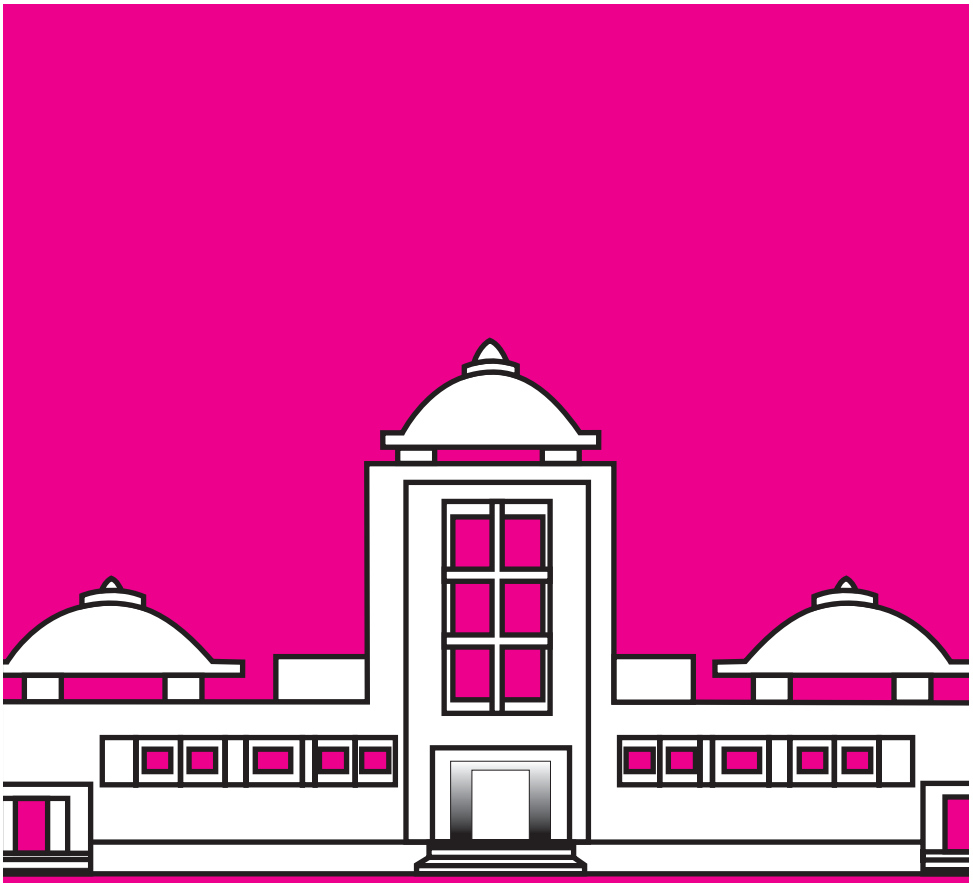
ISSN : 0973-6204

PHARMBIT

INTERNATIONAL JOURNAL INDEXING IN "CHEMICAL ABSTRACT"
Vol. XX. No. 2, Jul-Dec, 2009



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DEPARTMENT OF PHARMACEUTICAL SCIENCES
BIRLA INSTITUTE OF TECHNOLOGY
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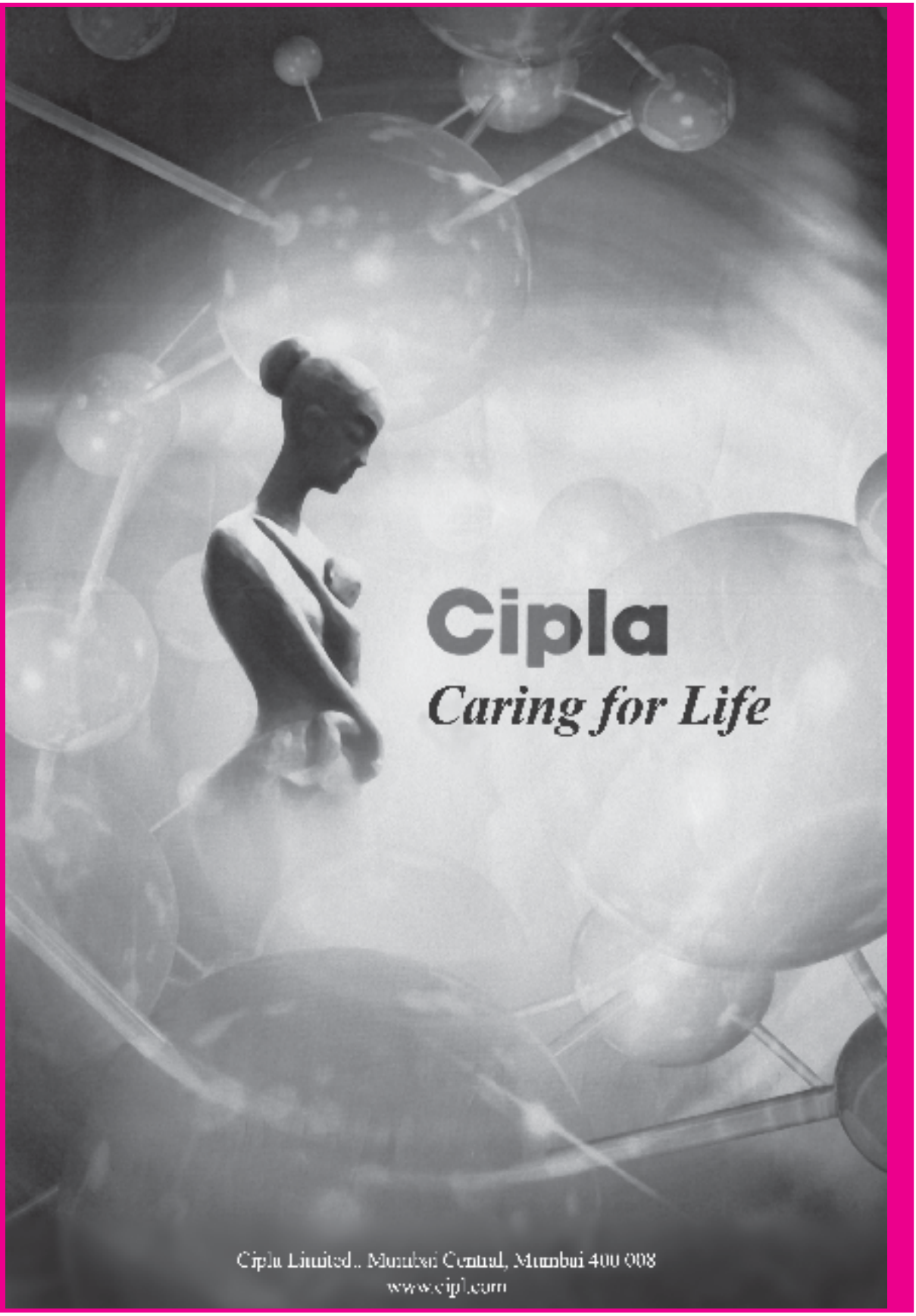
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From the Editor's Desk

It is a matter of great pleasure for us to bring out the XX, volume of PHARMBIT, biannual scientific journal in time and to inform that the journal has been accepted for indexing in Biotechnology & Bio engineering, Applied Microbiology, Neuroscience and Toxicology abstract under"Natural Science database" (www.csa.com) from Jan - June 2008 issue besides indexing in "Chemical Abstract" from Jan - June 2007issue.

In this issue 11 papers are being published covering papers on pharmacy practice, development of formulation, standardization and survey on plant drugs. It is known and established from ancient times that our country is rich in natural resources including medicinal plants. Now most of the research works and paper published in scientific journals are based on this topic. It is good sign for development but it is observed that many times only there is duplicity of work. It becomes a hard task for us to detect such papers. Here reviewer's role becomes much more vital. At the same time I would appeal to researchers and our pharmaceutical scientists fraternity to take research work oriented to application to patient and be cost effective in manufacture of those drugs & pharmaceuticals. Today the treatment cost is increasing leaps and bounds where medicine is also one of the components in treatment. Hence we as pharmacist a member of healthcare team having moral duty to provide output to masses, and then it would be true sense of Research.

As an editor of this journal, I am thankful to all Authors, Reviewers, Editorial Board Members, Advertisers, well wishers, Faculty members and Head,department of Pharmaceutical Sciences, Vice-Chancellor, BIT, Mesra and Vice-Chancellor Emeritus, BIT, Mesra for their support and encouragement in bringing out this edition of PHARMBIT. Lastly we invite suggestions from readers and well wishers to further improve the PHARMBIT.

Dr R. N. Gupta

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A Hospital Based Study on Some Clinico-Epidemiological Aspect of Bronchopneumonia Among Infants and Young Children

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ABSTRACT

A hospital based 6 months prospective study was carried out on children with clinical diagnosis of bronchopneumonia in paediatric department of RMMC, Chidambaram. The objective of the study was to find out the proportional case rate and the clinico-epidemiological features of the disease. The effectiveness of Nebulized salbutamol among bronchopneumonia children was also finding out. The proportional case rate was found to be 2.24%. Low birth weight (18%) mal-nutrition (23%) no immunization (17%), non-breast feeding (20%) and low socio-economic status (25%). Response to nebulized salbutamol was remarkably higher (70%) in 6 m to 12 m age group.

KEY WORDS : Bronchopneumonia, Low Birth Weight, Mal-Nutrition, Salbutamol, Socio-Economic Status.

INTRODUCTION

Bronchopneumonia refers to inflammation of the lung that is centered in the bronchioles and leads to the production of mucopurulent exudates that obstructs some of the small airways and causes patchy consolidation of the adjacent lobules. Bronchopneumonia is usually a generalized process involving multiple lobes of the lung. Acute respiratory infections namely pneumonia cause up to 5 million deaths annually among children less than 5 years old in developing nations. The estimated total of 12.9 million deaths globally in 1990 in children under 5 years of age, over 3.6 million were attributed to acute respiratory infections mostly due to pneumonia. This represents 28% of all deaths in young children and places pneumonia as the largest single cause of childhood mortality. Bronchopneumonia is the most important lower respiratory tract illness of infant and children .It is an acute viral syndrome characterized by hyperinflation, Wheeze and fine inspiratory crackles, mainly cause by respiratory syncytial virus. It is a clinical continuum that incorporates viral lower respiratory tract infection induced airway hyperresonsiveness.It accounts for about 8.5% of total respiratory diseases in hospitalized children. It is associated with significant short term morbidity which needs hospitalization. The incidence of bronchopneumonia varies with several epidemiological factors and with geographical areas. The risk factors for developing pneumonia include1) low weight for age 2) Lack of breast feeding 3) Failure to complete immunization 4) Presence of coughing sibling 5) Poor environmental factors 6) Malnutrition 7) Nasopharyngeal colonization. There is a lack in study in and around chidambarum.So in this present scenario, a prospective study was carried out with the objectives to find out proportional case rate of bronchopneumonia among infants and young children, its clinico-epide-epidemilogical features and effectiveness of nebulised salbutamol

MATERIAL & METHODS

One year prospective study was undertaken in the Department of Paediatric Medicine RMMC Chidambarum.The study was carried out from 1st November 2003 on children with clinical diagnosis of bronchopneumonia in OPD and indoor department. Clinical diagnosis criteria were the first

