Mechanism of Papaya and Neem Leaves in Dengue Fever Treatment

Papaya leaves increase platelet count. Vitamin C present in papaya leaves stimulates immune system. Antioxidants of papaya leaves eliminate excess toxins in blood. This preparation blocked the virus from entering cells. In cells where the virus had already entered, it prevented multiplication. The papaya leaf extract is used for the treatment of thrombocytopenia associated with dengue. It inhibits heat-induced and hypotonicity-induced hemolysis of erythrocytes obtained from both healthy individuals and individuals with dengue infection[21,22]. It is proved by studies on animals[23] and humans[24].

Neem leaves extract increase blood platelets and WBC and increase the immune system. Neem leaves consist Nimbin and Nimbidin which have anti-microbial properties[25]. Nimbolide show antimalarial activity by inhibiting the growth of Plasmodium falciparum[26]. Other constituents like Azadirachtin and Gedunin also have antimalarial activity. Nimbolide also shows antibacterial activity against S. aureus and S. coagulase[27,28]. Other constituents like Nimbidin, Mahmoodin and Margolone shows antibacterial activity. Gedunin isolated from neem seed oil, Cyclic trisulphide, cyclic tetrasulphide and nimbidin have been reported to possess antifungal acivities[29]. Neem protects against chemically induced carcinogens and liver damage by boosting antioxidant levels[30].

The combination of neem leaf and papaya leaf extract is an excellent remedy to treat dengue fever.

Precaution[31]

As no drug and vaccine available, vector control is the only method of choice. For personal, the followings are advisable; those are clothing to reduce exposed skin, using Insect repellent, especially in early morning and late afternoon, preferring of Bed netting, using Mosquito repellants (pyrethroid based) Coils and taken sanitation measures.

Reducing vector breeding sites, follows solid waste management; creating public education and awareness and removing empty water containers and cut weed/ tall grass in and around the residence are precautions for environmental.

Larvivorous fish (Gambusia), endotoxin producing bacteria (Bacillus) and copepod crustaceans (mesocyclops) are used as a biological origin to target larval stage of Aedes in large water storage containers. Thermal fogging-malathion, pyrethrum, Insecticide treatment of water containers, Space spraying (thermal fogs), Indoor space spraying(2% pyrethrum) and organ phosphorus compounds are used as a chemical for controlling the larval stage of Aedes in surrounding water storage areas.

Discussion

As we discussed earlier, proper medications are not available for dengue fever. Instead of getting fear on dengue virus, changes the mindset of people regarding cleanliness and proper garbage disposal and its health benefits is important. There is a need of proper program to monitor the resistance of vectors which are spreading the disease to the human community.

Conclusion

Prevention is better than cure. As we discussed earlier, No drug and vaccine is available. Vector control is the only method of choice. Avoiding the growth of vector is the best choice to prevent the dengue fever.

Acknowledgement

Authors are thanks to Dr. D. Sathis Kumar, Aditya Pharmacy College for his support and encouragement.

References

1. Available at http://www.who.int/mediacentre/factsheets/fs117/en/ retrieved on 8th July,2017.
2. Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L, and et.al., The global distribution and burden of dengue. Nature 496, 504-507.
3. Dwivedi, V.D., Tripathi, I.P., Tripathi, R.C., Bharadwaj, S., and Mishra, S.K. Genomics, proteomics and evolution of dengue virus. Briefings in functional genomics. 2017, 16(4), 217–227.
4. World Health Organization, Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, WHO, Geneva, Switzerland, 2009
5. Vaddadi Srinivas, Vaddadi Radha Srinivas. Dengue Fever: A Review Article. Journal of Evolution of Medical and Dental Sciences, 2015, 4(29), 5048-5058.
6. Armstrong, P.M., Rico-Hesse, R. Efficiency of dengue serotype 2 virus strains to infect and disseminate in Aedes aegypti. Am J Trop Med Hyg, 2003; 68 : 539-44.
7. Niyati Khetarpal, Ira Khanna. Dengue Fever: Causes, Complications, and Vaccine Strategies, Journal of Immunology Research, 2016, 2016, 1- 14.
8. Lum, L.C., Goh, A.Y., Chan, P.W., El-Amin, A.L., Lam, S.K. Risk factors for hemorrhage in severe dengue infections. J Pediatr. 2002, 140, 629-31.
9. Lye, D.C., Chan, M., Lee, V.J., Leo, Y.S. Do young adults with uncomplicated dengue fever need hospitalization? A retrospective analysis of clinical and laboratory features. Singapore Med J. 2008, 49, 476-9.
10. Available at http://epaper.timesofindia.com/Default/Scripting/ArticleWin.asp?-From=Archive&Source=Page&Skin=TOINEW&BaseHref=CAP/2013/09/19&PageLabel=2&EntityId=Ar00201&ViewMode=HTML retrieved on 4th August 2017.
11. Sharma, S., Sharma, S.K., Mohan, A., Wadhwa, J., Dar, L., Thulkar, S., and et al. Clinical profile of dengue haemorrhagic fever in adults during 1996-outbreak in Delhi, India. Dengue Bull. 1998, 22, 20-27 (WHO-SEARO).
12. Gupta, E., Dar, L., Kapoor, G., Broor, S. The changing epidemiology of dengue in Delhi, India. Virol J. 2006, 3, 92.
13. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva: World Health Organization; 2009; 4.
14. Available at www.livemint.com/science/fmbSpY4DthEK2LaqkkCxSM/over-18700-dengue-cases-reported-in-india-highest-in-kerala.html, retrieved on 12th July, 2017
15. Available at nvbdcp.gov.in/den-cd.html, retrieved on 14th August, 2017
16. Available at https://timesofindia.indiatimes.com/city/chennai/neem-papaya-juice-passes-dengue-test/articleshow/17686175.cms, retrieved on 14th August, 2017.
17. Rosmari Rodriguez-Roche, Ernest A. Gould, Understanding the Dengue Viruses and Progress towards Their Control, BioMed Research International, 2013, 2013, 1-20
18. Available at http://www.straitstimes.com/singapore/health/hsa-approves-worlds-first-dengue-vaccine-for-use-in-singapore retrieved on 18th August, 2017.
19. Available at https://www.organic facts.net/homeremedies/denguefever.html, retrieved on 2nd September, 2017.
20. Available at www.thehindu.com/todays.paper/tp-national/tp-tamilnadu/in-war-on-dengue-papaya-and-neem-juice-the-latest-warriors/article18724861.ece, retrieved on 10th September, 2017
21. Sarala, N., and Paknikar, S.S. Papaya Extract to Treat Dengue: A Novel Therapeutic Option?, Ann Med Health Sci Res. 2014; 4(3): 320–324.
22. Ranasinghe, P., Ranasinghe, P., Abeysekera, W.P, Premakumara, G.A, Perera, Y.S, Gurugama, P., et al. *In vitro* erythrocyte membrane stabilization properties of *Carica papaya* L. leaf extracts. Pharmacognosy Res. 2012, 4,196–202.

23. Patil, S., Shetty, S., Bhide, R., Narayanan, S. Evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in rats. J Pharmacogn Phytochem. 2013, 1, 57–60.

24. Subenthiran, S., Choon, T.C, Cheong, K.C, Thayan, R., Teck, M.B, Muniandy, P.K, et al. *Carica papaya* leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evid Based Complement Alternat Med 2013, 2013, 616737.

25. Kausik, B., Ishita, C., Ranajit, K.B., Uday, B., Biological activities and medicinal properties of neem (Azadirachta indica), Current Science, 2002, 82(11), 1336-1345.

26. Rojanapo, W., Suwanno, S., Somaree, R., Glinsukon, T. and Thebtaranonth, Y., Screening of Antioxidants from some Thia vegetables and herbs, J. Sci. Thailand, 1985, 11, 177–188.

27. Rochanakij, S., Thebtaranonth, Y., Yenjal, C. H. and Yuthavong, Y., Nimbolide, a constitute of Azadirachta indica inhibits plasmodium falciparum in culture, Southeast Asian J. Trop. Med. Public Health, 1985, 16, 66–72.

28. Khalid, S. A., Duddeck, H. and Gonzalez-Sierra, M., Isolation and characterization of antimalerial agent of the neem tree, Azadirachta indica, Journal of Natural Product, 1989, 52, 922–927.

29. Rao, B. S., Nazma and Rao, M.J, Antifungal activity of gedunin, Curr. Sci., 1977, 46, 714–716.

30. Sharma Pankaj, Tomar Lokeshwar, Bachwani Mukesh, Bansal Vishnu, Review On Neem (Azadirachta Indica): Thousand Problems One Solution, International Research Journal Of Pharmacy, 2011, 2(12), 97-102.

31. Guidelines For In tegrated Vector Management For Control Of Dengue / Dengue Haemorrhagic Fever, Govt of India, National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health & Family Welfare.

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Evaluation of Anti Urolithiatic Activity of Ethanolic Extract of Leaves of *Cyamopsis tetragonoloba* (L.,) Taub, in Ethylene Glycol Induced Urolithiasis in Rats

Prasnnadevi Bharathi Dasan*, Radha Ramalingam

Department of Pharmacognosy, Madras Medical College, Chennai, Tamilnadu, India

*E.mail: prasannadevi323@gmail.com

Received date: 16.05.2018 Accepted date: 05.06.2018

ABSTRACT

Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys and bladder. The development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate and phosphate. *Cyamopsis tetragonoloba* is commonly known as Guar "Cluster bean", which belongs to the family Fabaceae. It is an erect annual or perennial vegetative herb. The leaves of the plant are used as a traditional remedy for urolithiasis. The present aim of the study was evaluate anti urolithiatic activity of the ethanolic extract of leaves of *Cyamopsis tetragonoloba* against ethylene glycol induced urolithiasis in rats. Acute toxicity was performed by OECD guidelines. 0.75% v/v ethylene glycol induced urolithiasis in male albino rats for 28 days. The ethanolic extract of leaves of plant was administered orally from the 15th day as curative regimen. Urine analysis and serum analysis were performed. The results demonstrate that the ethanolic extract of leaves of plant has potent antiurolithiatic activity.

KEYWORDS: *Cyamopsis tetragonoloba*, Urolithiasis, Ethylene glycol, Serum and Urine analysis, Ethanolic extract.

INTRODUCTION

Urolithiasis is a worldwide problem, sparing no geographical, cultural or racial groups. It denotes stones originating anywhere in the urinary tract, including the kidneys and bladder [1]. Urinary calculi, if untreated, may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. Surgical operation, lithotripsy and local calculus disruption using high power laser are commonly used techniques to remove the calculi. However, these procedures are expensive and associated with the risk of acute renal injury leading to decrease in renal function. Moreover, an increase in stone recurrence is also observed[2].

Now-a-day, focus on plant research has increased all over the world and a large body of evidence collected shows immense potential of medicinal plants used in traditional systems. *Cyamopsis tetragonoloba* is commonly known as Guar "Cluster bean", which belongs to the family Fabaceae. It is an erect annual or perennial herb. Guar is cultivated in India for vegetable, fodder and green manure. The plant has many therapeutic benefits in treating diabetic, asthma, inflammation and used as laxative, cytotoxic agent, diuretic, anti-ulcer and hypolipideic agent. In view this, it is interesting to know that Cyamopsis tetragonoloba is claimed to be useful in the treatment of urinary stones in literature on traditional system, but the present study is an attempt confirm that urolithiatic activity of the plant which has not yet been proven scientifically. Therefore the present study was aimed at evaluating anti urolithiatic activity of ethanolic extract of leaves of *Cyamopsis tetragonoloba* against ethylene glycol induced urolithiasis in rats [3,4] .

MATERIALS AND METHODS

Plant collection, identification and extraction

Cyamopsis tetragonoloba was collected from Kancipuram, Tamilnadu, India. The leaves of *Cyamopsis tetragonoloba* plant material was botanically identified and authenticated by Dr. K.N. Sunil Kumar, R.O and HOD of Pharmacognosy, Siddha Central Research Institute, Arumbakkam Chennai-106. The leaves were dried under shade, powdered with mechanical grinder. The powder was packed in soxhlet apparatus and extracted with ethanol. The extract was concentrated using rotary evaporator and stored in air right container in a refrigerator.

Acute oral toxicity

Acute oral toxicity study was carried out according to the OECD guidelines- 423(OECD-2001). A limit test at one dose level of 2000mg/kg body weight was carried out with Wistar albino rat (three animals per step). Animals are observed individually at once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. If the test substance related mortality is produced further testing at the next lower dose will be carried out according to guidelines. The animals were observed for changes in skin, fur, eyes, mucous membranes, respiration, and central nervous system.[5]

Experimental Animal

The present study was conducted as per CPCSEA/IAEC approval no: 1917/ReBi/S/16/ CPCSEA/25.10.2016 and 10/AEL/IAEC/MMC, Date: 12.9.2017. The Wistar albino rats (150-200gm) used for this study were produced from Animal experimental laboratory, Madras Medical College, Chennai, India.

Ethylene glycol induced urolithiasis model

After a week of acclimatization, the rats were divided into five groups containing six animals in each.

Group I: Treated with Ethylene Glycol 0.75% v/v (0.75 ml ethylene glycol + 100 ml drinking water) for 28 days

Group II: Ethylene Glycol (0.75% v/v) for 28 days + standard drug Cystone 750 mg/kg bw,p.o (15-28th day).

Group III: Ethylene Glycol (0.75% v/v) for 28 days + Ethanolic extract of *Cyamopsis tetragonoloba* 200 mg/kg p.o (15-28th day)

Group IV: Ethylene Glycol (0.75% v/v) for 28 days + Ethanolic extract of *Cyamopsis tetragonoloba* 400 mg/kg, p.o (15-28th day)

Assessment of Antiurolithiatic Activity

Evaluation of Biochemical Parameters

i) Urine Analysis

On 28th day all animals which were kept in metabolic cages are taken and urine samples were collected. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40C. Urine was analyzed for urine volume, pH, calcium, phosphate, oxalate and magnesium content using available Kits (UV, Molybdate, Calmagite methods).

ii) Serum analysis

After the experimental period, blood was collected from the retro-orbital under anesthetic conditions. The blood was collected and serum was separated by centrifugation at 10,000 rpm for 10min. The serum supernatant was collected and then diluted within the ratio of 1:10. Aliquots of the diluted serum were used for the determination of serum constituents like creatinine and uric acid using the suitable method and serum enzyme activities.

Statistical analysis

Results are expressed as mean ± S.D. Differences among data were determined using one-way ANOVA followed by Dunnett's test. Differences between the data were considered significant at $p<0.05$. [6-11]

RESULTS AND DISCUSSION

Acute Oral Toxicity Study:

Acute toxicity study of ethanolic extract of *Cyamopsis tetragonoloba* leaves were performed according to OECD guidelines 423.

Table 1: Parameters of acute oral toxicity study

Serial No	Parameters	Observations
1.	Condition of fur and skin	Normal
2.	Mucus membrane	Normal
3.	Eyes dullness	Nil
4.	Breathing abnormalities	Nil
5.	Food intake	Normal
6.	Alertness	Normal
7.	Convulsions	Not observed
8.	Salivation	Nil
9.	Morbidity	Nil
10.	Mortality	Nil

From the **table.1** there was no mortality and no changes in general behavior of the animals at 2000 mg/kg of the ethanolic extract of *Cyamopsis tetragonoloba* leaves. The testing doses were selected as 200 mg/kg body weight and 400 mg/kg body weight respectively.

Evaluation Parameters

a. Urine analysis

Ethylene glycol (EG) is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/ aldehyde dehydrogenase to glycolic acid is oxidized to glyoxalic acid, which is further oxidized to oxalic acid by glycolate oxidase. High doses of ethylene glycol (>2500 mg/kg body wt) particularly when given as an oral bolus, cause the saturation dependent accumulation of glycolic acid in the plasma so glycolate oxidase is one of the rate limiting enzymes in the metabolism.

i. Estimation of urine volume and pH of urine

Table 2: Effects of treatment with *Cyamopsis tetragonoloba* leaves of ethanolic extract on urine volume and pH of urine

Parameters	Groups	Ethylene glycol induction				
		Before	After			
		0thday	7th day	Curative regimens		
				14thday	21th day	28th day
Urine volume	Disease control	2.52±0.19	2.39±0.21	2.04±0.17	1.66±0.16	1.25±0.22
	Standard	2.38±0.05	2.21±0.12^{ns}	1.97±0.09^{ns}	5.24±0.19****	5.71±0.16****
	Low Dose	2.36±0.04	2.31±0.07^{ns}	2.14±0.10^{ns}	4.76±0.20****	5.22±0.03****
	High Dose	2.55±0.31	2.37±0.08^{ns}	1.96±0.08^{ns}	5.98±0.18****	6.25±0.16****
pH of urine	Disease control	7.17±0.03	6.81±0.16	6.64±0.08	6.49±0.07	6.47±0.08
	Standard	7.15±0.02	6.94±0.20^{ns}	6.92±0.11^{ns}	7.26±0.08****	7.19±0.07****
	Low dose	7.25±0.02	7.05±0.08^{ns}	7.14±0.08*	7.27±0.03****	7.21±0.04****
	High dose	7.28±0.03	7.09±0.16^{ns}	6.73±0.16^{ns}	7.23±0.06****	7.32±0.02****

Values are expressed as Mean ± SEM, n=6

The statistical analysis was carried out by ANOVA followed by Dunnett's test. $^{*}P \leq 0.05$, $^{****}P \leq 0.0001$, ns - P >0.05 as compared to disease control.

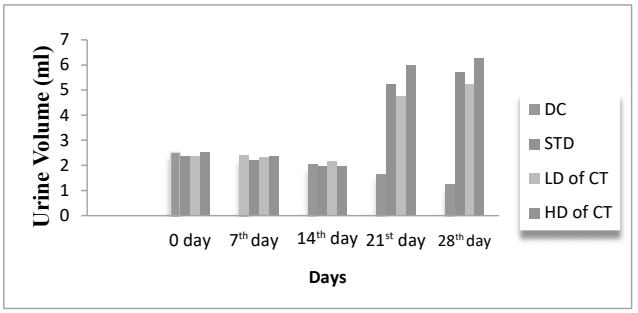


Fig.1: Estimation of Urine Volume

(For statistics details refer table 2.)

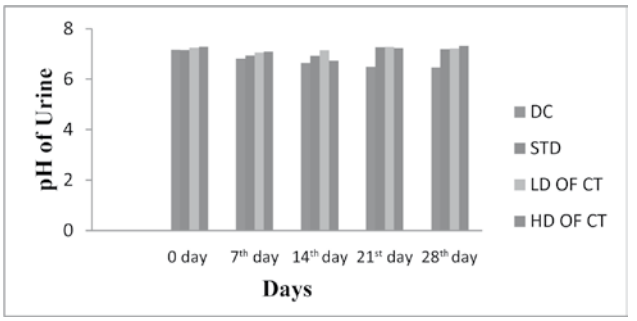


Fig.2: Estimation of pH of Urine

(For statistics details refer table 2.)

The glomerular filtration rate (GFR) is an important parameter for ensuring renal function and it gets decreased in urolithiasis due to the obstruction to the outflow of the urine by stones in urinary system, which leads to decreased in urine volume.

After administrations of ethylene glycol observed significant decrease in urine volume and urine pH (Fig. 1,2) as compared with those of before induction (0 day). Treatment of rats with cystone 750 mg/kg was found to increase urine volume and pH, where as a group receiving the plant extracts also found increase the urine volume and pH in a dose dependent manner.

ii. Estimation of calcium, phosphours, magnesium and oxalate in urine

Table 3: Effects of treatment with *Cyamopsis tetragonoloba* leaves of ethanolic extract on urine calcium, phosphours, magnesium and oxalate levels

Paramaters	Groups	Ethylene glycol induction				
		Before	After			
		0th day	7th day	Curative regimens		
				14thday	21th day	28th day
Calcium	Disease control	7.64±0.21	8.17±0.23	8.62±0.33	9.04±0.13	11.15±0.36
	Standard	7.66±0.22	8.69±0.03*	8.77±0.14ns	8.42±0.04*	7.79±0.04****
	Low dose	7.42±0.06	8.43±0.04ns	8.66±0.05ns	8.36±0.08**	8.12±0.25****
	High dose	7.46±0.06	8.32±0.16ns	8.53±0.10ns	8.12±0.16****	7.65±0.06****
	Disease control	4.77±0.12	6.14±0.13	7.21±0.18	7.94±0.10	8.45±0.11
	Standard	4.43±0.15	4.93±0.07****	5.34±0.09****	4.81±0.06****	4.47±0.08****
Phosphorus	Low dose	4.44±0.12	4.90±0.14****	5.23±0.09****	4.94±0.06****	4.65±0.10****
	High dose	4.61±0.19	4.98±0.13****	5.31±0.14****	4.91±0.13****	4.52±0.15****
Magnesium	Disease control	3.04±0.01	2.96±0.01	2.86±0.01	2.64±0.05	1.75±0.04
	Standard	3.07±0.01	2.89±0.01****	2.66±0.05****	2.79±0.02*	2.88±0.03****
	Low dose	3.06±0.01	2.97±0.01ns	2.88±0.01ns	2.92±0.01****	2.94±0.02****
	High dose	3.08±0.01	2.98±0.01ns	2.79±0.02*	2.94±0.02****	3.04±0.02****
	Disease control	1.37±0.05	2.19±0.25	3.03±0.07	4.41±0.06	5.35±0.05
	Standard	1.56±0.12	2.21±0.19ns	2.59±0.28ns	2.15±0.23****	1.56±0.16****
Oxalate	Low dose	1.48±0.08	1.97±0.07ns	2.05±0.06***	1.74±0.07****	1.61±0.07****
	High dose	1.55±0.06	1.94±0.08ns	2.11±0.11**	1.82±0.08****	1.46±0.06****

Values are expressed as Mean ± SEM, n=6.

The statistical analysis was carried out by ANOVA followed by Dunnett's test. $^{*}P \leq 0.05$, $^{****}P \leq 0.0001$, ns - $P > 0.05$ as compared to disease control.

The level of calcium, phosphours and oxalate were increased significantly after administration of ethylene glycol, inducing the nucleation and precipitation of calcium, oxalate and phosphate. But on administration of ethanolic extracts of leaves of C*yamopsis tetragonoloba* to the animals, the amounts of calcium, phosphours and oxalate in the urine were reduced significantly (Fig. 3,4,5) and magnesium level (Fig. 6) in urine showed a significant decrease in urolithiatic group upon administration of ethylene glycol when compared to before induction (0 day), the increase in urine magnesium level was recovered in animals that treated by plant extract as a consequence of the decrease of growth rate and nucleation of calcium crystals when compared cystone group.

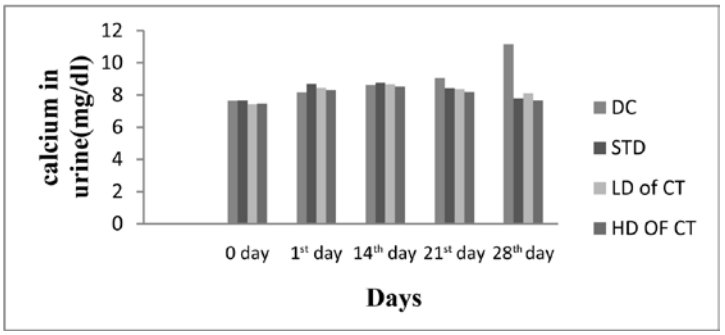
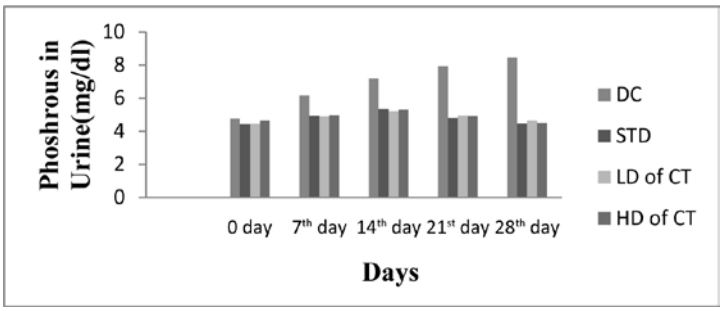


Fig.3: Estimation of Calcium in urine

(For statistics details refer table 3.)

Fig.4: Estimation of Phosphours in urine

(For statistics details refer table 3.)



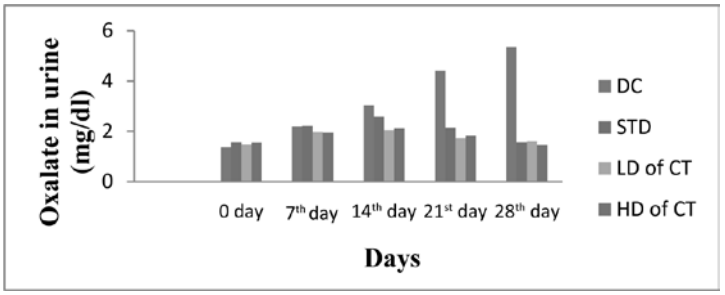
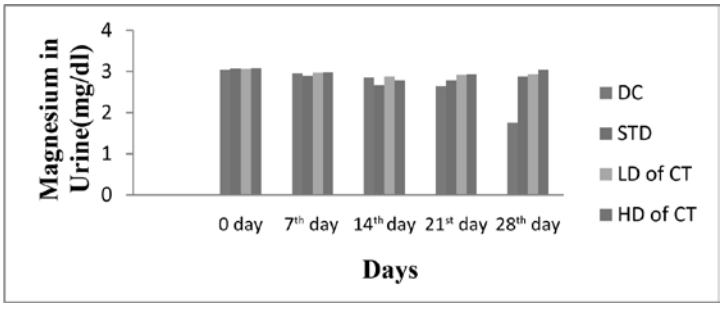


Fig.5: Estimation of Oxalate in urine

(For statistics details refer table 3.)