Table 1 : Age and sex wise distribution of Branchopneumonic Patients

Age (in months)	No. of Cases				Total	
	Male		Female			
	No	%	No	%	No	%
<3	68	34	16	8	84	16
3-12	29	14.5	25	12.5	54	24.5
Above 1 year (12 months)	26	8	36	18	62	52
All age group	123	61.5	77	38.5	200	100

Table 2 : Relation of some epidemiological factors with the severity of Bronchopneumonia [N-200]

No.	RISK FACTORS	CURED n=156		NOT CURED n=44		DEAD n=12		TOTAL (m=200)	
		No.	%	No.	%	No.	%	No.	%
1	Not breast fed	33	21.15	7	15.9	0	0	40	20
2	Low birth weight	30	19.23	6	13.63	0	0	36	18
3	Mal-nutrition	38	24.35	8	18.18	0	0	46	23
4	Low socio-economic status	39	25	11	25	0	0	50	25
5	Not vaccinated	32	20.51	2	4.54	0	0	34	17

episode of acute respiratory problems under 2 years of age with the clinical evidence of hyperinflation, wheeze, and fine in respiratory crackles (Table-1). Detalied history was taken regarding complaints, birth weight including prematurity, history of exclusive breast feeding and family history of allergy and bronchial asthma (Table-2). During physical examination emphasis was given to respiratory rate heart rate, respiratory distress, hyperinflation of chest air entry, wheeze and crackles severity of the disease was judged by respiratory rate, chest retraction, head nodding and cyanosis (Table-3). Mild bronchopneumonia were treated in out patients deparment.Mothers are also advised to bring back their children for follow-up immediately if anyone of following developed Viz. breathing becomes difficult/ breathing becomes fast/feeding becomes a problem/child becomes sicker. Children with moderate and severe bronchopneumonia were admitted and were clinically monitored for adequate air entry, breathing and circulation. treatment protocol for them were to keep on humidified oxygen therapy for central cyanosis/inability to drink /restlessness severe lower chest wall in drawing /grunting /respiratory rate more than 70 per minute. Breast feeding should be continued as long as possible. If dehydration developed it should be treated with usual line of treatment otherwise children with severe bronchopneumonia were treated with 2/3 rd volume of normal requirement maintenance fluid, withholding oral feed. Once respiratory distress settles, ryles tube /oral feeding started graduly.Antibiotics were usedin critically sick children or with associated secondary infection. Inhalation of nebulised salbutamol was tried once

Table 3 : Common clinical features observed in bronchopneumonia [N=200]

Clinical Features	CURED n=156		NOT CURED n=44		DEAD		TOTAL (n=200)	
	No.	%	No.	%	No.	%	No.	%
Fever	84	53.84	22	50	0	0	106	53
Cough	75	48.07	26	59.09	0	0	101	50.5
Tachycardia	6	3.84	7	15.9	0	0	13	6.5
Tachypnea	8	5.12	6	13.63	0	0	14	7
Severe Respiratory distress	2	1.28	1	2.27	0	0	3	1.5
Wheeze	80	51.28	46	104.54	0	0	126	63
Creptation	60	28.46	53	120.45	0	0	113	56.5
Rales & Crackles	2	1.28	20	45.45	0	0	22	11

and continued only if there was evidence of response to its initial administration.

Table 4 : Response of nebulization among infants suffering from bronchopneumonia [N=78]

Age (in months)	Total (n=78)	Response by nebulisation	
<6	48	21	43.75
6-12 months	30	21	70
Total	78	42	53.85

(X^2=4.9, D&F=1, P< 0.05 statistically significant)

RESULTS AND DISCUSSION

Total 2854 children were treated from paediatric department for different ailments during the study period of 1 year. Out of them, 625 patients (2.24%) were diagnosed as bronchopneumonia. From it 200 patients were selected for study purpose. In this study the incidence of bronchopneumonia was found highest in the month of September and October. From table I shows that maximum preponderance of cases (34%) were below 3 months of age with a slightly increased incidents in male sex. It may be probably be related to relatively smaller diameter of airway tube.

Analysis of table 2 shows that low socio-economic status, mal-nutrition and non-breast feeding were the contributory factors for severe bronchopneumonia in early age. Poor foetal nutrition leading to mal-nutrition operated to increase the severity of illness. Similarly non-vaccination practices are also a risk factor for severe bronchopneumonia in early age. This might be explained by the production of protective interferon produced by administration of OPV which protect other viruses also.

Thus by reducing the incidents of premature, LBW babies preventing mal-nutrition, promotion of exclusive breast feeding and timely primary immunization help to decrease the incidence of severe acute lower respiratory tract infections.

In relation of common clinical features (Table 3) observed in children suffering from bronchopneumonia it was found that feeding problem tachycardia, tachypnea, fever, cough and severe respiratory distress

were present insignificantly higher proportion. However, in relation to total bronchopneumonia cases, cough was present in 52.5%, respiratory distress in 0.5%, tachypnea in 8.5%, tachycardia in 10.5% and fever 56% cases.

Clinical symptomatology shows that fever, cough, tachycardia, tachypnoea and severe respiratory distress can be easily identified by health care providers even without instrumental aids.

From the analysis of table 4, it was observed that response to nebulization found to be higher in age group of more than 6 months (70%) whereas less response was found to be higher (75% in the age group of below 6 months). This might be due to immaturity of bronchiolar muscles, increased dynamic airway closure and greater degree of mucosal oedema with varying proportions. Good response to salbutamol indicates that wheezing was caused by bronchospasm and non-response indicates that wheezing is caused by mucosal oedema either or both situation may exist in bronchopneumonia.

Nebulized salbutamol was tried on 78 children of bronchopneumonia. Among them, 48 children were below 6 months and rests were between 6 to 12 months age. Responses were found only in 43% case of below 6 months of age where as response rate was remarkably higher (70%) in the age group between 6 to 12 month

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Ethnobotanical Study in A Remote District of West Bengal, India

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ABSTRACT

An ethnobotanical study was undertaken to collect and document the indigenous knowledge on the use of medicinal plants in a remote district of West Bengal, India during June 2008 to July 2009. The indigenous knowledge of local tribal people and the native plants used for medicinal purposes were collected through questionnaire and personal interviews during field trips.

In this present study, the Purulia district of West Bengal has been undertaken as the areas of research. We went through the rural areas, particularly among the tribal people who still have immense faith bestowed on the curability of herbal medicines. We searched out some of those medicines which are still in use among the rural population and have created a database for further investigation and research to find new leads.

The investigation showed that the remote district – Purulia of West Bengal, India is ecologically and climatically favourable for some medicinal plants. Many people in the studied area are still continued to depend on medicinal plants at least for the treatment of primary healthcare. But poverty, ignorance, illiteracy and less communication are major problems in this tribal zone which leaves many medicinal plants undiscovered to the local population.

The investigation revealed that, the tribal people of the studied area used 36 species of plants distributed in 34 genera belonging to 27 families to treat various diseases. The documented medicinal plants were mostly used to cure diarrhea, dysentery, poison bites, and stomachache.

INTRODUCTION

Plants have been used in traditional medicine for several thousand years[1]. In the developed countries, 25 per cent of the drugs are based on plants and their derivatives[2]. Ethno botany is not new to India because of its rich ethnic diversity. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine[3]. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world[4–8]. In India there are many reports on the use of plants in traditional healing by either tribal people or indigenous communities[9–16]. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future[3].

The Purulia district of the state West Bengal is a natural abode of large number of valuable medicinal plants. The remoteness and isolated living of the tribal peoples in this district resulted into the development of their own medico–religious health care system and living in their own style. It was seen that the tribal people of the rural population of this district are largely dependent on using some important medicinal plants for cure of various diseases (Table-1). It is unfortunate that their traditional knowledge has not been properly identified, standardized and documented for the better use by modern society. So a study is undertaken to survey this remote district of West Bengal to enlighten many of medicinal plants which

Table 1 : Medicinal plants used by the people from Purulia district of West Bengal

Plant	Scientific Name	Family	Parts and uses of the same
Rose apple, Jum	*Eugenia jambolana*	Myrtaceae	Young leaves' juice in dysentery
Karanja	*Caesalpinia crista*	Leguminosae	Leaves & barks are used in fever, asthma & as liver tonic; Seeds for wound healing and as anthelmintic
Harjora	*Cissus quadrangularis*	Vitaceae	Stem paste in bone fracture
Chorkanta	*Achyranthes bidentata*	Amaranthaceae	Roots in boils and acne; leaves in emesis
Vasaka	*Adhatoda vasica*	Acanthaceae	Juice of leaves in mild asthma, cough , piles and bleeding gum
Bael	*Aegle marmelos*	Rutaceae	Pulp of the fruits in indigestion, constipation and dysentery
Kulekhara	*Asteracantha longifolia*	Acanthaceae	Juice of leaves in anemia
Bar	*Ficus bengalensis*	Moraceae	Milky juice & bark paste are used in rheumatism & diabetes
Petari, Country mallow	*Abutilon indicum*	Malvaceae	Roots in boils and abscess.
Babla, Acacia	*Acacia nilotica*	Leguminosae	Juice of leaves in sore caused by water
Cucumber	*Cucumis sativa*	Cucurbitaceae	Fruits in indigestion
Lady's finger	*Hibiscus esculentus*	Malvaceae	Whole fruit or seeds in post-coital inflammation due to semen deficiency.
Jaba, China rose	*Hibiscus rosa-sinesis*	Malvaceae	Leaves in mild burns and boils.
Kamini	*Murraya paniculata, Murraya exotica*	Rutaceae	Bark paste is believed to be an antivenom and sex stimulant.
Sehgun, Teak	*Tectona grandis*	Verbenaceae	Oil of seeds with coconut oil to promote hair growth.
Radhachura	*Poiniciana pulcherrima Caesalpinia pulcherrima*	Leguminosae / Ceasalpiniaceae	Juice of leaves in abdominal pain and in oligomennorhoea or amennorhoea.
Hyacinth bean, Shim	*Dolichos lablab*	Fabaceae	Nausea,vomiting and abdominal pains.
Amada	*Curcuma amada*	Zingiberaceae	In mild diabetes

have been left undiscovered till date and to validate their therapeutic uses with proper knowledge. The ultimate motto of our project work is not limited in just creating a database for future study but also providing the scientifically justified information to the mass for the welfare of the society.

MATERIALS AND METHODS

Study area[17] : Purulia is the westernmost district (Figure1) of West Bengal with all-India significance because of its tropical location, its shape as well as function like a funnel. It funnels not only the tropical monsoon current from the Bay to the subtropical parts of north-west India, but also acts as a gateway between the developed industrial belts of West Bengal and the hinterlands in Orissa, Jharkhand, Madhya Pradesh and Uttarpradesh.