Fig.6: Estimation of Magnesium in urine

(For statistics details refer table 3.)



b. Estimation of serum constituents

i) Estimation of urea, uric acid, creatinine and LDH level in serum

Table 4: Effects of treatment with *Cyamopsis tetragonoloba* leaves of ethanolic extract on serum parameters

Paramaters	Groups	Estimation of urea, uric acid, creatinine and LDH(mg/dl) in serum				
		Ethylene glycol induction				
		Before	After			
		0th day	7th day	Curative regimens		
				14th day	21th day	28th day
Urea	Disease control	9.62±0.12	10.23±0.07	11.54±0.25	13.54±0.26	15.05±0.14
	Standard	9.74±0.21	10.08±0.21^{ns}	10.95±0.18***	10.39±0.19****	9.89±0.12****
	Low Dose	9.51±0.91	9.83±0.06^{ns}	10.22±0.06****	9.76±0.06****	9.58±0.10****
	High Dose	9.53±0.10	9.96±0.07^{ns}	10.13±0.04****	9.87±0.047****	9.51±0.14****
Uric acid	Disease control	2.42±0.03	2.86±0.03	3.15±0.03	3.56±0.07	4.16±0.19
	Standard	2.39±0.06	2.57±0.06**	2.82±0.04****	2.56±0.04****	2.35±0.04****
	Low Dose	2.47±0.08	2.77±0.03^{ns}	3.07±0.05^{ns}	2.85±0.08****	2.58±0.08****
	High Dose	2.46±0.03	2.81±0.03^{ns}	3.11±0.01^{ns}	2.98±0.05****	2.48±0.06****
Creatinine	Disease control	0.52±0.02	0.67±0.01	0.72±0.01	0.76±0.01	0.79±0.01
	Standard	0.61±0.01	0.64±0.01^{ns}	0.68±0.01^{ns}	0.61±0.06****	0.54±0.01****
	Low dose	0.57±0.01	0.61±0.01**	0.63±0.01***	0.59±0.01****	0.57±0.01****
	High dose	0.58±0.02	0.60±0.01**	0.63±0.01****	0.57±0.01****	0.52±0.01****
LDH	Disease control	173±0.58	182±0.60	198±1.08	234±1.58	258±2.06
	Standard	174±0.61	179±0.60^{ns}	185±0.96****	181±0.86****	176±0.76****
	Low dose	174±0.61	181±0.85^{ns}	187±0.97****	179±0.60****	174±0.67****
	High dose	172±0.57	182±0.60^{ns}	186±0.66****	178±0.33****	171±0.59****

Values are expressed as Mean ± SEM, n=6.

The statistical analysis was carried out by ANOVA followed by Dunnett's test. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{****}P \leq 0.0001$ and ns - $P > 0.05$ as compared to disease control.

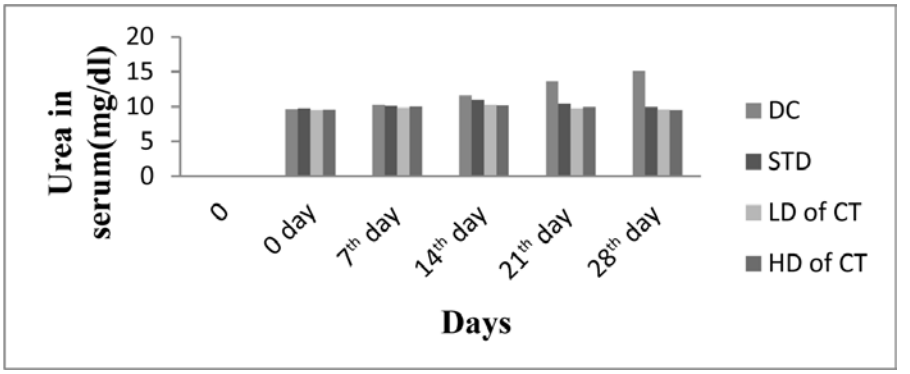


Fig.7: Estimation of Urea in Serum

(For statistics details refer table 4.)

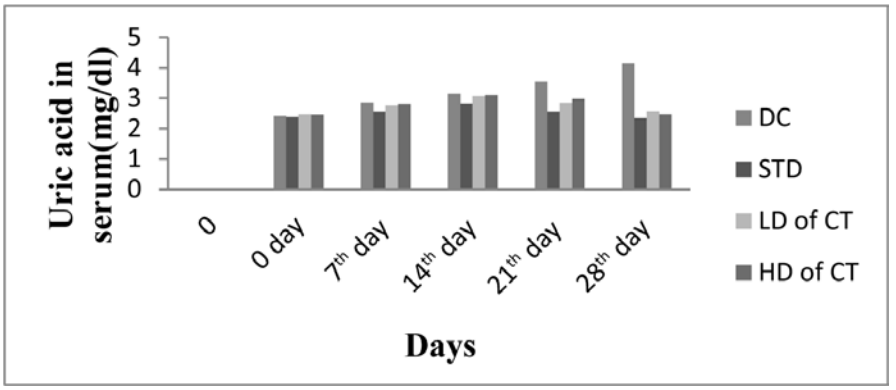


Fig.8: Estimation of Uric acid in Serum

(For statistics details refer table 4.)

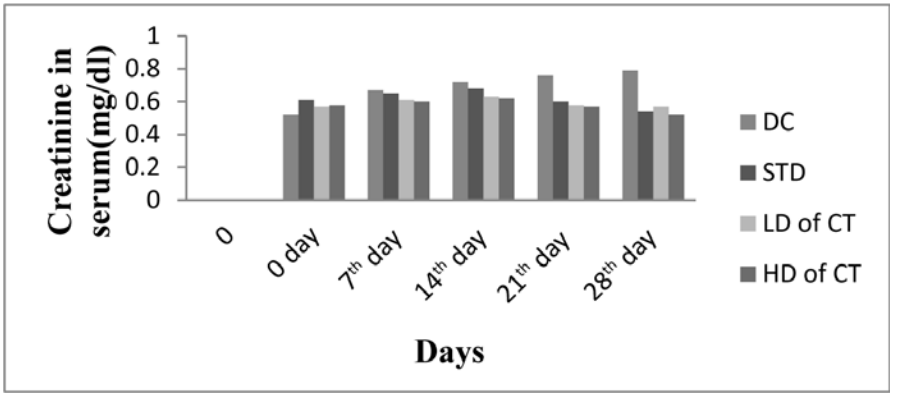


Fig.9: Estimation of Creatinine in Serum

(For statistics details refer table 4.)

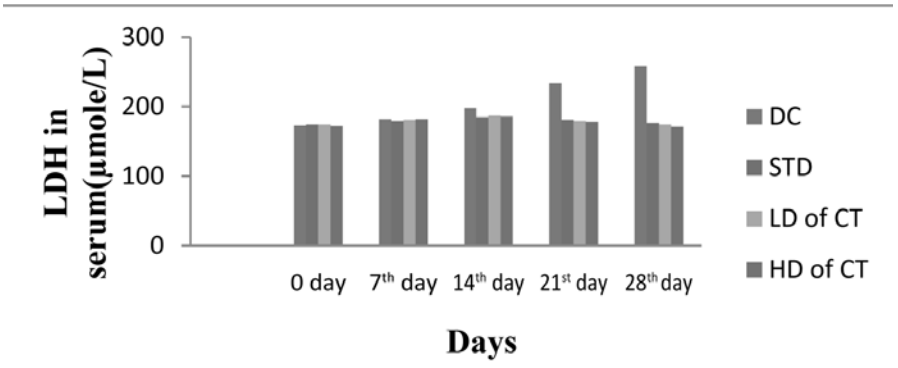


Fig.10: Estimation of LDH in Serum

(For statistics details refer table 4.)

In urolithiasis, there is a decrease in glomerular filtration due to the obstruction of urine flow by stones in urinary system. This causes impairment of renal function resulting in decreased excretion waste products, particularly nitrogenous substances such as urea, creatinine, uric acid and LDH with concurrent accumulation in blood.

After administration of ethylene glycol observed to significant elevation in serum urea, uric acid, creatinine and LDH compared with those before induction 0 day which indicates the renal damage (Fig. 7,8,9,10). However, treatment of rats with plant extracts decreased urea, uric acid, creatinine and LDH level compared with ethylene glycol which as in previous study suggested preventing impairment of renal function.

Conclusion

Urolithiasis is a complex process with the consequence of disturbance between promoters and inhibitors in the urinary tract. *Invivo* antiurolithiatic activity was carried out by ethylene glycol induced urolithiatic model using ethanolic extract. Two doses of the ethanolic extract i.e 200 mg/kg and 400 mg/kg were used for anti urolithiatic activity evaluation. The studies indicated that ethanolic extract at the dose of 400 mg/kg has significant anti urolithiatic activity which was comparable with that of the standard. Hence from these studies it was concluded that the ethanolic extract of leaves of *Cyamopsis tetragonoloba* possess significant anti urolithiatic activity.

Acknowledgements

I would like to acknowledge the Department of Biochemistry Siddha Central Research Institute, Arumbakkam Chennai-106 for allowing any time and laboratory facilities. I would like thank to Dr. R.Rajaraman Veterinary Officer, and also thank to Assistants of Animal Experimental Laboratory House, Madras Medical College, Chennai- 03 for providing animals to carryout Pharmacological studies. I take great pleasure in acknowledging my sincere thanks to all my professors Dr.R.Radha Dr.P.Muthusamy Dr.R.Vijayabharathi Dr.R.Vadivu Kumudhaveni of the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-03 for their valuable suggestions and moral support.

And special thanks to my family and friends for their suggestions and constantly supporting me throughout the work.

Refernces

1. Tiselius HG, Ackermann D, Alken P, Buck C, Conort P, Gallucci M; working party on Lithiasis, European Association of Urology, Guidelines on Urolithiasis, Eur Urol.2001 Oct; 40(4): 362-71.
2. Anil Tukaram Pawar, Niraj S. Vyawahares, Anti-urolithiatic activity of standardized extract of *Biophytum sensitivum* against zinc disc implantation induced urolithiasis in rats; Journal of Advanced Pharmaceutical Technology & Research, 2015; Vol 6(4): 177-182.
3. The Wealth of India, Raw materials, National Institute of Science Communication and Information Resources, Vol-2; Cl- Cy; 2001; 297-317.
4. Kritikar, K.R., & Basu, B.D. Indian Medicinal Plants International Book Distributors Vol-I, 2008, Page No- 706 to 707.
5. http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/ g1423.pdf.
6. P Soundararajan, R. Mahesh, T Ramesh and V Hazeena Begum, Biopotency of *Aerva lanata* on membrane bound ATPases and marker enzymes in urolithiatic Rats*, International journal of Biological Chemistry*, 2007;1: p.221-228.
7. R Sathish, K Natarajan and Mukesh Madhavrao Nikhad, Effect of *Hygrophila spinosa* T.anders on ethylene glycol induced urolithiasis in rats, *Asian Journal of Pharmaceutical and Clinical Research*, 2010;3(4):ISSN-0974-2441.
8. Abdullah H. AL-Gamil, Abeer A. A. Salama, Shayoub M. Elhassen, Zoahair M. Osman, W. EL-Eraky, Azza Hassan, Evaluation of Anti Urolithiatic activity of *Phonenix dactyleferae* seeds extract in ethylene glycol induced urolithiasis in rats. Ijppr. Human, 2017; Vol: 9 (2): 6-20.
9. Tania AV, Cristina DD, Ana PS, Maria TR, Antonio JL, Caden S, Evaluation of the antiurolithiatic activity of the extract of *Costus spiralis* Roscoe in rats, *J Ethnopharmacol*,1999.
10. Christina AJ, Ashok K, Packialakshmi M, Tobin GC, Preethi J, Murugesh N, Antiurolithiatic effect of *Asparagus racemosus* Willd on ethylene glycol-induced urolithiasis in rats, Methods Find Exp Clin Pharmacol 2005; 27: 633-638.
11. King. J, The dehydrogenase or oxidoreductase-N lactate dehydrogenase, In: Practical clinical enzymology, (1965a,b) 83-93.

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Estimation of Ledipasvir and Sofosbuvir by Vierdot's Method in Bulk and Dosage Forms

[1]Udaya Kumar.Thummala[1]*, Eswar Gupta.M[2], Prameela Rani.A[3]

[1]Associate Professor, Aditya College of Pharmacy, Surampalem, East Godavari District - 533437 India

[1]Research Scholar, Jntu Kakinada-533003

[2]Professor, Sir C.R.Reddy College of Pharmaceutical Sciences, Eluru-534007, W.G.District

[3]Anu College of Pharmaceutical Sciences, Nagarjuna University, Guntur

*Email- udayakumar.chowdary16@gmail.com

Received date: 21.02.2018 Accepted date: 14.06.2018

ABSTRACT

A simple, precise, accurate, rapid and specific UV spectroscopic method was developed for the simultaneous estimation of Ledipasvir and sofosbuvir in bulk and dosage form (tablet). The present study is based on Vierdot's method, in which 296 and 260 nm were selected for measuring absorbance of Ledipasvir and Sofosbuvir respectively. The developed method was validated as per ICH guidelines and the results were statistically validated. The method was linear in the range of 5-25µg/ml with r^2 value of 0.998 for both the drugs. Good recovery results were obtainedbetween 97 to 102%. The relative standard deviation for precision and ruggedness was less than 2.0%. The detection limit and quantification limit were found to be 0.00818 and 0.02729µgfor Sofosbuvir and 0.02846 and 0.09489µg for Ledipasvir respectively. The method was successfully applied to the assay of Ledipasvir and Sofosbuvir in tablet dosage form.

KEYWORDS: Ledipasvir, Sofosbuvir, Simultaneous Estimation, Vierdot's Method

INTRODUCTION

Hepatitis C is chronic viral disease of liver. Inhibitors of hepatitis C virus (HCV) limit the progression of infection by HCV. Ledipasvir and sofosbuvir are novel, potent anti-viral agents indicated for hepatitis C infection [1]. Ledipasvir(LPS) is a selective inhibitor of non-structural protein 5A (NS5A), which is involved in viral replication [2]. The chemical name of LPSis (1- { 3-[6-(9,9-difluoro – 7- { 2-[5-(2- methoxycarbonylamino-3-methyl-butyryl)-5-aza-spiro[2.4] hept-6-yl]-3H-imidazol-4-yl}-9H-fluoren-2-yl)-1H-benzoimidazol-2-yl]-2-aza-bicyclol-[2.2.1]heptanes-2-carbonyl}-2-methyl-propyl)-carbamic acid methyl ester. LPS is off-white to yellow coloured powder and exists in amorphous form [3-6]. The structure is shown in figure 1.Sofosbuvir(SOFO) is a potent inhibitor of non-structural protein 5B (NS5B), RNA dependent RNA polymerase required for viral replication. SOFO is chemically known as (S)-isopropyl 2-(((2R,3R,4R,5R) – 5-(2,4-dioxo-3,4-dihydropyrimdin-1(2H) -yl) - 4-fluoro3 hydroxy – 4 – methyltetrahydrofuran – 2 –yl)methoxy)(phenoxy)phosphoryl)amino)propanoate. SOFO is white to off-white powder and is in crystalline form [7-10]. The structure of SOFO is shown in figure 2.The fixed dose combination of LPS (90mg) and SOFO(400mg) was approved by USFDA in 2014 for the treatment of chronic infection of HCV genotype 1[10].

Various methods were reported in literature for estimation of LPS and SOFO individually and in combination using different analytical techniques such as UPLC-MS/MS[11,12], LC-MS/MS[13,14] and RP-HPLC[15-19]. Review of Literature revealed that there was no UV Spectroscopic, vierdot's method reported or available for simultaneous estimation of LPS and SOFO in tablet dosage form.

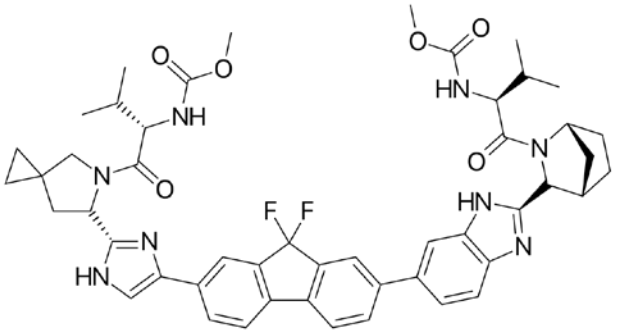


Figure No 1: Structure of Ledipasvir

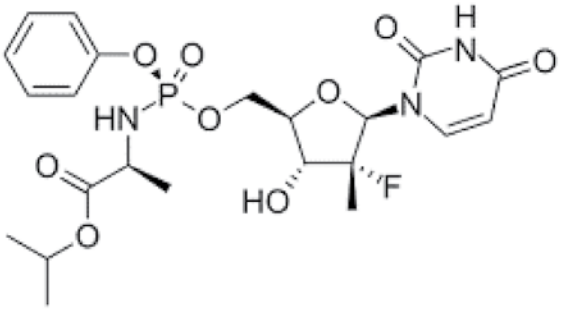


Figure No 2: Structure of Sofosbuvir.

Materials and Methods

An UV-VISIBLE spectrophotometer (UV 3000+, labindia), loaded with UV WIN software, having 1cm quartz matched cells with spectral band width of 190-1100 nm and wavelength accuracy of ±0.1nm is used for the study. The API reference standards were obtained as gift samples from Hetero labs limited. Optimized formulation which consist LPS 90mg and SOFO 400mg was used within shelf life. The solvents used such as methanol, Hydrochloric acid were purchased from Qualigens. All the chemicals used were of analytical grade.

Preparation of standard solutions

Accurately weighed 100mg of LPS and SOFO were transferred into two 100ml clean dry volumetric flasks individually. The drugs were dissolved in 25ml of methanol and volume is made upto 100ml methanol individually. 2.5ml of above each stock solution was diluted to 25ml with 0.1N HCl to obtain the concentration of 100µg/ml of LPS and SOFO respectively (solution B). Further dilutions were made with 0.1N HCl to obtain final concentration of 10µg/ml of LPS and SOFO respectively. The solutions were scanned in the UV range of 200 -400nm. The λ max for LPS and SOFO was found to be 296 nm and 260 nm respectively.

Calibration curve for LPS and SOFO

Appropriate aliquots of stock solution (solution B) of LPS and SOFO were taken into different 10ml volumetric flasks and diluted upto the mark with 0.1N HCl to obtain concentrations of 5-25µg/ml of LPS and SOFO respectively.

Analysis of marketed formulation

Optimized formulation tablet have 400mg of sofosbuvir and 90mg of Ledipasvir. Weight of powdered tablets equivalent to 400mg of SOFO and 90mg of LPS was accurately weighed and transferred into 100ml volumetric flask and dissolve with 30ml of methanol then made up the volume with methanol. The mixture was sonication for 10mins. The solution was filtered through 0.45µm filter. Further dilutions were made with 0.1N HCl to obtain the concentrations of 20 and 4.5µg/ml of SOFO and LPS respectively. The absorbance of this solution was measured at 260 nm and 296 nm. The result was calculated by using simultaneous equation method (Vierdot's method).

Method validation[20]

The developed method was validated according to ICH guidelines for the parameters specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness.

Specificity

Specificity of method is important to check the interference of excipients. A placebo solution with all tablet excipients except API was prepared in the same medium. The solution was scanned in the UV region of 200 – 400nm. The base line obtained was evaluated for the peaks.

Linearity

For linearity assessment five different concentrations(n=3) ranging from 5 – 25µg/ml of LPS and SOFO were analyzed. The absorbance was plotted on Y-axis against the concentration on X – axis. Linear regression equation was developed to evaluate the relation between concentration and response.

Accuracy

The accuracy of analytical procedure was established by analyzing (n=3) three concentrations of 5, 10,15 µg/ml solutions of LPS and SOFO in presence of placebo. The results were analyzed in terms of % recovery.

Precision

Repeatability and intermediate precision studies of LPS and SOFO were carried out by estimating the sample solution (10µg/ml) for 6 times on same day and consecutive day. The corresponding responses were estimated in terms of relative standard deviation. %RSD values were found to be within acceptable limits.

LOD and LOQ

The limit of Detection(LOD) and limit of Quantification(LOQ) were calculated based on calibration curve using the following formula:

$$LOD = 3.3\frac{\sigma}{S}$$

$$LOQ = 10\frac{\sigma}{S}$$

Where, σ = standard deviation of response(absorbance) and S = slope of calibration curve.

Robustness

Robustness of the method was assessed by analyzing (n=6) the test solutions prepared with the solvent of small variation in strength. 0.05N and 0.15N HCl solutions were used to study the robustness of method. The influence of this variation was determined by evaluating the %RSD.

Results

Vierdots method was proposed as a suitable method for the analysis of the drugs LPS and SOFO in dosage forms. A typical overlap spectrogram of standard LPS and SOFO and their mixture was shown in Figure 3. The λ_{max} was found to be 296 and 260 nm. The linearity equation for the LPS calibration curve was found to be y=0.00001x-0.0001(regression coefficient=0.997) and linear over Beer's range 5-25µg/ml. The linearity equation for the SOFO estimation was found to be y=0.00008x-0.00003(regression coefficient=0.997) and linear over Beer's range 5-25µg/ml. The linearity graph of LPS and SOFO mixture was shown in Figure 4. Table 1 was shown the linearity data. The developed method can be used for routine analysis because the linearity found in LPS and SOFO is nearing 1 that is 0.995 and 0.994 respectively which shows the good regression for linearity. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing the model mixtures containing 50%, 100% and 150% of sample solution of the drugs of LPS and SOFO along with 10µg/ml of placebo solutions within the linearity ranges. The mean percentage recovery for LPS was found to be 110.29%, 109.52% and 107.77% for 50%, 100% and 150% concentration respectively. The mean percentage recovery of SOFO was found to be 94.52%, 94.48% and 93.16% for 50%, 100% and 150% concentration respectively. The accuracy data was presented in Table 2. LOD for LPS and SOFO was found to be 0.0284µg and 0.00818 µg respectively. LOQ for LPS and SOFO was found to be 0.09489µg and 0.02729 µg respectively. The percentage purity of LPS and SOFO in tablet dosage form was 99.78±2.49% and 103.6±6.84% respectively. The precision and ruggedness of the optimized method was determined using % RSD of the absorbance for five replicate preparations of the mixture of drug. The % RSD was found to be less than 2. Precision data was represented in Table 3. The absorbance of 10µg/ml of Placebo solution were measured. The results shown that there was no interference at 260 and 296nm. Stability of the solution were determined by bench top stability. Five replicate of optimized concentrations were prepared and its absorbance were measured on 1st and 2nd day at room temperature. % RSD were calculated. The results of bench top stability shown that there is no changes in the solutions even after 2 days at room temperature. The % RSD was found to be less than 2. Robustness were performed by changing HCl molarity (±0.5M) using the same procedure. Absorbance of triplicate solutions using 0.05M HCl and another triplicate solution using 0.15M HCl were measured and % RSD were calculated. The results shows that %RSD was in within the criteria limit. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component is nearing 100%. Therefore this method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents.

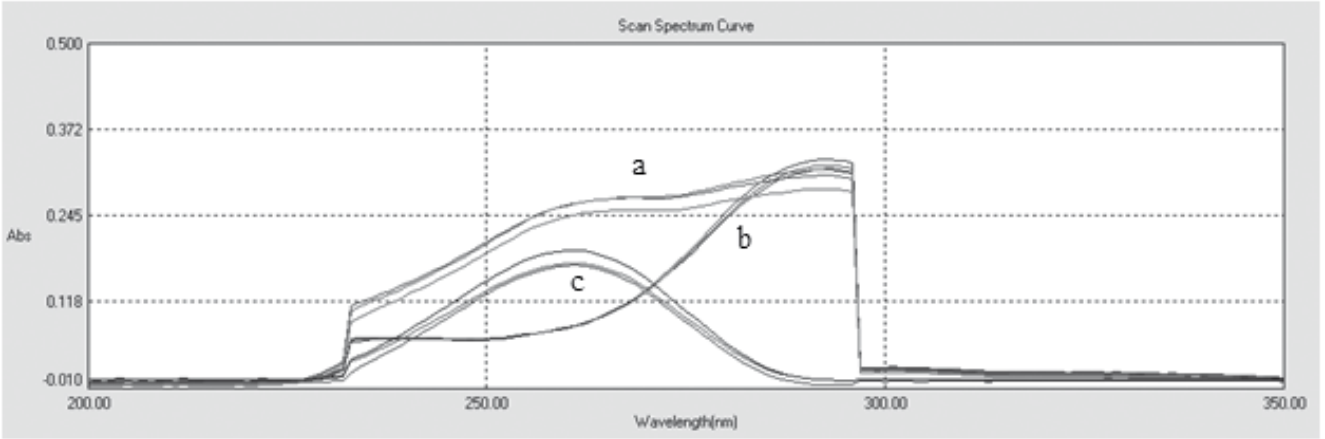


Figure No 3: UV absorbance spectra of LPS+SOFO mixture (a), LPS(b) and SOFO (c).

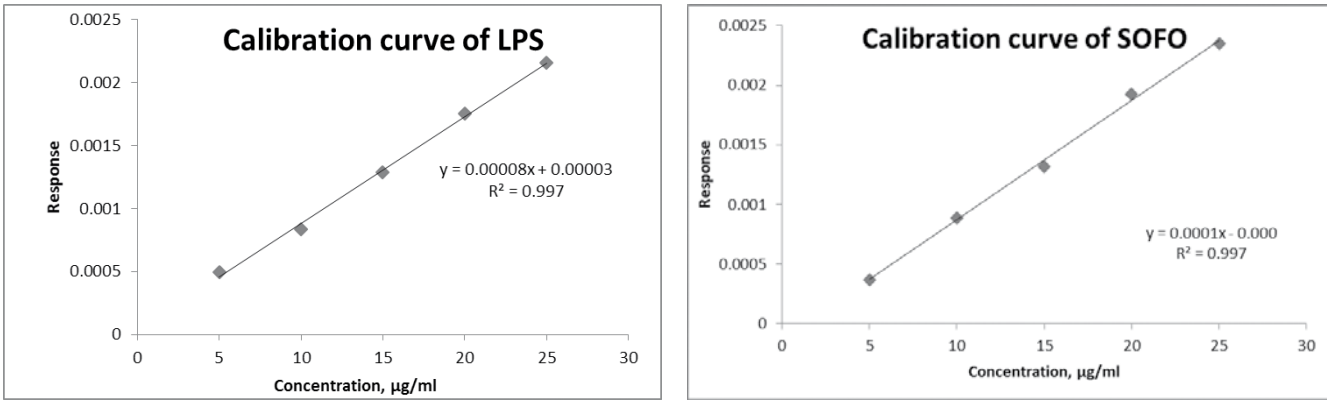


Figure No 4: Calibration curve

Table No 1: Linearity regression data

Parameters	LPS	SOFO
Linearity range (µg/ml)	5-25	5-25
Slope ± SD	0.000085 ± 0.000004	0.000100 ± 0.0000
Intercept ± SD	0.000032 ± 0.000081	-0.000131 ± 0.000027
r^2 ± SD	0.9985 ± 0.004394	±0.000441
R2= correlation coefficient; SD = Standard deviation; LPS= Ledipasvir; SOFO = Sofosbuvir		

Table No 2: Results of Recovery studies.

Concentration (µg/ml)	LPS % Recovery (mean±SD)	SOFO %Recovery (mean±SD)
5	110.299±2.817	94.528±5.357
10	109.521±2.091	94.488±5.175
15	107.770±1.406	93.101±2.886
LPS= Ledipasvir; SOFO = Sofosbuvir; SD= standard deviation		

Table No 3: Data for precision of LPS and SOFO

Concentration	Absorbance at 296 nm	Absorbance at 260 nm	Concentration of LPS	Concentration of SOFO
Mixture 1	0.35	0.281	0.000839	0.000896
Mixture 2 0.343 0.275			0.00082	0.000878
Mixture 3	0.35	0.275	0.00081	0.000897
Mixture 4	0.341	0.273	0.000814	0.00873
Mixture 5	0.334	0.267	0.000795	0.000855
Average	0.34360	0.27420	0.00082	0.00088
SD	0.00673	0.00502	0.00002	0.00002
%RSD	1.95883	1.83077	1.97330	1.98697
SD= Standard Deviation %RSD= Percentage Relative standard Deviation; LPS= Ledipasvir; SOFO = Sofosbuvir				

Table No 4: Summary of validation parameters.