Table 1 : Medicinal plants used by the people from Purulia district of West Bengal (continued)

Plant	Scientific Name	Family	Parts and uses of the same
Oleander, Karabi	*Nerium odorum*	Apocynaceae	Bark paste as cardiotonic
Arjuna	*Terminalia arjuna*	Combretaceae	Bark in chest pain and boiled bark in liver disorder
Kul, Common jujube	*Ziziphus jujuba*	Rhamnaceae	Leaves as astringent, antidirrhoeal and anthelmintic.
Shatamuli	*Asparagus racemosus*	Liliaceae	Root paste in scalding of urine, cough, abortion, bronchitis and tuberculosis.
Tulsi	*Ocimum sanctum*	Labiatae	Whole plant in seminal weakness & rheumatism; Leaves in cough
Custard apple	*Annona squamosa*	Anonaceae	Seeds are used to destroy lice and as abortifacient
Vinca, Periwinkle	*Catharanthus roseus*	Apocynaceae	Leaves of white variety in blood sugar and violet variety in asthma
Marigold	*Tagetas erecta*	Asteraceae	Juice of leaves to arrest bleeding.
Papaya	*Carica papaya*	Caricaceae	Fruit as digestive, Gum of stem in cough.
Nim	*Azadirachta indica*	Meliaceae	Leaves, bark, fruits, flowers, seeds & twigs are used in skin diseases, as antiseptic; stem as tooth-brush in pyorrhea
Tamarind	*Tamarindus indica*	Ceasalpiniaceae	Seeds in blood dysentery
Sisoo	*Dalbergia sisoo*	Fabaceae	Leaves as analgesic and antipyretic.
Palash	*Butea monosperma*	Fabaceae	Leaves in boils & pimples; Seeds in convulsions and skin diseases
Til	*Sesamum indicum*	Pedaliaceae	Boiled extract of leaves and roots in alopecia.
Thankuni	*Centella asiatica*	Apiaceae	Leaves in dysentery
Akanda	*Calotropis procera*	Asclepiadaceae	Roots in nocturnal eneuresis
Sal	*Shorea robusta*	Dipterocarpaceae	Seeds are used in diarrhoea and dysentery.
Bhui-amla	*Phyllanthus niruri*	Euphorbiaceae	Plant fresh juice & roots are used as diuretic, in troubles of gentio- urinary tracts, jaundice, & dysentery.

Purulia lies between 22.60 degree and 23.50 degree north latitude and 85.75 degree and 86.65 degree east longitude. The total geographical area of the district is 6259 sq. kms (Census 2001). Out of which the Urban and Rural areas are 79.37 sq. kms (1.27%) and 6179.63 sq. kms (98.73 %) respectively. This district is encircled by Bankura, Midnapore and Burdwan district of West Bengal and Hazaribag, Singbhum, Dhanbad, Ranchi, Jamshedpur and Bokaro of Jharkhand state.

The district is characterized by undulating topography with rugged hilly terrains. The main rivers passing through or bordering the district are Kangsabati, Kumari, Darakeswar & Subarnarekha. Due to undulated topography nearly 50% of the rainfall flows away as run off.

Cultivation of this district is predominantly monocropped. About 60 % of the total cultivated land is upland. Out of the total agricultural holding about 73 % belongs to small and marginal farmers

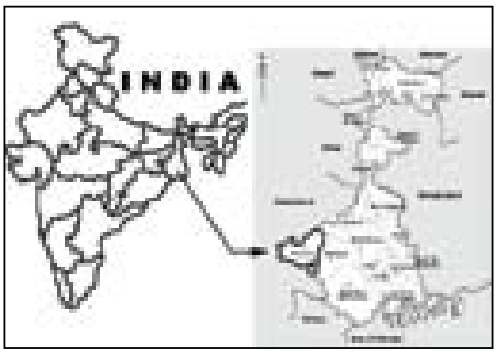

Figure 1 *: Location of the study are*

having scattered and fragmented smallholding. Paddy is the primary crop of the district.

Method of collection of information & taking of interview

The ethnobotanical study was carried out as a field survey in 14 villages of Purulia district of West Bengal primarily during the period of January, 2007 to April, 2008. Ethnomedicinal data were collected through general conversations with the village people in general and were verified, precised and summarized by the village Ayurvedic physicians. The questionnaires were used to obtain information on medicinal plants with their local names, parts used, mode of preparation and administration. In this regard the help of the villagers and the physician or the Baidya (vernacular designation of the physician) were taken in the identification of the plant. The persons mostly above the age of 60 have accurate information regarding their old traditions.

Generally two types of interviews were taken, firstly of individuals and secondly of groups. Of individuals, persons were selected at random on the way or entering a hut finding out knowledgeable individuals from the village or also the Pahan (village priest) or the Headman. In group interviews more than one individual were approached, our purpose explained and interviews taken. They were requested to collect specimens of the plants they knew or to show the plant species on site.

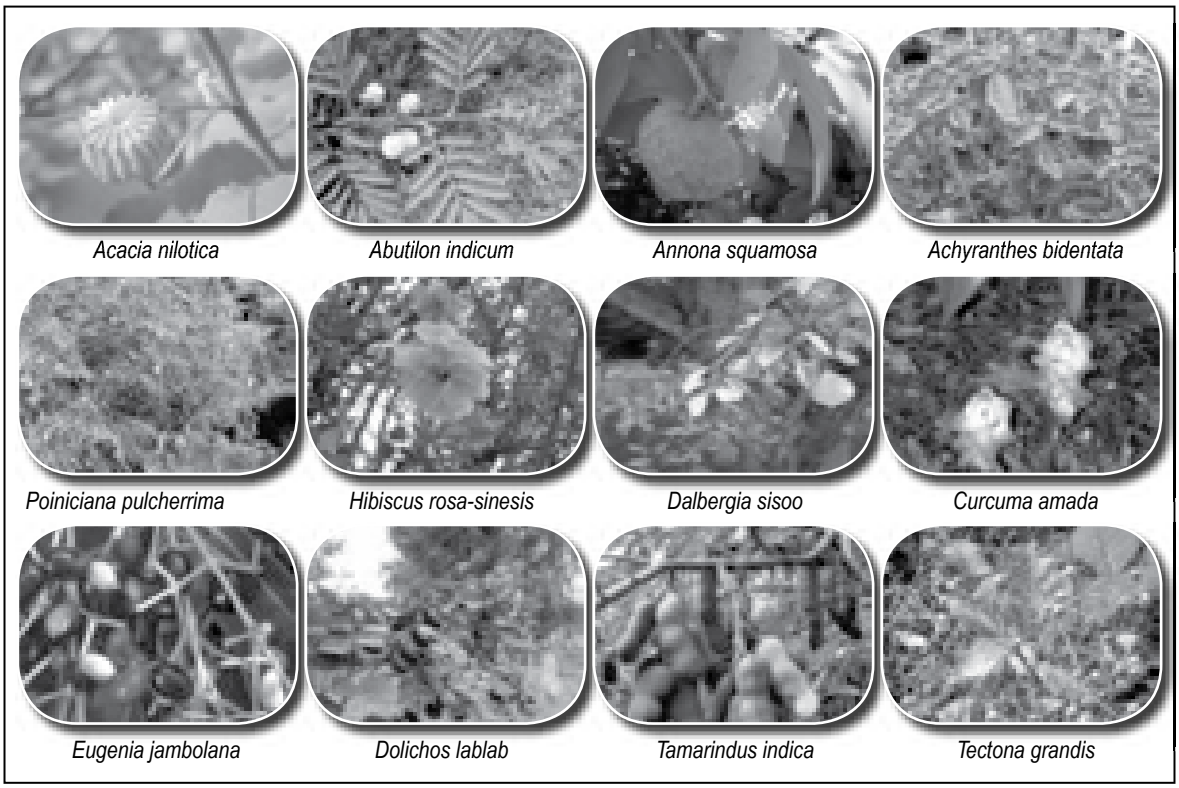

Figure 2 *: Some photographs of the collected specimens*

The identified plants were photographed and some plants without a genuine name were photographed, collected in intact and herbarium of the same was prepared. Thereafter the samples were identified by the help of The Botanical Gardens, Shibpur, West Bengal.

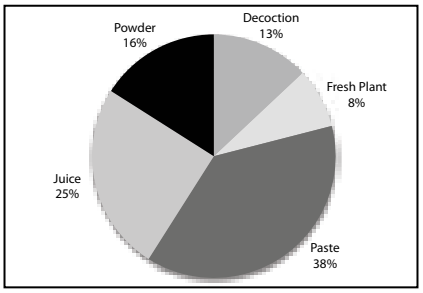

***Figure 3** : Methods of preparation*

RESULTS AND DISCUSSION

The rural area of West Bengal which was our study area is a potential source of traditional medicines. The medicine varies according to the symptoms and secondary effects and with the tribe and place. During the course of investigation it has been observed that a particular plant is sometime prescribed for different ailments in different localities and some Vaidya's (Ayurvedic physicians) apply a mixture of plants for remedy of a diseases. That means all these plants are useful for curing the ailments. The plants are enumerated in (Table-1& Figure-2) with their botanical name, family, parts along with their ethnomedicinal uses. Different parts of medicinal plants were used as medicine by the local traditional Baidyas. Among the different plant parts, the leaves were most frequently used for the treatment of diseases followed by whole plant parts, stem, fruit, root, seed, flower and latex. The methods of preparation fall into four categories, viz.: plant parts applied as a paste (38%), juice extracted from the fresh plant parts (25%), powder made from fresh or dried plant parts (16%), some fresh plant parts (8%) and decoction (13%) (Figure 3). External applications (mostly for skin diseases, snake bites and wounds) and internal consumption of the preparations were involved in the treatment of diseases. It was observed that, most of the remedies consisted of single plant part and more than one method of preparation. However, many of the remedies consisted of different parts of the same plant species to treat single or more diseases.

The Ethnobotanical data presented in this paper has been collected from the practitioners of Ayurveda (Vaidya's) with great difficulty because they do not want to divulge the secret of the identity of plant crude drug of traditional reputation. The use of the plant parts as a crude drug by various communities, local people and Vaidya's in Purulia for various ailments no doubt indicates that the plant must have medicinal properties in it. Therefore, the plant requires a thorough screening of its bio-active properties for the reported efficacies. The wealth of medicinal plant knowledge among the people of this district is based on hundreds of years of beliefs and observations. This knowledge has been transmitted orally from generation to generation; however it seems that it is vanishing from the modern society since younger people are not interested to carry on this tradition. It is hoped that our effort will not only provide additional support to the earlier findings recorded in the literature, but also provide clues for new materials having medicinal potentiality for traditional Indian system of medicine.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the honest villagers & practitioners of Ayurveda (Vaidya's) of the Purulia district for sharing their knowledge on medicinal plants of traditional reputation. The authors also pay their heartfelt thanks to the botanists of the Botanical Garden, Shibpur, who helped in identifying some of the important collected samples.

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Development and Validation of A HPLC Method for the Simultaneous Analysis of Abacavir Sulphate and Lamivudine in Combined Tablet Dosage Form

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KEY WORDS : Abacavir Sulphate and Lamivudine, HPLC, Method development and validation.

ABSTRACT

A simple and sensitive HPLC method has been developed for the simultaneous analysis of abacavir sulphate (ABS) and lamivudine (LMD) in combined dosage form. The method utilizes sample preparation followed by separation on a Waters Nova-pak HR silica column 300 mm length, 3.9 mm inner diameter with 6 μm particle size. Analytes were monitored by UV detection at 254 nm using an isocratic mode with acetic acid (0.2% v/v): methanol in the ratio of 65:35, v/v as mobile phase. The flow rate was set at 2 mL/min and sample volume injected were 20 μl, respectively. Calibration curves for ABS and LMD were found linear over concentration range of 7.5-120 μg/mL and 3.75-60 μg/mL with correlation coefficients 0.9996 and 0.9991, respectively. The recovery of ABS and LMD in tablet dosage form was found in the range from 98.28 to 99.96 %. The developed method was successfully employed in the analysis of ABS and LMD in tablet dosage form.