Parameter	LPS	SOFO
Linearity range (µg/ml)	5 – 25	5 – 25
Correlation coefficient ± SD	0.9985 ± 0.004394	0.9986 ± 0.000441
LOD (µg/ml)	0.0284	0.00818
LOQ (µg/ml)	0.0948	0.02729
% Recovery	107 – 110%	93-94%
Precision (% RSD)		
Intraday	1.958	1.83
Interday (ruggedness)	1.872	1.332
Robustness	Robust	Robust
Assay(% w/w)	99.78	103.66
Bench top stability (%RSD)		
First day	1.95	1.83
Second day	2.06	1.96
SD = standard deviation; %RSD = Percentage relative standard deviation; %w/w = Percentage weight/weight; LPS= Ledipasvir; SOFO = Sofosbuvir		

Discussion

The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods[11-19] consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like HCl, methanol and distilled water, which are very simple, economical and also easily available. Also, our proposed method requires less time for the determination of LPS and SOFO simultaneously compared to other methods [11-19] and these methods also required reagents which are costly and time consuming.

Conclusion

The developed method was found to be specific, linear, robust, ruggedness, accurate, precise and economical. The method can be used for routine quality control analysis of LPS and SOFO in tablet dosage form.

Acknowledgements

The authors are grateful to Hetero labs limited, Nakkapally for providing the standard drugs of Ledipasvir and Sofosbuvir. The authors would like to thank The Principal, Dr. K. Ravi Shankar and management of Aditya College of Pharmacy for providing the necessary facilities to carry out the work. The authors extend their gratitude to Dr.D.SathisKumar, Aditya Pharmacy College for his copious help in the present study.

References

1. Chal B, Mogalian E, Oliyan R, Pakdaman R, Stefanidis D, Vahid Zia, Combination formulation of two antiviral compounds, US 14/168, 264.
2. Ivan Gentile, Antonio Riccardo Buonomo, Federico Borgio, GluseppeCastaldo and GuglielmoBorgio. LPSipasvir: A novel synthetic antiviral for the treatment of HCV infection. Expert opinion on Investigational drugs, 2014; 23(4),561-571.
3. Available from URL: https://www.google.co.in/patents/US20140212487.
4. Available from URL: https://pubchem.ncbi.nlm.nih.gov/compound/Ledipasvir.
5. Mohamed El-Kassem M Hassouna[1]*, Maha Mohammed Abdelrahman[2] and Mahmoud Abdelfatah Mohamed[3]:Assay and dissolution methods development and validation for simultaneous determination of Sofosbuvir and Ledipasvir by RP-HPLC method in tablet dosage forms, J Forensic Sci & Criminal Inves 2017; 1(3): 001-0011.
6. Available from URL: https://www.drugbank.ca/drugs/DB09027.
7. Harmeet Kaur Bhatia, Harmanjit Singh, Nipunjot Grewal and Navreet Kaur Natt. Sofosbuvir: A novel treatment option for chronic hepatitis C infection. Journal of pharmacology and pharmacotherapeutics, 2014; 5(4): 278-284.
8. SuryawanshiRajana,ShindeNitin,Tudkar Ganesh et al. development and validation of simple UV spectrophotometric method for the determination of Ledipasvir in bulk form and stress degradation studies. Inventi Rapid: Pharm Analysis and Quality Assurance, 2016;(3):1-5.
9. Available from URL: https://www.drugbank.ca/drugs/DB08934.
10. Available from URL: https://pubchem.ncbi.nlm.nih.gov/compound/sofosbuvir.
11. ChenweiPana, YongpingChenb, WeilaiChenc, GuangyaoZhoua, lingxiangJina, Yi Zhenga, Wei lina, ZhenzhenPanb. Simultaneous determination of Ledipasvir, sofosbuvir and its metabolite in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study. Journal of Chromatography B, 2016; 1008: 255-259.
12. Mamdouh R. Rezk, Emad B. Basalious, Iman A. Karim. Development of a sensitive UPLC-ESI-MS/MS method for quantification of Sofosbuvir and its metabolite, GS-331007, in human plasma: Application to a bioequivalence study. Journal of Pharmaceutical and Biomedical Analysis,2015;114:97-104.
13. Ehab F. Elkady, Ahmed A. Aboelwafa. A rapid and optimized LC-MS/MS method for the simultaneous extraction and determination of sofosbuvir and Ledipasvir in human plasma. Journal of AOAC international, 2016; 99(5):1252-1259.
14. Nebsen. M, Eman S. Elzanfaly. Stability-Indicating method and LC-MS-MS characterization of forced degradation products of sofosbuvir. Journal of Chromatographic science, 2016;54(9):1631-1640.
15. BakhtZaman, Faisal Siddique, Waseem Hassan. RP-HPLC method for simultaneous determination of sofosbuvir and Ledipasvir in tablet dosage form and its application to in vitro dissolution studies. Chromatographia, 2016;79(23):1605-1613.
16. Devilal. J, Durga Prasad. J, Narottam pal and SrinivasaRao.A. New method development and

validation for the determination of Ledipasvir in bulk drug form by using reverse phase HPLC technique. World journal of pharmacy and pharmaceutical sciences, 2016;8(5):1312-1321.

17. Mohan vikas. P, Satyanarayana. T, Vinod kumar. D, Mounika. E, Sri latha. M,Anusha. R and Sathish. Y. Development and validation of new RP-HPLC method for the determination of sofosbuvir in pure form. World journal of pharmacy and pharmaceutical sciences,2016; 5(5):775-781.
18. Raj Kumar.B, Subrahmanyam. KV. A new validated RP-HPLC method for the simultaneous determination of simeprevir and sofosbuvir in pharmaceutical dosage form. Indo American Journal of Pharmaceutical Research, 2016; 6(02):4508-4520.
19. Ravi Kumar Vejendla, SubramanyamCVS, VeerabhadramG. Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP-HPLC. Int J Pharm,2016;6(2):121-127.
20. Available from URL: http://www.gmp-compliance.org/guidelines/gmp-guideline/ich-q2r1-validation-of-analytical-procedures-text-and-methodology.

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Evaluation of in Vitro Antimicrobial Activity of Flower Extract of Albizia Saman

Murali R, Anton Smith A*, Parimalakrishnan S, Jelin Jaralda G and Vinothini N

Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, Tamil Nadu.

*Corresponding Author e-mail: auantonsmith@yahoo.co.in

Received date: 04.06.2018 Accepted date: 18.06.2018

ABSTRACT

To evaluate *in vitro* antimicrobial activity of flower extract of *Albizia saman* (Leguminosae; Family) against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis* and *Bacillus subtilis*. The antimicrobial sensitivity testing was conducted by the agar disc diffusion method. The results obtained from the *in vitro* studies clearly reveals that the flower extracts have great potential in antimicrobial activity. The ethanolic extract of *Albizia saman* flower showed maximum antibacterial activity against *Bacillus subtilis.*

KEYWORDS: *Albizia saman,* Antimicrobial activity, Pathogenic bacteria, Disc Diffusion Method.

INTRODUCTION

Antibiotics are the one of the most important weapons in fighting bacterial infections. Due to the raising prevalence of microorganisms showing resistance to antibiotics, there is a need to develop new antimicrobial compounds. Plants play a significant role in maintaining human health and improving the quality of human life. They serve valuable components of food, such as seasonings and beverages as well as in cosmetics, dyes, and medicines to humans. In fact, many plant extracts prepared from plants have been shown to exert biological activity *in vitro* and *in vivo*, which justifies research on traditional medicine focused on the characterization of antimicrobial activity of these plants [1]. Many plants possess antimicrobial activities and are used for the treatment of different diseases [2]. The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually plants or their extracts and many of these herbal remedies proved successful [3]. Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compound[4]. The ability of plants to grow on different type of soils which are rich in microorganisms possess as a result of their potential to produce wide range of selective antibacterial compounds that are capable of wading off potential microbial invaders [5]. Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002). It is estimated that there are 2,50,000 to 5,00,000 species of plant on earth [6].

Albizia saman formerly called *Samanea saman* (Leguminosae; family), a fast budding tree normally used as pasture for ornamental purposes. The flowers, bark, leaves, roots, seeds and pods of the tree are so far used as medicine from the traditional system[7]. The alcoholic extract of selected leaves inhibit *Mycobacterium tuberculosis*, the alkaloid portion of leaves is active on CNS and as laxative. Seeds are masticated for stinging throat. A decoction from the fresh leaves and inner bark are used for colds, diarrhea, and intestinal problem[8]. The literature search indicates that *Albizia saman* contains alkaloids, glycosides, terpenoids etc., the extracts of flowers possess antioxidant activity[9] and anti cancer activity[10]. The tiny flowers (12–25 per head) are massed in pinkish heads 5-6 cm (2-2.4 inch) across and about 0000004 cm (1.6 inch) in height[11]. The mature pods of

Albizia saman are black brown, almost oblong, clumpy, 10 to 20 cm long, 15 to 19 mm wide, 6 mm thick, slightly curved, eventually cracking irregularly, and filled with brownish pulp which is sticky, sweet and edible. The bottom most line is that pods of *Albizia saman* tree which are rarely used as a plant source for herbs. Knowledge on phytochemical constituents of plant parts is mandatory in understanding the basis for any therapeutic effect. Recently, isolated flavonoids were reported to exhibit antimicrobial activity. In addition to that the flavonoids through their free-radical scavenging activity have evoked multiple biological functions including vasodilatory, anti-carcinogenic, anti-inflammatory, anti-bactericidal, immune stimulatory, anti-allergic and anti-viral functions[8].

There is an urgent need to revolutionized research in herbal medicine and isolated drug discovery, considering the presence of incurable diseases such as HIV AIDS and the threat of new emerging disease such as SARS, bird flu etc. Plants extracts and fractionated plant extracts have been a good source of herbal medicine and natural products/ phytochemicals over the years[12]. Hence we selected the flower of *Albizia saman* to investigate the anti-microbial property against *Escherichia coli* (gram -ve), *Klebsiella pneumoniae (*gram-ve), *Proteus mirabilis* (gram -ve), *Staphylococcus aureus* (gram +ve), *Pseudomonas aeruginosa* (gram -ve), *Enterococcus faecalis* (gram +ve) and *Bacillus subtilis* (gram +ve).

Materials and Methods

Plant Collection and Identification

The fresh *Albizia saman* flowers were collected from Annamalai Nagar, Chidambaram during the month of January to March. Selected samples were taxonomically identified and authenticated by Botanical Survey of India (BSI), Palayamkottai, Tirunelveli District.

Processing of Plant Materials

The flowers of *Albizia saman* were cleaned, shade dried, segregated, pulverized by a mechanical grinder and passed through a Sieve #40. The powdered plant materials were stored in a clean air tight container until needed for analysis with proper labelling.

Preparation of Plant Extracts

Crude plant extract was prepared by Soxhlet extraction method. The flowers were shade dried at room temperature for 10 days. The dried flowers were stored in an air-tight container for future use. About 175g of powdered plant material was uniformly packed into a thimble and extracted with different solvents separately. Solvent used were petroleum ether, ethyl acetate and ethanol as per polarity. The process of extraction continues for 24 hours. The petroleum ether, ethyl acetate and ethanolic extracts were separately concentrated using rotary evaporator and then preserved individually at 5°C in air tight containers until used for the Anti-bacterial activity.

Test Organism Used

Test organisms used were received from microbiology division of Raja Muthiah Medical College Hospital. The stains used were *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 2453), *Proteus mirabilis* (MTCC 1771), *Klebsiella pneumonia* (MTCC 3384), *Staphylococcus aureus* (MTCC 3160), *Enterococcus faecalis* (MTCC 439), *and Bacillus subtilis* (MTCC 441).

Sub Culture:

The inoculation needle was heated to red hot condition in the blue zone of the Bunsen burner flame. The needle was cooled by holding it within the zone of sterility of the flame. The inoculation needle is inserted into the specimen culture tube containing actively growing bacteria. The growth on the surface of the agar slant was touched with the tip of the needle. There is no need to dig or scrape. Too vigorous handling may result in extraneous contaminants finding access into culture. After inoculation the tubes were incubated at 37°C for 24 hours.

Determination of Anti-Microbial Activity

The anti-microbial sensitivity testing was conducted by the agar disc diffusion method. The sensitivity medium (Muller-Hinton agar) was prepared by adding 3.8g of Muller-Hinton agar powder to 100 ml distilled water and autoclaved at 121°C for 15 minutes at 15 lbs, and poured in sterile Petri plates to a uniform thickness of

approximately 4mm and the agar was allowed to set at ambient temperature before use.

The bacterial isolates were suspended in peptone broth and incubated at 37°C for 3-4 hours before used as inoculums. The turbidity of the growth culture was adjusted to McFarland's standard 0.5[13]. The turbidity standard provides an optical density comparable to the density of a bacterial suspension 1.5x 10^8 colony forming units (CFU/ml). A sterile cotton swab inserted into the bacterial suspension, rotated, and then compressed against the wall of the test tube to remove any excess fluid. The swab was then streaked on the surface of the Muller-Hinton agar plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface.

To test anti-bacterial activity of *Albizia saman* plant extract, it was first dissolved in a Dimethyl Sulfoxide (DMSO), and then varying concentrations of the extracts (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml) were soaked on autoclaved discs of Whatman filter paper. These filter paper discs were placed on a streaked Muller-Hinton agar plate surface. The plates were incubated overnight at 37° C for 24 hours. The antimicrobial activity was detected by measuring the zones of inhibition.

To test antibacterial activity of the synthetic antibiotics standardized discs of Ciprofloxacin (5µg), Erythromycin (15µg), Norfloxacin (10µg), Cotrimoxazole (25µg), Ceftriaxone (30µg) and Gentamicin (10µg) were tested by the agar disc diffusion method by placing on a streaked Muller-Hinton agar plate surface. The antimicrobial activity was also detected by measuring the zones of inhibition[14].

Results and Discussion

The results obtained from the *in vitro* studies performed using flower extract of *Albizia saman* against the selected microorganisms are shown in Table 1-4. The ethanolic extract of flower exhibit highest antibacterial activity against *Bacillus subtilis* (14.2 mm inhibition zone) and its lowest antibacterial activity was detected against *Klebsiella pneumoniae* (13.6 mm inhibition zone) at 100mg/ml.

Table No. 1: Mean zones of inhibition (in mm) for different concentrations of *Albizia Saman* flower in ethanolic extract

Bacterial Test Strains	Concentrations of flower extract (mg/ml)				
	100	50	25	12.5	6.25
Escherichia coli 13.8mm 12.6mm			11.7 mm	9.8 mm	8.6 mm
Klebsiella pneumoniae	13.6 mm	12.9 mm	10.0 mm	10.3 mm	8.9 mm
Proteus mirabilis	13.9 mm	12.8 mm	11.3 mm	10.8 mm	7.5 mm
Staphylococcus aureus	14.1 mm	13.2 mm	12.1 mm	10.3 mm	8.3 mm
Pseudomonas aeruginosa	13.7 mm	13.0 mm	12.4 mm	11.2 mm	10.0 mm
Enterococcus faecalis	13.8 mm	13.1 mm	11.2 mm	10.1 mm	9.2 mm
Bacillus subtilis	14.2 mm	13.7 mm	12.2 mm	11.0 mm	10.5 mm

Table No. 2: Mean zones of inhibition (in mm) for different concentrations of *Albizia Saman* flower in petroleum ether extract

Bacterial Test Strains	Concentrations of flower extract (mg/ml)				
	100	50	25	12.5	6.25
Escherichia coli	10.0 mm	9.8 mm	9.6 mm	9.4 mm	8.6 mm
Klebsiella pneumoniae	10.2 mm	10.0 mm	9.9 mm	9.5 mm	8.7 mm
Proteus mirabilis	9.8 mm	9.6 mm	9.6 mm	9.2 mm	8.3 mm
Staphylococcus aureus	10.2 mm	10.0 mm	9.8 mm	9.4 mm	8.9 mm
Pseudomonas aeruginosa	10.2 mm	10.0 mm	9.8 mm	9.5 mm	8.7 mm
Enterococcus faecalis	10.0 mm	9.8 mm	9.7 mm	9.5 mm	8.9 mm
Bacillus subtilis	10.8 mm	10.6 mm	10.2 mm	9.7 mm	9.5 mm

Table No. 3: Mean zone of inhibition (in mm) for different concentrations of *Albizia Saman* flower in ethyl acetate extract

Bacterial Test Strains	Concentrations of flower extract (mg/ml)				
	100	50	25	12.5	6.25
Escherichia coli	12.8 mm	11.5 mm	10.2 mm	10.8 mm	8.0 mm
Klebsiella pneumoniae	12.6 mm	12.3 mm	9.5 mm	10.3 mm	9.2 mm
Proteus mirabilis	13.3 mm	11.4 mm	10.0 mm	9.7 mm	8.9 mm
Staphylococcus aureus	12.5 mm	11.8 mm	10.8 mm	9.5 mm	7.7 mm
Pseudomonas aeruginosa	12.7 mm	11.6 mm	9.7 mm	9.2 mm	10.8 mm
Enterococcus faecalis	12.4 mm	11.7 mm	10.3 mm	9.4 mm	7.6 mm
Bacillus subtilis	13.2 mm	12.6 mm	10.6 mm	9.9 mm	10.0 mm

Table No. 4: Mean zones of inhibition (in mm) for different Antibiotics

Bacterial Test Strains	Antibiotic Concentrations in (µg /disc)					
	CIP	E	NOR	CO	CEF	G
Escherichia coli	16.9 mm	7.5 mm	9.4 mm	10.7 mm	12.0 mm	13.0 mm
Klebsiella pneumoniae	15.8 mm	10.0 mm	9.0 mm	6.4 mm	6.8 mm	12.0 mm
Proteus mirabilis	27.0 mm	6.4 mm	19.0 mm	13.0 mm	21.0 mm	12.0 mm
Staphylococcus aureus	21.7 mm	12.4 mm	14.0 mm	13.4 mm	13.0 mm	10.0 mm
Pseudomonas aeruginosa	28.0 mm	8.6 mm	18.9 mm	14.0 mm	15.5 mm	10.0 mm
Enterococcus faecalis	16.7 mm	11.7 mm	10.0 mm	9.0 mm	13.8 mm	10.0 mm
Bacillus subtilis	17.6 mm	12.6 mm	11.1 mm	10.9 mm	14.7 mm	11.9 mm

CIP = Ciprofloxacin (5 µg); CO = Cotrimoxazole (25 µg); E= Erythromycin (15 µg); CEF = Ceftriaxone (30µg); NOR = Norfloxacin (10 µg); G= Gentamicin (10 µg)

Albizia saman flowers possess good antibacterial activity confirming the great potential of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. In this study, we have shown that ethanolic extract of flower exhibit high level of action when different concentrations are used.

The crude extract of this flower was very effective against *Bacillus subtilis and Staphylococcus aureus*. The study of antimicrobial activities of ethanolic extracts of *Albizia saman* flower at various concentrations against pathogenic bacteria was carried out and the results were compared with various antibiotics. They found that the ethanolic extract showed maximum inhibition on *Pseudomonas aeruginosa*, *Staphylococcus aureus and Bacillus subtilis* in an ascending order.

Conclusion

The study showed that the flower extract of *Albizia saman has* a potent anti-microbial activity against various stains of microorganisms. Hence the level of anti-microbial activity of the ethanolic extract of *Albizia saman* flowers can be concluded to be highly effective against *Bacillus subtilis.* It is recommended to isolate and separate the bioactive compounds for the anti-microbial activity using advanced scientific techniques.

Conflict of Interest

The authors declare no conflicts of interest in this research

References

1. M.J.Martinez, A.Jauregui, Screening of some Cuban medicinal plants for antimicrobial activity, Journal of Ethnopharmacology 1996, 52(3), 171-174.
2. S.Daljit, S.Arora and Jasleen Kaur, Antimicrobial activity of species, International Journal of Antimicrobial Agents 1999, 12(3), 257-262.
3. E.A.Sofowora, Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd., Hoboken, 64-79: 1982.
4. A.J.Aladesanmi, A.Sofowora, J.D.Leary. Preliminary Biological and Phytochemical Investigation of two

Nigerian Medicinal Plants, International Journal of Crude Drug Research 1986, 24(3), 147-153.

5. B.P.A.Cammue, M.F.C.De Boue, F.R.G.Terras, P.Proost, et al., Isolation and characterization of a novel class of plant antimicrobial peptide from Mirablilis jalapa. Seeds, Journal of Biological Chemistry 1992, 267, 2228-2233.
6. R.P.Borris, Natural Product Research: Perspectives from a major pharmaceutical company, Journel of Ethnopharmacology 1996, 51, 29-38.
7. S.Thippeswamy, P.Praveen, D.C.Mohana, K.Manjunath, Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman Merr*. against some human and plant pathogenic bacteria and fungi, International Journal of Pharmaceutical research and Bio Science 2011, 2(2), 443-452.
8. T.Kirithika, Preliminary phytochemical screening and antioxidant property of various extracts of *Albizia saman* leaves, International Journal of Pharmaceutical Research and Bio Science, 2013, 2(1), 315-323.
9. M.Sundar, A.Anton Smith, J.Amutha Iswarya Devi, A.Kottai Muthu and S.Parimalakrishnan, Phytochemical investigation and antioxidant activities of *Albizia saman* flowers, Journal of Chemical and Pharmacetical Sciences. 2016, 9(4), 2952-2955.
10. R.Murali, A.Anton Smith, S.Parimalakrishnan, G.Jelin Jaralda and N.Vinothini, Evaluation of *in vitro* anticancer activity of ethanol and ethyl acetate extracts of *albizia saman* flowers, International Journal of Pharmaceutical, Chemical and Biological Sciences 2018, 8(2), 244-249.
11. Afia Ferdous, Mohammad Zafar Imam and Tajnin Ahmed, Antioxidant, Antimicrobial and Cytotoxic Activities of Samanea saman (Jacq.) Merr, Stamford Journal of Pharmaceutical Sciences, 2010, 3(1), 11-17.
12. R.C.Jagessar, M.Shang, R.W.Scheidt, D.H.Burns. Neutral ligands for selective chloride ion complexation (Cis)-5, 10, 15, 20-Tertrakis (2arylurea) phenyl porpheyins, Journal of American Chemical Society 1998, 120(45), 11684 -11692.
13. J.McFarland, Nephelometer: An Instrument for Estimating the Number of Bacteria in Suspensions Used for Calculating the Opsonic Index and for Vaccines. Journal of the American Medical Association, 1907, 14, 1176-1178.
14. A.Hala Mohammed and A.Al Fadhil Omer. Antibacterial Activity of Azadirachta indica (Neem) Leaf Extract against Bacterial Pathogens in Sudan, American Journal of Research Communication, 2015, 3(5), 246-251.

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Pattern of Self-Medication usage among the Health Sciences Students of Sri Ramachandra Medical College and Research Institute

Seenivasan P, Rajanandh MG, Karthik S*, Vaishnavi S, Senthil Kumar P

Department of Pharmacy Practice, Faculty of Pharmacy, Sri Ramachandra Medical College and Research Institute, Deemed to be University, Porur, Chennai – 600 116.

* Corresponding author email: karthiksjn19@gmail.com

Received date: 18.06.2018 Accepted date: 25.06.2018

ABSTRACT

Self-medication is the own selection and use of medicines by an individual to treat his/her self-recognized illnesses or symptoms. It is practiced significantly worldwide even though its type, extent and reasons for its practice may vary. No data is available on the current status of self-medication practices among health sciences students of Sri Ramachandra Medical College and Research Institute (SRMC&RI), Deemed to be University. A descriptive cross-sectional study was conducted on 200 health sciences students in SRMC&RI. Pre-tested questionnaire was used as a tool for data collection. Simple random sampling technique was employed. Among the 200 students, 80 were males and 120 were females. The prevalence of self-medication in this study was higher in females (61%) than male. The overall prevalence of self-medication among health science students was high. The major reason given for self-medication was their sufficient pharmacological knowledge. Analgesics and antipyretics were the most frequently used. Most of them have taken self-medication for Flu / Cough / Cold. Though one-fourth of the students do not recommend their friend for self-medication, they continue to the practice of self-medication. Thus there is a need to sensitize the health science students about the concepts and principles of self-medication.

Key words: Self-medication, Health science students, SRMC&RI.

INTRODUCTION

Self-medication can be defined as the use of drugs to treat self-diagnosed disorders or symptoms, or the intermittent or continued use of a prescribed drug for chronic or recurrent disease or symptoms[1, 2]. Developing countries like India and a few developed countries experience the problem of self-medication very commonly. In developing countries, the prevalence of this problem is found to be between 12.7-95%[3]. The prime reason behind this large number in our country is that medicines, including all classes of drugs, are easily available in chemist shops everywhere as over the counter products without a prescription[4]. The legal requirement of having a prescription to purchase a drug is no longer strong or even valid in many parts of the country.

The use of self-medication has a few benefits like it is economical, convenient and medical resources for minor illnesses do not get wasted[5]. At the same time, on the other hand, there are many drawbacks like the diagnosis of the disease may not be correct, there is a delay in getting the doctor's appointment, the harmful side effects of the drugs are not known, because of irrational and inappropriate use of antibiotics the patients may experience drug resistance, and unknowingly taking the same drug with different brand names may lead to drug interactions and at times drug addiction [6-8]. The pattern of taking self-medication is influenced by factors like gender, age, level of education, society, family, medical knowledge, perception of illness and advertisements on drugs [9].

Beside there is emergent concern about irrational drug use that can be fostered by self-medication practices in

the society as well as in health care workers. Previous studies have reported very high prevalence rates of taking self-medication in different countries like 68% in European countries, 31% in India [10], 59% in Nepal [11], with rates going as high as 92% in the adolescents of Kuwait [12]. Self-medication practices are more common in women and in those who live alone, have a lower socioeconomic status, have more chronic ailments, have psychiatric conditions, are of younger age and in students [13].

Possible risk of self-medication practices include: incorrect self-diagnosis, delays in seeking medical advice when needed, infrequent but severe adverse reactions, serious drug interactions, wrong administration, wrong dosage, wrong choice of therapy, masking of a severe disease and risk of dependence and abuse [14]. The present study is conducted to assess the prevalence of self-medication among the health science students of Sri Ramachandra Medical College in Chennai and to assess the students' perception and attitude regarding the practice of self-medication.