INTRODUCTION

Multi-drug therapy has become the standard treatment for acquired immunodeficiency syndrome (AIDS)[1]. The situation is imposed by the need to delay the development of resistance by the human immunodeficiency virus (HIV), the causative virus of AIDS, to single anti-HIV drugs and to minimize potential dose dependent side effect[2]. The current typical regimen for treating HIV infection is to use a combination of at least two drugs, a practice known as 'highly active antiretroviral therapy' (HAART)[3].

Abacavir sulphate is chemically known as {(1S,4R)-4-[2-amino-6-(cyclopropylamino)9H-purin-9-yl] cyclopent-2-enyl}methanol sulphate[4] and lamivudine (Fig 2), is chemically known as (2R,5S)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1H)-pyrimidinone[5]. These systemic antiretroviral drugs extensively used in human immunodeficiency virus (HIV) infected patients. Abacavir sulphate is novel nucleoside reverse transcriptase inhibitor (NRTI) that is an HIV-1 reverse transcriptase inhibitor and a potent in vivo and in vitro inhibitor of HIV-1, the causative agent of the acquired immunodeficiency syndrome (AIDS). It differs structurally from other NRTIs in that it is a carbocyclic nucleoside analogue rather than a dideoxynucleoside analogue. Abacavir sulphate was approved by the FDA in 1998 for use in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and children, based on observed beneficial effects of abacavir on surrogate markers of HIV-1 activity.

Lamivudine is a nucleoside analog having potent in-vitro and in-vivo inhibitory activity against HIV reverse transcriptase[6]. Lamivudine specifically refers to the (–) enantiomer of the cis-racemate. This

antiretroviral drug combination is official only in Indian Pharmacopoeia[7]. Literature survey reveals several methods that have been used for the quantitative determination of the two drugs individually or in combination with other drugs in human plasma by high performance liquid chromatography[8, 9], LC/MS/MS[10], HPLC-MS[11], MALDI-TOF/TOF[12] etc. RP-HPLC method with solid phase extraction procedure has been reported for simultaneous determination of six nucleoside analog reverse transcriptase inhibitors and HIV suppressing drugs of which ABS and LMD are a part. Besides, simultaneous quantification of several antiretroviral agents including these two drugs has been reported by a solid-liquid extraction procedure using RP-HPLC system[13-16] .

HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active components. The aim of the work was to develop a relatively simple HPLC method for simultaneous quantification of ABS and LMD in antiretroviral FDCs without the necessity of sample pre-treatment. This paper describes the development and validation of reliable, simple, stable and economic reverse phase HPLC assay, using UV detection for the simultaneous analysis of ABS and LMD in FDC tablets. The method appears to be suitable for quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference.

EXPERIMENTAL

Chemicals and reagents

Standard material of abacavir sulphate and lamivudine was provided by Cipla Ltd, Mumbai, India, as gift sample. Abacavir and Lamivudine 600/300 (Abacavir sulphate 600 mg + lamivudine 300 mg) tablets manufactured by Cipla Ltd, was purchased from local market.

All chemicals and reagents used were of HPLC grade. Methanol was purchased from Merck Chemicals, Mumbai, India and acetic acid was purchased from SLR laboratories, Delhi, India. HPLC grade water was prepared using Millipore Purification System (Millipore, Molsheim, France, Model Elix-10).

Instruments

The instrument Waters Liquid Chromatography equipped with a pump (Waters 600), Autosampler (Waters 717), UV Detector (Waters 486) in isocratic mode was used. The system was connected with the help of a Millennium 32 Software in a Computer System for data collection and processing. Beckman DU-640B spectrophotometer is used for determination of absorbance in different wave length and λmax. A Microprocessor pH system was used for pH measurements.

Chromatographic conditions

The mobile phase consists of 0.2% v/v acetic acid in water and methanol was used in the ratio of 65:35, v/v and filtered through 0.45 μm nylon membrane filter before use. The column used was Waters Nova-pak HR silica of 300 mm length, 3.9 mm inner diameter and 6 μm particle size. The injection volume was injected 20 μL with a flow rate of 2 mL/ min. The wavelength of detection selected was 254 nm, as both the drugs showed optimum absorbance at that wavelength. Analysis was performed at controlled column temperature at 40°C.

Preparation of Standard Solutions

Approximately 60 mg of ABS and 30 mg of LMD reference standards were accurately weighed and transferred to a 50 mL volumetric flask. The volume was filled to the mark with methanol, to obtain a

concentration of 1200 μg/mL of ABS and 600 μg/mL of LMD. The solution was further diluted to obtain solutions in the range 7.5-120 μg/mL of ABS and 3.75- 60 μg/mL of LMD.

Preparation of Sample Solution

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed portion of the powder equivalent to about 60 mg of ABS and 30 mg of LMD, was transferred to a 50 mL volumetric flask. This content was then mixed with methanol, sonicated for 20 mins and diluted with the same solvent. The resultant mixture was filtered through 0.45 μm nylon filter and filtrate was diluted to have a concentration of 15 μg/mL of ABS and 7.5 μg/mL of LMD.

METHOD VALIDATION

System Suitability

The system suitability was evaluated by six replicate analysis of ABS/LMD mixture at a concentration of 15 μg/mL of ABS and 7.5 μg/mL of LMD. The acceptance limit was 2% for the percent relative standard deviation (%RSD) of the peak area and the retention time of ABS and LMD.

Detection and quantitation limits (sensitivity)

Limits of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD is defined as the lowest concentration resulting in a peak area of three times the base line noise. LOQ is defined as the lowest concentration that provides a signal to noise ratio higher than 10.

Linearity (calibration curve)

The calibration curves were constructed with concentrations (simultaneously prepared) ranging from 7.5 to 120 μg/mL and from 3.75 to 60 μg/mL for ABS and LMD, respectively. Calibration curves were constructed by plotting the ratio of the mean peak area of either ABS or LMD versus the concentration. The linearity was assessed by linear regression analysis, which was calculated by the least square method.

Accuracy and precision

Precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) for 3 consecutive days. Three different concentrations of ABS and LMD were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). Every sample was injected in triplicate. The accuracy of the method, which is defined as the nearness of the true value and found value, was evaluated by the following equation :

$$\% \text{ accuracy} = \text{observed concentration} / \text{nominal concentration} \times 100$$

Specificity

The specificity of an analytical method may be defined as the ability to detect the analyte peak in the presence of the analyte by-products, or other inactive components, such as dosage form excipients or impurities. This Study is performed to demonstrate to non interference from degradation products that are formed during acid stress, base stress and oxidative stress, on the test sample. The method will be stability indicating, if the degradation products do not interfere with the analyte peaks.

Stability

The stability of the drug solution was determined by analyzing samples after short term storage at controlled room temperature (20-25°C) for 12 h. The long-term stability was determined by analyzing

samples stored at 4°C for 30 days. The auto sampler Stability was determined by analyzing the samples after 24 h of storage in the auto sampler.

Recovery

The pre-analyzed samples were spiked with extra 80, 100 and 120 % of the standard abacavir sulphate and lamivudine were analyzed by the proposed method. The experiment was conducted in triplicate. This was done to check the recovery of the drug at different levels in the formulations.

Assay procedure

Drug contents were calculated by comparison with the appropriate standard solution of the drug. No interferences due to excipients was observed in the spectra or chromatograms produced.

RESULTS AND DISCUSSION

Method development and optimization

The chromatographic conditions were optimized for the simultaneous determination of ABS and LMD within a short analysis time and an acceptable peak resolution (> 2). To accomplish these objectives, the chromatographic column was first chosen based on peak shapes and resolution. In preliminary experiments, the sample retention time increased with an increase in column length. In order to avoid long run times, a C18 column was initially used. This, however, resulted in peak overlap between ABS and LMD, with a consequent peak shape inconsistency during elution. Therefore, a silica column of 300 mm length and 3.9 mm inner diameter with 6 μm particle size was used which permits the use of high flow rate with consequent low back-pressure, was subsequently used for better resolution, reduce elution time, and obtain sharp peaks. With regards to the mobile phase, the use of salts in the mobile phase can affect the lifespan of the column. In order to avoid this drawback, a mobile phase containing various ratios of water and methanol was initially used. Acetic acid was used instead of water, and found better result by using 65% of 0.02% acetic acid in water.

Finally the influence of flow rate of mobile phase and column temperature were studied. The retention times decreased when the flow rate increased. Hence, flow rate of 2 mL/min was selected. Column temperature influenced the chromatogram and selected 40°C as optimum column temperature. In all cases, satisfactory chromatographic resolution (>2) between peaks were reported. The proposed method was found to be selective for the estimation of two drugs as recoveries of the drugs were not affected by the excipients and the excipient blend did not show any absorption in the range of analysis.

Validation of the proposed method

The method was validated with respect to the following parameters given below as per ICH guidelines[17]:

System suitability

This step was accomplished under the conditions stated earlier. The %RSD of peak area and retention time for ABS and LMD were within 2%. This indicates the suitability of the system to analyze ABS and LMD simultaneously (Table 1). The retention time were 11.09 and 6.21 for ABS and LMD, respectively (Fig 1 and 2).

Detection and quantitation limits (sensitivity)

The LOD was found to be 0.1028 and 0.1168 μg/mL and LOQ was 0.3115 and 0.3541 μg/mL for ABS and LMD, respectively.

Linearity (calibration curve)

The linearity of the calibration curves for ABS and LMD was calculated and constructed by least square regression method as illustrated previously. Table 2 shows the outcome of the statistical analysis. The correlation coefficient (r^2) for the standard calibration curves for ABS and LMD were 0.9996 and 0.9991, respectively. This indicates linearity of the peak area ratio of ABS or LMD in the range of 7.5–120 μg/ml and 3.75–60 μg/ml, respectively (Fig 3 and 4).

Accuracy and precision

Accuracy and precision during the intra and inter-day run are given in Table 3. The data were found within the acceptance criteria (i.e. %RSD is <2). Inter and intra-day accuracy (expressed as %RSD) ranged from 0.55 to 1.93 and from 0.84 to 1.96 for ABS and LMD, respectively.

Specificity

In order to confirm the specificity of the method for ABS and LMD in the presence of excipients, three methanolic solutions of the tablets after filtration through 0.45 μm filter were injected into the HPLC. As discussed above, the specificity of an HPLC method is the ability to detect the analytes in the presence of other ingredients. Peak identification was performed under the experimental conditions stated previously. The excipients present in the sample did not exhibit any peaks, and therefore no interferences were detected as indicated by the absence of signals in the chromatograms.

Similarly, Study is performed to demonstrate to non interference from degradation products that are formed during acid stress, base stress and oxidative stress on the test sample (Table 4). The method will be stability indicating, if the degradation products do not interfere with the analyte peaks.

Stability

The data for short-term, long-term, and the autosampler stability of the ABS and LMD solutions are given in Table 5. The %mean concentration was found within the acceptable limit (90–110%).