Methodology

Study Site

Constituent colleges of Sri Ramachandra Medical College and Research Institute, Deemed to be University. Human Institutional Ethics Committee approval was obtained prior to the commencement of the study.

Study design

A cross-sectional study

Study tool

A pre-tested questionnaire was used for data collection. It consisted of five sections: The first section was about the demographic details of the study participants. The next one was on their practice of using self-medication. The third part was on the reasons why they had used the self-medication. Last but one part was on their attitude towards self-medication. The final section was on their knowledge about self-medication.

Study Population and sampling

Students of Health Sciences such as Medical, Pharmacy, Dental, Allied Health Science and Nursing were included for the study. As there was no previous study conducted in Sri Ramachandra Medical College and Research Institute on the current topic, a 50% expected prevalence of self-medication was considered. Random sampling was used to collect the data.

Study procedure

A self-administered, structured questionnaire was distributed amongst the students from various constituent colleges of Sri Ramachandra Medical College and Research Institute, after explaining the purpose of the study. Inclusion criteria for the study was the students of age group 18-21 years, both genders and those voluntarily willing to participate in the study. The full details of the study were explained to them and a written informed consent was obtained from all before initiating the survey. The filled questionnaire was collected back and checked by the investigator for any missing responses.

Data entry, analysis, and interpretation

The Analysis was with manual calculator and Vassar stats (statistical tables' calculator) and also SPSS Software package. A statistical significance level of 0.05 was used to determine the association between variables. The results were presented in percentages as depicted in Tables, Figures, and Charts.

Results and Discussion

A total of 200 students were assessed regarding their practice, attitude and perception regarding self-medication behavior, out of which 45% (n= 90) were males and 55 % (n= 110) were females. The mean age of the respondents was 19.5 years. The prevalence of self-medication among the study participants was 82.5% (n =165). A proportionately larger number of females were self-medicating (n= 100, 61%) than males (n=65, 39.3%). Self-medication was proportionately common in Allied Health Science and BDS students. The distribution of self-medication practice according to the various constituent colleges is shown in Table 1.

Table 1. Characteristics of the study population (N = 200)

Variables	Self-medication n (%)	No self-medication n (%)	Total N
Gender			
Male	65 (39.3%)	15 (42.8%)	80
Female	100 (61%)	20 (57.1%)	120
Various constituent colleges			
BDS	54	27	81
Allied Health Science	42	23	65
MBBS	22	12	34
Pharm.D	12	08	20

The majority of the students self-medicated because of the illness being too trivial for consultation (39.3%), followed by their confidence about the pharmacological knowledge (72.7%). 60% used old prescriptions for the same illness as a source for information about the drug. The characteristics of the study subjects indulged in self-medication is shown in Table 2.

Table 2. Characteristics of study subjects indulged in self-medication (N = 165)

Reasons for self-medication	n (%)
Illness too trivial for consultation	65 (39.3)
Sufficient pharmacological knowledge	120 (72.7)
Privacy	09 (5.45)
Old prescription for same illness	99 (60)
Academic knowledge	104 (63.03)
Pharmacist	46 (27.8)
Friends	32 (19.3)
Drug advertisement / Internet	22(17.6)

Most of the participants (n= 182, 91%) checked the expiry date on the drugs before self-medication. Analgesics were the most common class of drugs self-medicated by the majority of the participants (73.9%), of which, Paracetamol followed by Ibuprofen were used commonly by the student followed by Antipyretics (61.8%) and Anti histamine (59.3%). It was also observed that 58.7% of the participants reported to have self-medicated themselves with Antibiotics (Table 3).

Table 3. Categories of drugs commonly self-prescribed (N = 165)

Categories	N (%)
Analgesics	122 (73.9)
Antipyretics	102 (61.8)
Antihistamines	98 (59.3)
Antibiotics	97 (58.7)
Antiulcer	42 (25.4)
Antidiarrheal	33 (20)
Folic acid / Iron supplement	32 (19.3)
Vitamins	28 (16.9)

Mostly with amoxicillin, fluoroquinolone antibiotics frequently self-medicated. Among the various indications for self-medication reported by the students, flu/cold/cough (96.9%) was the most common followed by vomiting (81.8.), Fever (61.8), Sore throat (55.7%), Rashes (33.3%) and ulcer in mouth (24.2%) was the most common indication for self-medication with antibiotics (Table 4). (Table 5) shows the attitude of the students towards the practice of self-medication. About 61% of the participants had positive opinion on self-medication which was a part of self-care and it needs to be encouraged. About 67% they continue with self-medication and 63.5% was opted “NO” to advice to their friends regarding self-medication.

Table 4. Indications for self-medication (N = 165)

Indications	N (%)
Flu / Cough / Cold	160 (96.9)
Vomiting	135 (81.8)
Fever	102 (61.8)
Sore throat	92 (55.7)
Rash/allergies	55 (33.3)
Ulcer in mouth	40 (24.2)

Table 5. Attitude of the students towards self-medication (N=200)

Items	Yes n (%)	No n (%)	Not sure n (%)
Self-medication is a part of self-care	122 (61)	78 (39)	10 (05)
Continue with/start self-medication	134 (67)	50 (25)	16 (08)
Advice self-medication to friends	73 (36.5)	127 (63.5)	0

The International Pharmaceutical Federation defines self-medication as the use of non-prescription medicines by people on their own initiative [15]. Self-medication is a widely practiced health seeking behavior. A total of 200 students were assessed regarding their practice, attitude and perception regarding self –medication behavior. The mean age of the study participants was 19.9 years, which was the expected age because of the inclusion criteria which said college students aged 18 and above were included in the study. In our study a proportionately larger number of females were self-medicating than males. Students from various medical and allied science back ground were included in our study. The extent of self-medication was found to be (61%) in our study. This result is similar to that seen in other studies, wherein the value of use of self-medication amongst students has ranged from 43.24% to 98%. [16,17]. The Reasons for self-medication in our study was having sufficient pharmacology knowledge (72.7%) followed by Academic knowledge (63.03%). A similar report was reported in a study from Visakhapatnam [18]. In other study from Punjab [19] also followed similar report as our study but after sufficient pharmacology knowledge (42.86%) followed by self-confidence (34.29%).But in other study in Tamilnadu [20] itself the reason for self-medication vary from our study illness too trivial for consultation (70.5%) followed by use of old prescription for same illness (53.1%) were the majority reason for self-medication which is quite contrast to our study.

Analgesics (73.9%) followed by Antipyretics (61.8%) were the most common class of drugs self-medicated by majority of the participants in our study. In the present study, paracetamol followed by ibuprofen drug were used commonly by the student A similar study were reported by Iran,[21] Mozambique,[22].

Flu/Cough/Cold was the most common indication for self-medication in our study which was similar to observations made in studies from Western, [23] and Southern part of India, [24] however in studies from Tamilnadu [20] and Visakhapatnam [18] and Ethiopia [25]. Fever and headache were the most commonly reported symptoms that led to self-medication, followed by cough and common cold.

In our present study 67% of the participants reported to continue with or start with self-medication followed by (61%) reported Self-medication is a part of self-care which was quite differ from other studies from Visakhapatnam [18], Ethiopia [25] and Karachi [26] they reported self-medication was a part of self-care than with continue with or start with self-medication.

Conclusion

The prevalence of self-medication among health science students was high. The major reason given for self-medication was their sufficient pharmacological knowledge. Analgesics and antipyretics were the most frequently used. Most of them have taken self-medication for Flu / Cough / Cold. Though one-fourth of the students do not recommend their friend for self-medication, they continue to the practice of self-medication. Thus there is a need to sensitize the health science students about the concepts and principles of self-medication.

Conflict of interest

The authors declare no conflicts of interest in this research

References

1. World Health Organization (WHO). Guidelines for the regulatory assessment of Medicinal Products for use in self-medication. http://apps.who.int/medicinedocs/en/d/Js2218e/

2. Awad A, Eltayeb I, Matowe L, et al. Self-medication with antibiotics and antimalarials in the community of Khartoum State, Sudan. J Pharm Pharm Sci 2005,12,8(2),326-31.

3. Wijesinghe PR, Jayakody RL, Seneviratne RA. Prevalence and predictors of self-medication in a selected urban and rural district of Sri Lanka. WHO South-East Asia J Public Health. 2012,1(1),28-41.

4. Kamat VR, Nichter M. Pharmacies, self-medication and pharmaceutical marketing in Bombay, India. Soc Sci Med. 1998, 47(6), 779-94.

5. Calabresi P, Cupini LM. Medication-overuse headache: similarities with drug addiction. Trends Pharmacol Sci. 2005, 26(2), 62-8.

6. Hughes CM, McElnay JC, Fleming GF. Benefits and risks of self medication. Drug Saf. 2001, 24(14), 1027-37.

7. Bauchner H, Wise PH. Antibiotics without prescription: "bacterial or medical resistance"? Lancet. 2000,355(9214),1480.

8. Greenhalgh T. Drug prescription and self-medication in India: an exploratory survey. Soc Sci Med. 1987,25(3),30718.

9. Afolabi AO. Factors influencing the pattern of selfmedication in an adult Nigerian population. Ann Afr Med. 2008,7,1207.

10. Deshpande SG, Tiwari R, Self medication—a growing concern. Indian J Med Sci, 1997,51,93-6.

11. Shankar PR, Partha P, Shenoy N, Selfmedication and non-doctor prescription practices in Pokhara valley, Western Nepal: a questionnaire-based study. BMC Fam 2002 , 3, 17.

12. Abahussain E, Matowe LK, Nicholls PJ, Self reported medication use among adolescents in Kuwait. Med Princ Pract, 2005,14, 161-4.

13. Figueiras A, Caamaño F, Gestal-Otero JJ, Sociodemographic factors related to selfmedicationin Spain. Eur J Epidemiol, 2000, 16, 19-26.

14. Ruiz ME, Risks of self-medication practices. Curr Drug Saf, 2010, 5, 315-23.

15. Joint Statement by the International Pharmaceutical Federation and the World Self-MedicationIndustry. 2013 Feb 27.

16. Sawalha AF. Assessment of self-medication practice among University students in Palestine: therapeutic and toxicity implications. Islamic Univ J. 2007, 15, 67-82.

17. Gutema GB, Gadisa DA, Abebe Z, Berhe DF, Berhe AH, Hadera MG, et al. Self medication practices among health sciences students: the case of Mekelle University. J Appl Pharam Sci. 2011, 01(10), 183-189.

18. Evaluation of the knowledge, attitude and practice of self-medication among b.sc nursing students Gajendra Naidu. J1, Vamsi Krishna2, Rao B. 2015, 4(46), 8054-8060

19. Gupta V, Bansal P, Manhas R, Singh Z, Ghaiye P (2011) Preferred system of medicine and reasons of self-medication among college students in Malwa region of Punjab. J Drug Deliv and Ther 2011,1(2), 27–29.

20. Kayalvizhi S, Senapathi R (2010) Evaluation of the perception, attitude and practice of self medication among business students in 3 select cities, South India. IJEIMS2013, 1 (3) 40– 44.

21. Sarahroodi S, Maleki-Jamshid A, Sawalha AF, Mikaili P, Safaeian L Pattern of self-medication with analgesics among Iranian University students in central Iran. J Family Community Med 2012, 19 (2), 125–129.

22. Lucas R, Lunet N, Carvalho R, Langa J, Muanantatha M, et al. Patterns in the use of medicines by university students in Maputo, Mozambique. Cad SaudePublica 2007, 23 (12) 2845–2852.

23. Banerjee I, Bhadury T Self-medication practice among undergraduate medical students in a tertiary care medical college,.West Bengal J Postgrad Med 2012,58 (2),127–131.

24. Badiger S, Kundapur R, Jain A, Kumar A, Pattanshetty S, et al. Self-medication patterns among medical students in South India. Australas Med J 2012, 5 (4), 217–220.

25. Abay SM, Amelo W Assessment of self-medication practices among medical, pharmacy, and health science students in Gondar University, Ethiopia. J Young Pharm 2010, 2 (3), 306–310.

26. Zafar SN, Syed R, Waqar S, Irani FA, Saleem S Prescription of medicines by medical Students of Karachi, Pakistan: a cross-sectional study. BMC Public Health 2008, 8, 162.

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

Assessment of Relevant Information in Prescriptions of Outpatient Clinics and Community Pharmacies in Malappuram

Aswathi Janardhanan[1], Akhil Reghu[2], M.S. Shijin[3],
E. Sunusha[4], K. K. Thabsheera[5], Siraj Sundaran[6]*

[1,2]Pharm. D, [3,4]M. Pharm, [5]B. Pharm,[6*]Professor, Department of Pharmacy Practice,
Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala- 673634.
*E.mail: tsirajsundaran@gmail.com

Received date: 11.05.2018 Accepted date: 06.06.2018

ABSTRACT

Many studies have been conducted to investigate on the relevant information present in prescriptions and have found that inappropriate prescription writing has lead to medication errors. Objective: Present study aimed to investigate on the prescription writing process among prescribers in the outpatient clinics and community pharmacies in Malappuram, Kerala.

Method: This cross-sectional observational study was conducted for three months with randomly collected prescriptions from the outpatient clinics and pharmacies in Malappuram, Kerala. A total of 90 prescriptions were collected and compared with the standard prescription checklist prepared by the investigator. Three major domains studied using the prescription checklist were prescriber information, patient information, and drug related information.