Recovery

The proposed method when used for extraction and subsequent estimation of ABS and LMD from pharmaceutical dosage form after spiking with 80, 100 and 120 % of additional drug afforded recovery of 98-102% as listed in Table 6.

Assay of ABD and LMD in tablets

The chromatograms of ABS and LMD standard and sample are given in Fig 5 and 6. The assay results obtained are presented in Table 7.

CONCLUSION

The developed HPLC method was found suitable for the analysis of abacavir sulphate and lamivudine in marketed tablet dosage form. Statistical analysis proves that the method is repeatable and selective for the analysis of abacavir sulphate and lamivudine simultaneously. This method might be employed for quality control analysis. This method has the advantage of being specific for both drugs without the need for additional sample preparation, such as the extraction of the active constituents.

Table 1. System suitability parameters for the analysis of ABS and LMD

Parameters	ABS (15μg/mL)		LMD(7.5μg/mL)	
	Retention time (min)	Peak area	Retention time (min)	Peak area
Average	11.09	302920.8	6.21	107204.7
SD	0.06	1977.12	0.36	2052.89
%RSD	0.54	0.65	0.57	1.91

Table 2. Regression characteristics of the ABS and LMD determined by the HPLC

Drug	Range, μg/mL	Regression equation	r^2	LOD, μg/mL	LOQ, μg/mL
ABS	7.5-120	AABS= 18515x+12383	0.999	0.1028	0.3115
LMD	3.75-60	ALMD= 14105x+2083	0.999	0.1168	0.3541

Table 3. Intra-day and inter-day precision and accuracy results of ABS and LMD(n = 6)

Nominal concentration (μg/mL)	Day 1			Day 2			Day 3		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
ABS(7.5)	7.62	0.15	1.93	7.42	0.14	1.83	7.52	0.11	1.44
ABS(60)	60.03	0.64	1.07	60.06	0.58	0.97	60.04	0.84	1.40
ABS(120)	120.29	0.77	0.64	120.27	0.67	0.55	120.84	0.67	0.55
LMD(3.75)	3.95	0.03	0.84	3.78	0.08	1.99	3.85	0.08	1.96
LMD(30)	30.84	0.60	1.95	30.45	0.50	1.64	30.28	0.48	1.57
LMD(60)	60.17	0.53	0.89	60.43	0.60	1.00	60.03	0.97	1.62

Table 4. Specificity study (Forced Degradation Studies)

Test Preparation		abacavir sulphate		lamivudine	
		Test mg	Assay %	Test mg	Assay %
As such	Strength	300.34	100.0	150.95	100.0
Base Treated	0.1 N NaOH	300.54	97.3	150.56	97.1
Acid Treated	0.1 N HCl	300.87	97.8	150.86	96.8
Peroxide Treated	5% H_2O_2	300.43	97.5	150.90	96.5

Table 5. Short-term, long-term, &auto-sampler stability for ABS and LMD combined solns.(n = 6).

Nominal concentration (μg/mL)	Short term stability			Long term stability			Auto sampler stability		
	Mean*	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
ABS(7.5)	99.43	0.76	0.76	100.54	0.87	0.87	103.54	0.93	0.90
ABS(60)	100.54	0.84	0.84	102.65	1.37	1.33	102.87	1.82	1.77
ABS(120)	101.65	0.67	0.66	100.86	0.67	0.66	98.98	0.96	0.97
LMD(3.75)	100.06	0.08	0.08	98.97	1.95	1.97	100.58	1.87	1.86
LMD(30)	99.35	0.48	0.48	99.65	1.38	1.38	98.36	1.07	1.09
LMD(60)	100.98	0.97	0.96	102.76	1.54	1.50	101.98	1.39	1.36
* Expressed as percentage of nominal concentration									

Table 6. Recovery studies for the determination of abacavir sulphate (ABS) and lamivudine (LMD) by the proposed HPLC method

Drug	Amount Added (mg)	Amount Recovered (mg)	% Recovery
ABS	480	478.5	99.69
	600	598.1	99.68
	720	711.4	98.8
LMD	240	239.9	99.96
	300	295.1	98.37
	360	353.8	98.28

Table 7. Assay results obtained in ABS and LMD tablet dosage form

Tablet[a]	Drug[b] mg/tablet	Drug found ± SD, %
Abacavir sulphate 600 mg + Lamivudine 300 mg	ABS, 600 mg	98.66 ± 0.21
	LMD, 300 mg	99.83 ± 0.35

[a] n = 3.

[b] ABS = Abacavir sulphate; LMD = Lamivudine

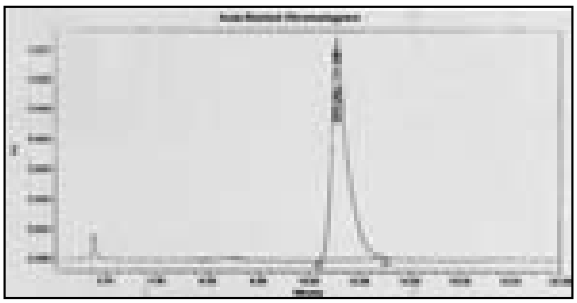

Fig 1. *Representative HPLC chromatogram for identification of abacavir sulphate*

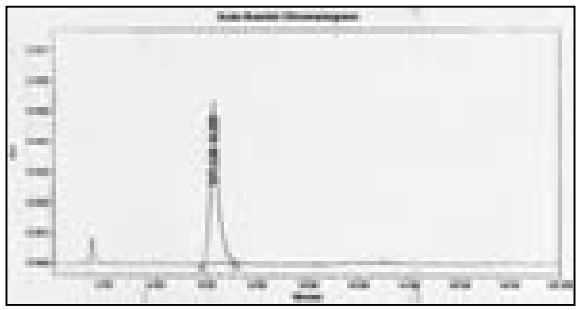

Fig 2. *Representative HPLC chromatogram for identification of lamivudine*

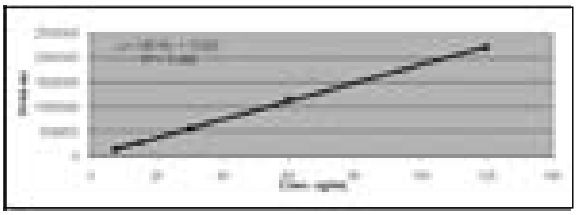

Fig 3. *Linearity curve of abacavir sulphate*

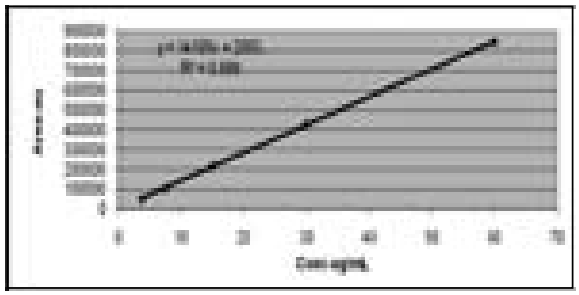

Fig 4. *Linearity curve of lamivudine*

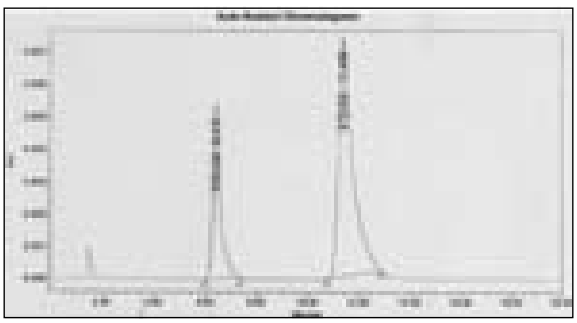

Fig 5. *A representative HPLC chromatogram of ABS and LMD (standard)*

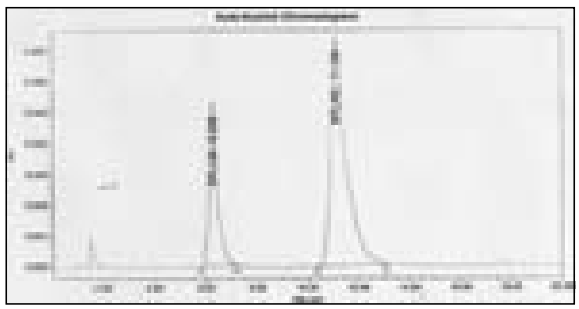

Fig 6. *A representative HPLC chromatogram of ABS and LMD (sample)*

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Quantitative Estimation of Stigmasterol from *Leucas Clarkei* by HPTLC Method

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ABSTRACT

A simple, precise, accurate and reliable high performance thin layer chromatographic (HPTLC) method has been developed for the quantification of stigma sterol in whole plant of Leucas clarkei. Chromatographic analysis was performed using chloroform and Methanol extract of Leucas clarkei, on silica gel GF 254 HPTLC plates, using a solvent system, comprising of chloroform: methanol 9:1 (v/v). Detection and quantification of stigma sterol was achieved at 365 nm. The total content of stigma sterol estimated in chloroform and ethanol extract was 3.82%, 3.91% respectively.

INTRODUCTION

Leucas clarkei[1,2] (Lamiaceae) grows as annual herbs in most tropical countries and is widely distributed throughout India. The Tribal of Orissa uses this plant for the treatment of pain caused by obstruction in menstrual flow, epilepsy, sore throat and infectious diseases. It is widely used for healing of wounds[3]. The preliminary phytochemical screening[4] shows the presence of active ingredients such as fatty acid, flavonoids, steroids, terpenoids and phenolic compounds.

The aim of present study is to develop a simple, rapid, precise and accurate HPTLC method for quantification of stigma sterol in *Leucas clarkei* whole plant extracts.

MATERIAL AND METHODS

Collection of Plant Material

The Plant *Leucas clarkei* were collected from Bolangir in the month of October. It was identified and authenticated by Dr. (Mrs.) Uma Devi, Head of Department of Botany, Govt. Women's College, Sambalpur, Orissa, having authenticated voucher no GWC/B-315/09 and voucher specimens deposited there for future reference .

PREPARATION OF EXTRACT

The collected leaf of *Leucas clarkei* were shade dried, coarsely powdered and stored in air tight container at 27°C,1500gm of powdered drug extracted with Hexane, Chloroform, Methanol, Purified Water by using Soxhlet apparatus. The extracts were evaporated by Rota evaporator to dryness.

INSTRUMENT

CAMAG (Switzerland) HPTLC system equipped with Linomat IV sample applicator was used for application of test sample. CAMAG TLC Scanner, controlled by win CATS Software was used for scanning the plates. CAMAG twin trough glass chamber was used for developing the plates.

REAGENT AND OTHER MATERIALS

Analytical grade chloroform and methanol were obtained from S.D. Fine chemicals (Mumbai). Standard stigma sterol as marker compound obtained from RRL (IMMT), BBSR.

STANDARD PREPARATION

Standard stock solution (1000 μg/ml) of stigma sterol was prepared by dissolving 10mg of stigma sterol in a minimum quantity of chloroform and diluting to volume with the same solvent up to 10ml in a volumetric flask. Further standard dilutions were prepared by dilution of this stock solution.

SAMPLE PREPARATION

Preparation of sample for Chloroform Extract

50mg of extract was dissolved in a minimum quantity of chloroform and then total soluble extract was poured to a 25ml volumetric flask and the volume was adjusted by adding chloroform.

PREPARATION OF SAMPLE FOR METHANOL EXTRACT

50mg of extract was dissolved in a minimum quantity of chloroform and then total soluble extract was poured to a 25ml volumetric flask and the volume was adjusted by adding chloroform.

CHROMATOGRAPHIC CONDITIONS

Stationary Phase	-	TLC precoated silica gel 60F254
Mobile Phase	-	Chloroform: Methanol (9:1 v/v)
Development	-	Camag twin trough glass chamber (20 X 10 cm)
Separation	-	Ascending development
Spotting Volume	-	2.5, 7.5, 10, 15, 17.5, 20 mcl for standard12 mcl for sample of each extract
Distance Developed	-	85mm
Detection Wavelength	-	366 nm
Lamp used	-	Deuterium lamp
Slit Dimension	-	5.0 x 0.45mm

SCANNING OF SPOT

The spots were scanned by TLC scanner, as per the chromatographic conditions as application mode, mobile phase saturation time and band width to achieve the best resolution and peak shape.

LINEARITY AND CALIBRATION[5, 8]

An essential step for any new developed instrumental technique is to détermine the range in which the detector exhibits linear response for the analyste. Standard and Sample solutions were applied as per the spotting volume mention in chromatographic Condition, by using Linomat-IV applicator. The chromatoplate was then developed in a pre-saturated twin trough chamber containing mobile phase. The developed plate was scanned at 366nm using a scanner and peak areas were measured. A calibration curve was constructed by plotting peak area (Y-axis) against concentration of drug (X-axis) The corrélation coefficient, slope and intercept were found to be 0.9994, 918.2 and 940.2 respectively.

RESULTS AND DISCUSSION

Result

From the above diagram the following results were obtained.

i) 24μg Chloroform: Ethanol (1:1) extract contain 918.2ng of stigmasterol.

ii) 24μg Methanol extract contain 940.2ng of stigmasterol.

CALCULATION

i) Percentage of stigma sterol was present in chloroform extract

$$= \frac{0.9182}{24} = 3.82\%$$

ii) Percentage of stigma sterol was present in Methanol extract

$$= \frac{0.9402}{24} = 3.91\%$$

CONCLUSION

From the above experiment, it was found that chloroform extract contain 3.82% and the Methanol extract 3.91% of stigmasterol.

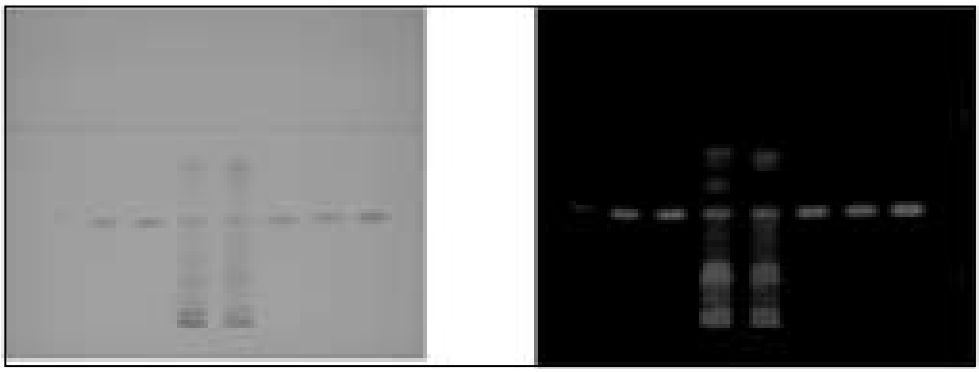

Fig. I *After Sulfuric acid spray (5%)*

Fig. II *Visible after Sulphuric acid spray in 366nm*

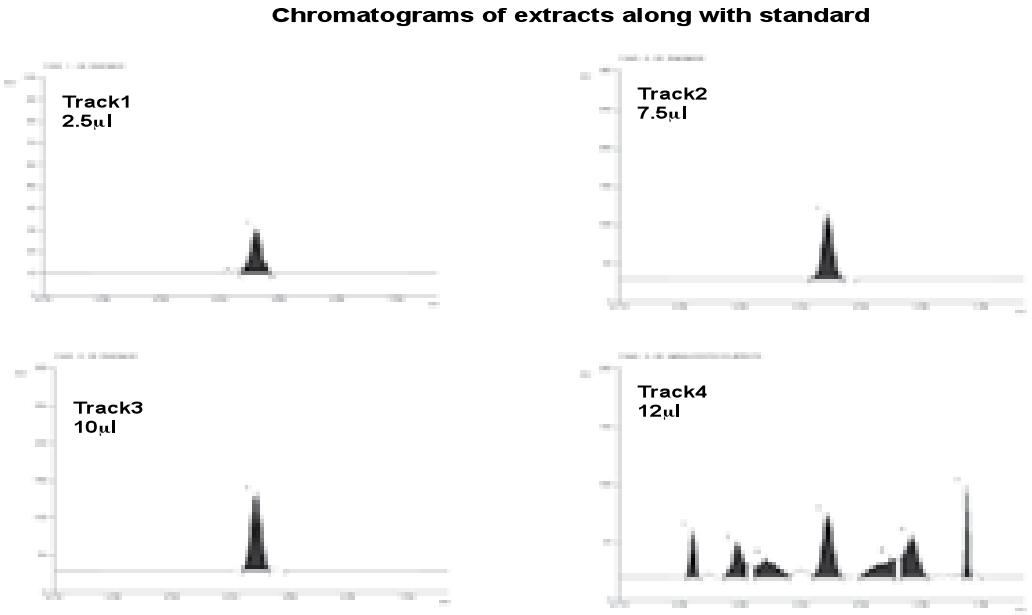

Fig. III

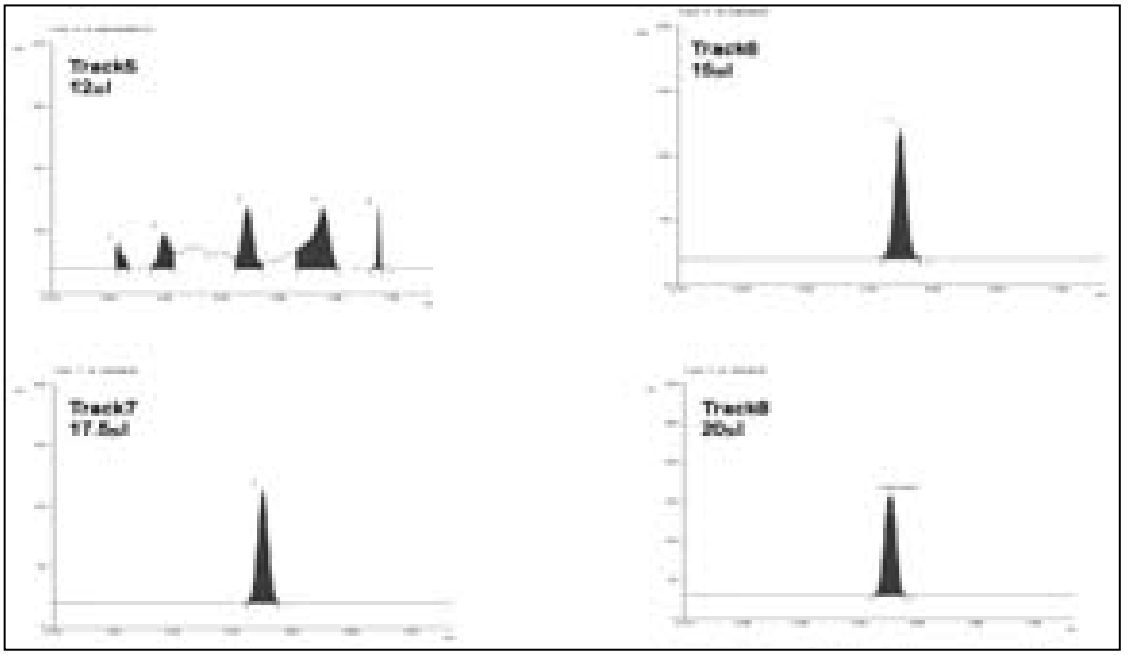

Fig. IV

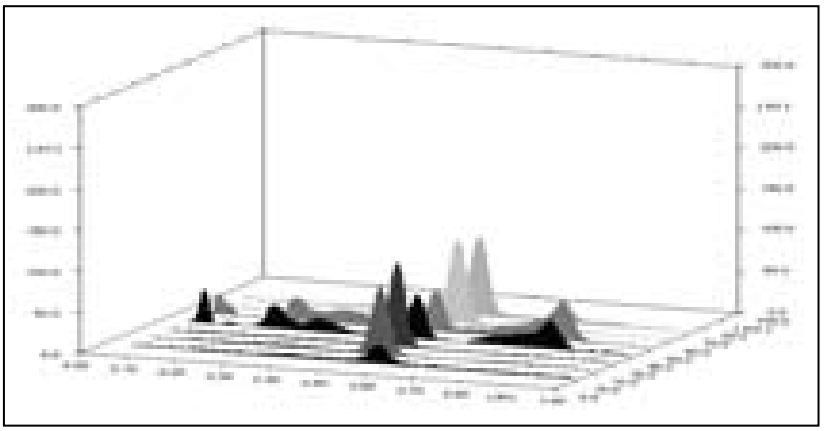

Fig. V

3D-View of Extract and Stigma sterol

T1, T2, T3, T6, T7, T8: Standard

T4 : Sample 1, T5 : Sample 2

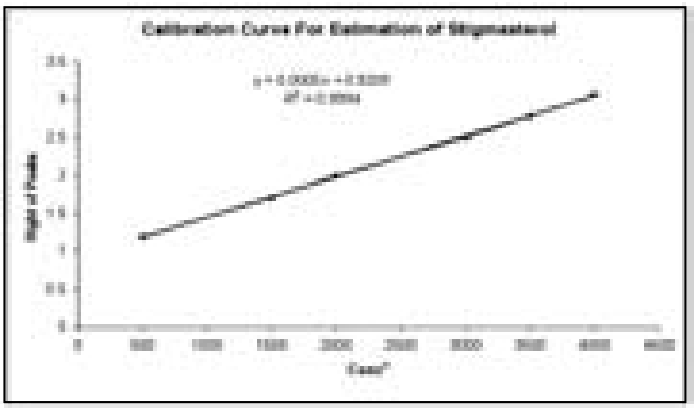

Fig. VI

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Dr. A. Sree, Scientist, E-II, IMMT (RRL), Bhubaneswar, Orissa.

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Simultaneous Estimation of Simvastatin and Ezetimibe in Solid Dosage Form by UV Spectrophotometry

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KEY WORDS : Simvastatin, Ezetimibe, Methanol, Absorption correction method, Absorbance ratio method, UV Spectrophotometry.