Results: Study found that only 5.56% of the prescriptions had diagnosis mentioned in it. Signatures of prescriber were only found in 30% of prescriptions. Legibility was found in 38.89% of the prescriptions. Only 54.44% of prescriptions had patient gender specification. Body weight and communication address of the patient was not mentioned in any of the prescriptions. All the prescriptions had the name of the drugs prescribed where 98.89% were in trade names. Dose was mentioned in all prescriptions. Dosage form was mentioned in 95.56% of prescriptions, frequency of drug administration was present in all the prescription and total quantity of drugs to be dispensed in 92.22% of the prescriptions. The instructions for use of the drugs were only present in 10% of prescriptions.

Conclusion: The study concludes with the need for physician to emphasize on certain prescription writing standards in accord with World Health Organization guidelines.

Key words: Prescriber, Patient, Drug, Prescription Information Checklist

INTRODUCTION

Prescription is an order for medication issued from an authorized prescriber who may be a physician, dentist, veterinary surgeons or any other licensed medical practitioner.[1] Prescription is in effect three types of document in one- it is a clinical document, a legal document and an invoice. Law may require some of the information on the prescription and other some is to ensure that the patient receives the correct medicine. A prescription contains direction for both the pharmacist and patient. When taking a written prescription from a customer, the pharmacist should first review it to make sure that it looks legitimate.

Many studies have been conducted to investigate about the writing habits and the relevant information present in the prescription. These studies found that there was vast of medication errors that arise due to inappropriate prescription writing process. The extent of such errors varied from 2.6% to 15.4%. Studies have shown that 15% to 21% of prescription contain at least one prescribing errors.[2] These studies were able to give an input to the prescribers to follow a genuine and standard guideline for writing a prescription which has very much minimized the prescribing errors. But not many studies of this kind have been conducted in the Indian scenario. The present study wanted to assess on the relevant information's in the prescriptions collected from selected outpatient clinics and community pharmacies in Malappuram, South India.

Material and Methods

The study was conducted in the outpatient clinics and community pharmacies in Malappuram, Kerala, South India for a period of three months, from October 2013 to December 2013. The study followed prospective cross–sectional observational study design where prescriptions were randomly collected. The obtained prescriptions were verified using the self prepared prescription information checklist to identify any deficiencies. This checklist was categorized into three major domains namely prescriber information related domain, patient information related domain and drug information related domain.

Prescriber information related domain verified the information such as name of the prescriber, prescriber's specialization, address of the clinic, license or registration number of the prescriber, diagnosis given by the prescriber, legibility of the prescription, date of prescribing and prescriber's signature. The information in the domain are important for identifying a patient's prescriber, locating the clinic, whether the prescriber is a specialist in the case or a second opinion is advisable or not. Diagnosis helps the pharmacist to see to it that whether the prescribed drugs pertain to the case or not. Date of the prescribing helps in the record maintenance and information retrieval. Signature of the prescriber is for legality and confirmation that the prescriber is the one who has written the prescription.

Patient information related domain verified the name of the patient, age, gender, weight and address of the patient. This information in this domain are helpful for the identification of patient, and the age, sex and weight helps in dose calculation if inevitable. Address of the patient aid in any medical record purpose, information collection of the case, patient counseling, monitoring, etc.

Drug information related domain verified here are the drug's name in the prescription (whether trade name or generic name), dose, dosage form, frequency of administration, instruction for use and total quantity to be dispensed. This information help in dispensing the right medication in right quantity in right dosage form at right dose at right time. This information will also serve for the billing procedure, patient counseling and intervention if necessary.

All the data obtained were analyzed in the Microsoft excel and result was formatted. Since a descriptive study the results were expressed as the percentage values of the data.

Results

A total of 90 prescriptions were collected, verified using the checklist and analyzed. The following are the findings of the study.

Prescriber information domain: Among the 90 prescriptions verified using the prescription checklist it was observed that about 95.56% has prescriber's name on it and it was in the printed format in the prescription

pad. Name of the clinical was mentioned in 75.56% of the prescriptions and the address of the clinic in 94.44% of the prescriptions. Diagnosis of the prescriber was only there in 5.56% of the prescriptions. The legibility was observed only in 38.89% of prescriptions. The findings are represented in table 1. Overall it was that the prescriptions information on diagnosis which is one of the essential information in concern with care and safety was very low when compared to any other information in the prescriber's information domain. This was followed by the missing of signature which would be a confirmation of the prescriber's writing and it is considered for legality. The legibility was also a concern as per the observation in this study.

Patient information domain: Among the 90 prescriptions studied all the prescriptions had the name of the patients in it. Age of the patient was mentioned in all the prescriptions. Gender of the patient was only mentioned in 54.44% of the prescriptions verified. Weight and address of the patients were missing in all the prescriptions. Findings are represented in table 2. The missing information of weight is of important concern for dose calculations if required in the patients especially in case of special populations or major concerns such as hepatic or renal problems.

Drug information domain: All the prescription had the drug names in it among which 98.89% were trade names and only 1.11% prescription had generic names. Dose of the drugs were mentioned in all the prescriptions. Dosage forms of the drugs to be dispensed were given in 95.56% of the prescriptions. Frequency of administration was given in all the prescriptions. Instructions on the use of the drugs were only given in 10% of the prescriptions. Quantity of the drugs to be dispensed was given in 92.22% of the prescriptions. Instructions for the use of drugs were more silent in the drug related domain which is an essential element in concern to the drug use by the patients.

Table No. 1: Prescriber information domain verified

Sl. No.	Prescriber information elements	Number (%)
1.	Name of the Prescriber	86 (95.56%)
2.	Prescriber's Specialization	82 (91.11%)
3.	Name of the Prescriber's Clinic	68 (75.56%)
4.	Address of Prescriber's Clinic	85 (94.44%)
5.	Prescriber's Registration or License number	83 (92.22%)
6.	Diagnosis made by the Prescriber	5 (5.56%)
7.	Legibility of the Prescription	35 (38.89%)
8.	Date of Prescribing	90 (100%)
9.	Signature of the Prescriber	27 (30%)

Table No. 2: Patient information domain verified

Sl. No.	Patient information elements	Number (%)
1.	Name of the patient	90 (100%)
2.	Age	90 (100%)
3.	Gender	49 (54.44%)
4.	Weight	0
5.	Address of the patient	0

Table No. 3: Drug information domain verified

Sl. No	Drug information elements	Number (%)
1.	Drug name	90 (100%)
2.	Dose of the drugs in the prescription	90 (100%)
3.	Dosage form of the drugs in the prescription	86 (95.56%)
4.	Frequency of drug administration	90 (100%)
5.	Instruction for use of the drugs	9 (10%)
6.	Total quantity of drugs to be dispensed	83 (92.22%)

Discussion

In case of the prescriber's domain diagnosis was one of the essential elements in concern with care and safety was very low when compared to any other information. The legibility was also a concern as per the observation in this study. The findings of prescriber information domain were similar to the work conducted by Sharif et al and Sawalha et al.[3,4]

The patient information domain had missing of elements such as weight of the patient and their place identity. The weight could be of use in situation where there is need for dose calculations. The findings showed similarity to Irshaid et al and Sawalha et al.[4,5]

Instructions for the use of drugs were more silent in the drug related domain which was an essential element in concern to the drug use by the patients. All the prescriptions had the name of the drugs prescribed where more 98% were written as trade name or brand name. Similar kind of finding was observed in the study of Tayem et al.[6] The information on the dose, dosage form, and frequency of drug administration was similar to the studies conducted by Sawalha et al, and Bawazir.[5,7] The information regarding the total quantity of drug to be dispensed was similar to the study conducted by Sharif et al.[3]

Conclusion

The study has observed the deficiencies in some essential information in prescriptions such as diagnosis of patients, gender of the patient, weight of the patient, address of the patient and prescriber's signature, instruction for the use of drugs was the important element missing. Illegibility of the prescription was another observation. All these information flaws can add to more number of medication errors. The need for the physician education on appropriate prescription writing is obvious and educational training about the prescription writing according to World Health Organization Guidelines for the prescriber's would be helpful. The study can be further customized by employing intervention methods such as administrative monitoring and educational intervention and then evaluate the effect on the information domains.

References

1. Remington- The science and practice of pharmacy by, 21st edition, volume II, Lippincott, Williams and Wilkins publishers, Pg No: 1823-5.
2. Jeetu G and Girish T. Prescription drug labeling medication errors: A big deal for pharmacists, J Young Pharm, 2010; 2(1):107-11.
3. Suleiman I Sharif, Alya H Alabdouli, Rubian S Sharif. Drugs prescribing trends in a General Hospital in Sharjah, United Arab Emirates, American Journal of Pharmacological Science, 2013; 1(1):6-9.
4. Sawalha A F, Sweileh W M, Zyoud S H, Al-Jabi S W, Bni Shamseh, Odah A A. Analysis of prescriptions dispensed at community pharmacies in Nablus, Palastine, East Mediterr Health J, 2010; 16(9):788-92.
5. Irshaid Y M, Al Homrany M, Hamdi A A, Adjepon-Yamoah K K, Mahfouz A A. Compliance with good practice in prescription writing at outpatient clinic in Saudi Arabia, East Mediterr Health J, 2005; 11(5-6):922-8.
6. Tayem Y I, Ibrahim M A, Qubaja M M, Shraim R K, Taha O B, Abu Shkhedem. Compliance with guidelines of prescription writing in central hospital in West Bank, East Mediterr Health J, 2013; 19 (9):802-6.
7. Saleh A Bawazir. Prescribing patterns of Ambulatory care physicians in Saudi Arabia, Annals of Saudi Medicine, 1993; 13(2):172-7.

SUBSCRIPTION ORDER
INTRNATIONAL JOURNAL OF PHARMACY RESEARCH

PSG COLLEGE OF PHARMACY

Coimbatore - 641 004

E-mail - editorinijpr@gmail.com

Yes I / We wish to subscribe for IJPR

Enter my / our subscription for

For India

₹ 1600 per Year

For Outside India

$75 per Year

$140 per / 2 Years

Name :

Organization :

Address :

Pin code/Zip code :

Country :

Payment enclosed By Demand Draft(DD)

Details :

DD should be taken in favour of **The Principal, PSG College of Pharmacy, Payable at Coimbatore.**

Copy Right Form

Manuscript Title : .....

Authors Name : .....

I/we certify that I/we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have my/our name listed as a contributor. I/we believe the manuscript represents valid work. Neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter. I/we certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. I/we attest that, if requested by the editors, I/we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, for examination by the editors or their assignees. Financial interests, direct or indirect, that exist or may be perceived to exist for individual contributors in connection with the content of this paper have been disclosed in the cover letter. Sources of outside support of the project are named in the cover letter.

I/We hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership, including any and all rights incidental thereto, exclusively to the Journal, in the event that such work is published by the Journal. The Journal shall own the work, including 1) copyright; 2) the right to grant permission to republish the article in whole or in part, with or without fee; 3) the right to produce preprints or reprints and translate into languages other than English for sale or free distribution; and 4) the right to republish the work in a collection of articles in any other mechanical or electronic format.

We give the rights to the corresponding author to make necessary changes as per the request of the journal, do the rest of the correspondence on our behalf and he/she will act as the guarantor for the manuscript on our behalf.

All persons who have made substantial contributions to the work reported in the manuscript, but who are not contributors, are named in the Acknowledgment and have given me/us their written permission to be named. If I/we do not include an Acknowledgment that means I/we have not received substantial contributions from non-contributors and no contributor has been omitted.

	Name	**Signature**	**Date signed**
1	.....	.....	.....
2	.....	.....	.....
3	.....	.....	.....
4	.....	.....	.....
5	.....	.....	.....
6	.....	.....	.....

(up to 6 contributors)

PSG COLLEGE OF PHARMACY

(An ISO 9001:2008 Certified Institution)
Accredited with 'B' Grade by NAAC (1st Cycle)
Coimbatore - 641 004. Tamilnadu, India

Our Mission

"To establish a 'Center of Excellence' for Pharma Education & Research."

Our Vision

"To impart theoretical and practical knowledge in Pharma Sciences so that those who come out from Institution will have an exact mind set up for rational thinking and scientific innovations."

COURSES OFFERED		Seats
1.	B.Pharm	- 60
2.	M.Pharm	
	◆ Pharmaceutics	- 15
	◆ Pharmaceutical Analysis	- 15
	◆ Pharmacology	- 12
	◆ Pharmacy Practice	- 15
3.	Pharm.D	- 30
4.	Pharm.D (Post Baccalaureate)	- 10

INTERNATIONAL JOURNAL OF PHARMA RESEARCH (IJPR)

CONTENTS

Vol.9 • Issue 1 January - June 2018

Open Access JOURNALS

http://www.oajournals.com

Indexed in Google Scholar, Open Access, Academic Keys, SJIF#, Scientific Indexing Services, Research bible, GIF#, Directory of Research Journal Indexing, Index Copernicus International, Indian Citation index, Ulrich's Web#, Jour Info#, Cite Factor#, World Cat

SJIF#, GIF#, Ulrich's Web#, Jour Info#, Cite Factor# - Under evaluation

INTERNATIONAL JOURNAL OF PHARMA RESEARCH
THE RESEARCH PUBLICATION FROM PSG COLLEGE OF PHARMACY
Peelamedu, Coimbatore 641 004, Tamil Nadu, INDIA
Phone : +91-422-2570170 - Extn.5841 Fax : +91-422-2594400
E-Mail : editorinijpr@gmail.com Website : www.psgpharma.ac.in

Published by : Principal, PSG College of Pharmacy, Peelamedu, Coimbatore
Printed at : Ace Data Prinexcel Private Limited, Peelamedu, Coimbatore, Ph:0422-2561500
Chief Editor : Dr. M. Ramanathan, PSG College of Pharmacy, Peelamedu, Coimbatore
Price per copy : Rs.800/-