ABSTRACT

Simple, precise and accurate spectrophotometric methods were developed for the simultaneous estimation of simvastatin and ezetimibe in tablets using absorption correction method and Q Analysis spectrophotometric methods. In the first method, simvastatin and ezetimibe in methanol had λ_{max} at 237.6 nm and 260 nm, respectively showing linearity in the concentration range of 4- 24 μg/ml for both simvastatin and ezetimibe. The absorbance of the mixed standard and sample solutions were measured at 260 nm and the content of ezetimibe was determined using its E (1%, 1cm) value, where the absorbance of simvastatin is zero. The absorbance of the final dilutions was measured at 237.6 nm and the absorbance of ezetimibe was corrected at 237.6 nm. The content of simvastatin was then calculated using E (1%,1cm) value. In the second method, the amounts of simvastatin and ezetimibe were calculated on the basis of measurement of the absorbance at 242 nm an isoabsorptive point for both the drugs and the other at 237.6 nm, λ_{max} of simvastatin. In these wavelengths, both the drugs were showing the linearity in the concentration range of 4- 24 μg/ml. The amount of simvastatin and ezetimibe from the tablets were calculated by using the above two methods. Results of analysis of both the methods were validated statistically and by recovery studies.

INTRODUCTION

Simvastatin[1,2] (SIM) is an oral anti - lipidaemic agent and chemically it is butanoic acid, 2,2-dimethyl- 1,2,3,7,8,8a - hexa hydro - 3,7-dimethyl- 8-[2-(tetra hydro - 4 - hydroxy - 6 – oxo-2H - pyran - 2 - yl) - ethyl] -1 - naphthalenyl ester. SIM is official in BP[3]. Literature describe second derivative UV spectrophotometry[4], HPLC[5,6] and LC-MS[7] methods for its determination in pharmaceutical preparations when present alone. Ezetimibe1 (EZE) is also an oral anti - lipidaemic agent and is chemically 1 - (4 - fluorophenyl) - (3R) - [3 - (4 - fluorophenyl) - 3S) -hydroxyphenyl] - 4S - (4 - hydroxyphenyl) - 2 - azetidinone. It is not official in any pharmacopoeia. HPLC[8] and LC-MS[9] methods have been reported for the estimation of EZE in pharmaceutical formulations and in plasma. Also First derivative spectrophotometric method[10] and HPLC[11,12] methods were reported for the estimation of SIM and EZE in combination.

The review of literature revealed that no method is yet reported for the simultaneous estimation of SIM and EZE in fixed dose combination products by absorption correction method and absorbance ratio method (Q analysis). Hence the present paper describes simple, rapid, accurate and reproducible methods for the simultaneous estimation of SIM and EZE in tablet formulation using absorption correction method and absorbance ratio method.

MATERIALS AND METHODS

Materials

SIM and EZE were gift samples from Micro labs Bangalore. The commercial fixed dose combination product (SIMVAS-EZ® containing 10 mg of SIM and 10 mg of EZE) was procured from the local market. Methanol AR Grade was procured from Qualigens Fine Chemicals, Mumbai.

Equipments

A Shimadzu UV- 1700; UV-Visible spectrophotometer using 1cm matched quartz cells were used for the experimental purpose. Shimadzu - AX - 200 electronic balance was used for weighing the samples. Calibrated glassware was used for analysis.

Method 1- Absorption correction method

Overlain spectra of the drugs in methanol indicates that EZE shows λ_{max} at about 233.2 nm but it also shows significant absorption at 260 nm, but SIM has zero absorbance at that wavelength. However both the drugs have strong absorption at 237.6 nm. The Beer's law obeyed in the concentration range of 4-24 μg/ml at 237.6 nm for SIM and EZE and by EZE over 4- 24 μg/ml at 260 nm. Absorbances of both the drugs are practically additive at 237.6 nm. These absorbances were plotted against concentration to obtain the calibration graph. Both the drugs obey Beer's law with the above concentration range. The absorptivity values were calculated. The absorption ratio of EZE at 260:237.6 nm was calculated which is practically constant. The estimation of EZE has been done at 260 nm using its absorptivity value free from the interference of SIM. An accurate estimation of SIM at 237.6 nm has been achieved after correction of absorption by EZE on the basis of its absorption ratio.

Analysis of synthetic mixture of SIM and EZE

Solutions containing various proportions of SIM and EZE were prepared in nethanol and absorbance were measured at 260 nm and 237.6 nm and the concentrations were calculated by absorption correction method.

Analysis of tablet formulation

Twenty tablets were weighed accurately, the average weight was determined and then ground to a fine powder. A quantity equivalent to 20 mg of SIM was transferred to 100 ml volumetric flask. The contents were shaken with methanol to dissolve the tablet powder and made to volume with methanol. The solution was then sonicated for 10 minutes. Filtered through Whatman filter paper no.41. The filtrate was diluted with methanol to give the dilution within the Beer's law range (10 μg/ml). The sample solution was then measured at 260 nm and the content of EZE was calculated using its absorptivity value. The absorbance of the solution was measured at 237.6 nm and corrected absorbance of SIM at 237.6 nm was calculated using following formula.

AcSOL (237.6nm) = Ao SOL (237.6nm) – AEZE (260 nm) / 10 x R

Where,

AcSOL (237.6nm) = Corrected absorbance of solution of SIM at 237.6 nm

Ao SOL (237.6nm) = Observed absorbance of solution of SIM at 237.6 nm

AEZE (260 nm) = Absorbance of EZE at 260 nm

R = Absorption ratio of EZE at 260: 237.6 nm = 1.698

The content of SIM was then calculated using E (1%, 1cm) value. Further, the precision of the method was confirmed by carrying out the analysis at three different intervals in the same day and on three successive days.

RECOVERY STUDIES

To determine the accuracy of the method, recovery study was performed using the method of addition. To the preanalysed marketed formulation powder equivalent to 20 mg of SIM, an accurately weighed quantity of raw material was added at 4 levels viz., 50%, 75%, 100%, 125% for both the drugs and the total drug contents were determined as described for formulation. The percentage recovery was determined. Each level was repeated for six times.

METHOD 2- ABSORBANCE RATIO METHOD

From the overlain spectrum of SIM and EZE, two wavelengths were selected one at 242 nm which was isoabsorptive point of both drugs and other at 237.6 nm the λ_{max} of SIM. Serial dilutions of different concentrations of both the drugs were prepared and absorbance were measured at their selected wavelengths. Both the drugs obeyed in the concentration range of 4-24 μg/ml. The absorptivity values for both the drugs at the selected wavelengths were calculated. The method employs Q Analysis. The concentrations of drug in sample solutions were determined by using the following formula.

For SIM $$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} X \frac{A}{a_1}$$

For EZE $$C_1 = \frac{Q_0 - Q_1}{Q_2 - Q_1} X \frac{A}{a_2}$$

Where $$Q_0 = \frac{\text{Absorbance of sample at 237.6 nm}}{\text{Absorbance of sample at 242 nm}}$$

$$Q_1 = \frac{\text{Absorptivity of simvastatin at 237.6 nm}}{\text{Absorptivity of simvastatin at 242 nm}}$$

$$Q_2 = \frac{\text{Absorptivity of ezetimibe at 237.6 nm}}{\text{Absorptivity of ezetimibe at 242 nm}}$$

A = Absorbance of sample at isoabsorptive point.

a_1 and a_2 = Absorptivities of SIM and EZE, respectively at isoabsorptive point.

Analysis of synthetic mixture of SIM and EZE:

Solutions containing various proportions of SIM and EZE were prepared in methanol and absorbance were measured at 242.0 nm and 237.6 nm and the concentrations were calculated by absorbance ratio method.

ANALYSIS OF TABLET FORMULATION

Twenty tablets were weighed accurately; the average weight was determined and then ground to

a fine powder. A quantity equivalent to 20 mg of SIM was transferred to 100 ml volumetric flask. The contents were shaken with methanol to dissolve the tablet powder and made to volume with methanol. The solution was then sonicated for 10 minutes. Filtered through Whatman filter paper no.41. The filtrate was diluted with methanol to give the dilution within the Beer's law range. The sample solution was then measured at 242 nm and 237.6 nm. The content of SIM and EZE were calculated by applying Q analysis. The precision of the method was confirmed by carrying out the analysis at different intervals in the same day and on successive days.

RECOVERY STUDIES

The accuracy of the method was confirmed by recovery experiments using method of standard addition. To the marketed formulation powder equivalent to 20 mg of SIM, an accurately weighed quantity of raw material is added at four levels – 50%, 75%, 100% and 125% for both the drugs using methanol as the solvent. The added amount was calculated and the percentage recovery was calculated. Each level was repeated for six times.

RESULTS AND DISCUSSION

Simple, precise and accurate spectrophotometric methods were developed for the simultaneous estimation of SIM and EZE in tablets using absorption correction method and Q Analysis spectrophotometric methods. In absorbance correction method, the working standard solutions of both the drugs were scanned from 200 to 400 nm to select the wavelength for estimation (figure.1). The overlain spectra of the drugs in methanol indicate that EZE shows λ_{max} at about 233.2 nm but it also shows significant absorption at 260 nm, while SIM has zero absorbance at that wavelength. However both the drugs have strong absorption at 237.6 nm. Hence 260 nm and 237.6 nm were selected as analytical wavelengths. Both SIM and EZE obeyed Beer's law in the concentration range of 4 - 24 μg/ml. The E (1%, 1cm) values were found to be 211.94 and 359.81 for EZE at 260 and 237.6

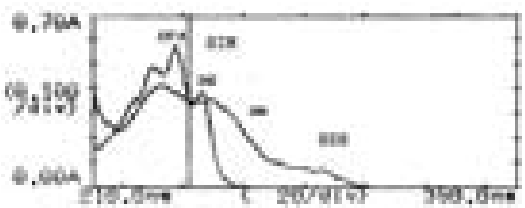

Fig. 1 *: Overlain spectra of SIM (10 mcg/ml) and EZE (10 mcg/ml) in methanol.*

Table 1 : Absorptivity Values (E1% 1μm) of SIM and EZE at 242 nm (Isoabsorptive Point) and 237.6 nm (λ_{max} of Sim) and 260 nm

S.No	Absorptivity at 237.6 nm		Absorptivity at 242 nm		Absorptivity at 260 nm	
	SIM	EZE	SIM	EZE	SIM	EZE
1	520.00	350.00	317.50	331.25	-	205.00
2	533.75	357.50	332.50	335.83	-	212.50
3	537.50	365.00	334.17	335.63	-	215.00
4	537.50	360.00	334.38	336.00	-	211.88
5	538.50	363.00	335.00	335.83	-	213.50
6	537.08	363.33	335.00	332.84	-	213.75
Mean	534.06	359.81	331.43	322.50	-	211.94

nm, respectively and 534.06 for SIM at 237.6 nm (Table 1). Moreover the absorption ratio of EZE at 260:237.6 nm has been found to be 1.698 which is practically constant. To study the mutual interference, if any, in the simultaneous estimation of SIM and EZE, synthetic mixtures containing various proportions of SIM and EZE were prepared and the contents were estimated by the proposed method. The amount recovered indicated no mutual interference up to the ratio of 14:6 for both the drugs. Analysis of synthetic mixture is shown in table 2. The stability of solutions of formulation was determined by measuring the absorbance at 260 nm for EZE and 237.6 nm for SIM and EZE, respectively at periodic intervals. There was no change in the absorbance at these wavelengths up to 4 hours indicating that the solution was stable for at least 4 hours. Commercial formulation containing SIM and EZE was analysed by proposed method. Six replicate analysis of formulation was carried out and the mean SIM content was found to be 10.11 mg/tablet and the mean content of EZE was found to be 10.10 mg/tablet. The corresponding standard deviation was found to be 1.1286 for SIM and 1.4948 for EZE indicating that the method has required precision. This is shown in table 3. The accuracy of the method was determined by recovery studies. SIM and EZE were added to pre analysed tablet powder at four different levels viz., 50%, 75%, 100% and 125%. Six replicate analysis was carried out at each level. The recovery was found to be in the range of 98.76% to 100.68% for SIM and from 99.01% to 100.86% for EZE with %RSD values are less than 2%. This indicated that the method was accurate. The results of analysis are shown in table 4. To study the robustness of the method, analysis of formulation was carried out the repeatable analysis on the same day and on three different days. The validation results are given in table 5. The results are proved the reliability of the method.

Method II is graphical absorbance ratio method. This method is based on the selection of isoabsorptive point of two drugs. i.e. the wavelength of equal absorptivity of the two components of the mixture. The isoabsorptive point was found to be 242 nm.The other wavelength selected is the absorption maximum of one of the components. In this case, 237.6 nm was selected. It is the absorption maximum of SIM. Different concentrations of serial dilutions were prepared and the absorbance were measured at

Table 2 : Analysis of Synthetic Mixtures of SIM and EZE

Method	Concentration of SIM and EZE	Calculated concentration of SIM	Calculated Concentration of EZE
Absorption correction method	6+14	6.05	13.98
	8+12	8.13	11.97
	10+10	10.11	10.13
	12+8	8.01	12.06
	14+6	14.01	6.05
Absorbance ratio method	6+14	6.08	14.01
	8+12	8.12	12.08
	10+10	10.27	9.97
	12+8	12.11	8.05
	14+6	14.11	5.92

Individual Concentrations of SIM and EZE from their Standard Mixtures in Methanol

Table 3 : Results of Analysis of Formulation

Method	Drug	Standard drug added (%)	Total amount of drug (mcg/ml)	Amount Recovered (mcg/ml)	% Recovery	SD	%RSD
Absorption correction method	SIM	50	15.12	15.05	99.54	0.7892	0.7917
		75	17.62	17.74	100.68		
		100	20.12	19.87	98.76		
		125	22.62	22.56	99.73		
	EZE	50	15.11	15.24	100.86	0.7672	0.7670
		75	17.61	17.62	100.01		
		100	20.11	19.91	99.01		
		125	22.61	22.66	100.22		
Absorbance ratio method	SIM	50	15.19	15.26	100.46	0.6938	0.6909
		75	17.69	17.90	101.19		
		100	20.19	20.09	99.50		
		125	22.69	22.80	100.48		
	EZE	50	15.08	15.15	100.46	0.7375	0.7391
		75	17.58	17.63	100.28		
		100	20.08	19.85	98.86		
		125	22.58	22.47	99.51		

242 nm and 237.6 nm. Both the drugs obeyed in the concentration range of 4 - 24 μg/ml. The absorptivity values were calculated (Table 1). The absorptivity value at 237.6 nm for SIM and EZE was found to be 534.06 and 359.81, respectively. At 242 nm it was found to be 331.43 for SIM and 332.84 for EZE. To study the mutual interference, the synthetic mixtures containing various proportions of SIM and EZE were prepared and the contents were estimated by the proposed method. The amount recovered indicated no mutual interference up to the ratio of 14:6 for both the drugs (Table 2). The stability of solutions of formulation was determined by measuring the absorbance at 242 nm and 237.6 nm for SIM and EZE at periodic intervals. There was no change in the absorbance at these wavelengths up to 4 hours indicating that the solution was stable for at least 4 hours. Commercial formulation containing SIM and EZE was analysed by proposed method. Six replicate analysis of formulation were carried out and the mean SIM content was 10.19 mg/tablet and the mean content of EZE was 10.08 mg/tablet. The corresponding standard deviation was found to be 0.7848 for SIM and 1.4339 for EZE indicating that the method has required precision (Table 3). The accuracy of the method was determined by recovery studies. SIM and EZE were added to pre analysed tablet powder at four different levels viz., 50%, 75%, 100% and 125%. Six replicate analysis was carried out at each

Table 4 : Recovery Studies

Method	Drug	Label claim (mg/tab)	Amount found* (unit)	% Label claim*	SD	%RSD
Absorption correction method	SIM	10	10.11	101.18	1.1286	1.1154
	EZE	10	10.10	101.00	1.4948	1.4791
Absorbance ratio method	SIM	10	10.19	101.90	0.7848	0.7702
	EZE	10	10.08	100.80	1.4339	1.4225

*Mean ± SD of six observations

Table 5 : Intraday and Inter Day Precision in the Analysis of SIM and EZE

Method	Drug	Concentration (µg/ ml)	SD		% RSD	
			Intraday	Interday	Intraday	Interday
Absorption correction method	SIM	10	0.0416	0.07	0.4152	0.6856
	EZE	10	0.0721	0.0709	0.7048	0.7106
Absorbance ratio method	SIM	10	0.0793	0.0953	0.7789	0.9361
	EZE	10	0.1509	0.1609	1.5024	1.5918

level. The percentage recovery was found to be 100.34 ± 0.6938 and 99.78 ± 0.7375 for SIM and EZE, respectively. The percentage relative standard deviation was less than 2% for both the drugs indicating that the developed method was found to be accurate (Table 4). To study the robustness of the method, analysis of formulation was carried out the repeatable analysis on the same day and on three different days(Table 5). The results proved the reliability of the method. In repeatable analysis of formulation, the low % RSD values indicated the precision of both the methods. The results of recovery study showed that there is no interference due to the excipients used in formulation. Hence, the methods were found to be accurate.

HPLC methods were already reported, which require highly sophisticated instrument, costly solvents and are time consuming process but the sensitivity of the instrument is high. Required sensitivity was achieved even in UV spectrophotometric methods for the estimation of SIM and EZE in combination products. This was confirmed by high absorptivity values of SIM and EZE at their selected wavelengths. A derivative spectrophotometric method has also been reported for the simultaneous estimation of both the drugs in combination. For measuring the absorbance in derivative spectrophotometry, each solution should be scanned to the entire wavelength range every time and the zero order spectra should be derivatised to first order, then only absorbance can be measured. Hence, when compared to derivative method, these two methods are easy, time consuming and are giving the reliable results. This was confirmed by validation of these methods.

Thus, the developed methods are simple, precise and accurate, and can be effectively used for the routine analysis of SIM and EZE simultaneously from combined tablet dosage forms.

ACKNOWLEDGEMENTS

The authors wish to thank Arulthiru Amma and Thirumathi Amma, Adhiparasakthi charitable medical, educational and cultural trust for providing the necessary facilities to carryout this work. Also thankful to Micro Labs, Bangalore for providing gift samples of SIM and EZE.

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Preparation and Invitro Evaluation of Verapamil Hydrochloride Microcapsules

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ABSTRACT

The main objective of the present study is to prepare Verapamil hydrochloride (VH) loaded microcapsules prepared with some naturally active mucoadhesive polymers like natural polysaccharides and evaluate their efficacy in retarding the release of the drug from the microcapsules prepared by Orifice Gelation technique. A series of batches was prepared to optimize the parameters like the polymers: drug ratio, polymer: polymer ratio. The microcapsules were evaluated for physical characteristics like particle size by optical microscopy, surface morphology by Scanning Electron Microscopy (SEM), FTIR spectroscopy, drug entrapment efficiency and in vitro drug release characteristics. The microcapsules showed a mean particle size of 71.57-1000μm.Electron microscopy studies revealed that the microcapsules were spherical with nearly smooth surface. FTIR spectroscopic studies revealed that there was no polymer-drug interaction. Application of in vitro drug release data to various kinetic equations indicated Zero order release from the microcapsules.

KEY WORDS : Verapamil Hydrochloride, Microcapsules, Orifice Gelation technique.

INTRODUCTION

Oral administration of short half life drugs having low bioavailability is a challenge to the formulators[1]. So in this respect many strategies are employed to improve the bioavailability and prolong the half life of the drugs. The use of natural biocompatible polymers in the design of drug delivery devices for improving bioavailability of drugs is gaining importance[2].In the formulation of many oral multiunit dosage forms like microcapsules/microspheres, the residence time of the device is increased by incorporation of mucoadhesive polymers which enhances the bioavailability of the drugs[3]. Some widely used polymers like Sodium alginate, Gelatin, Guar gum, Acacia have been reported to have bioadhesive properties[4]. Verapamil Hydrochloride (VPH) is the first calcium channel blocker to be approved by FDA in 1981 is currently the major Ca-antagonist and is widely used for treatment of hypertension, angina pectoris, supraventricular tachycardia and myocardial infarction. It is completely absorbed from the GIT (90%), but low bioavailability of 22$\pm$8%. The low bioavailability is owing to the rapid biotransformation in liver with a biological half life of 4.0$\pm$1.5 hours. The short half life and poor bioavailability of the drug favors the development of controlled release formulations[5, 6].

The present study mainly deals with the prolongation of the release rate of the VH using a combination of various mucoadhesive polymers by using the Orifice Gelation technique[7].

EXPERIMENTAL

Materials

Sodium alginate was purchased from Loba Chemie(Mumbai),Verapamil hydrochloride was kindly gifted by Glenmark Pharmaceuticals(Mumbai), Gelatin, Guar gum, Tragacanth, Acacia were purchased

from Loba Chemie(Mumbai). Calcium Chloride, was purchased from Central drug house, New Delhi. All other chemicals and solvents were of laboratory reagent grade.

Preparation of microcapsules

Verapamil Hydrochloride(VPH) loaded microcapsules were prepared using Orifice gelation Technique and different combinations of the formulations were prepared using polymers namely Gelatin, Acacia, Tragacanth, Guar gum and Sodium alginate of different concentrations by dissolving in purified water to form a homogenous drug-polymer mixture which was kept untouched for 20-30 minutes to make it bubble-free, which was passed through 24 gauge syringe into 100 ml of 3% Calcium chloride solution drop wise so that the drops congealed into spheres and were retained in the Calcium chloride solution for 30min for the completion of the curing reaction and rigidization. The microcapsules so formed were collected by decantation and washed with purified water repeatedly for three times to remove any traces of Calcium Chloride and then dried at room temperature for 24 hours.

Table 1 : Composition of Microcapsules

Formulation Code	Drug(VPH)	Polymers				
		Sodium alginate	Tragacanth	Acacia	Gelatin	Guar Gum
F1	40mg	350mg	---------	---------	--------	100mg
F2		350mg	--------	---------	150mg	---------
F3		400mg	--------	100mg	--------	----------
F4		400mg	100mg	---------	--------	--------

SIZE AND SHAPE OF MICROCAPSULES[8]

Microcapsules were observed under the optical microscope and approximately around 100 microcapsules were counted for particle size using a calibrated optical microscope (Olympus NWF 10X,Educational Scientific Stores, India) fitted with an ocular micrometer and stage micrometer. The surface morphology of the optimized batches of microcapsules were determined by Scanning Electron Microscopy (SEM) using a SEM sample stub using double sided sticking tape and coated the microcapsules with gold film (thickness 200nm) under reduced pressure (0.001 mm) of Hg (JEOL-JSM 6390LV) as shown in Fig 1.

SWELLING STUDIES[9]

Swelling rate of the microcapsules was measured as a function of percent of water uptake by the beads. The beads were incubated in phosphate buffer of pH 6.8 at 37°C and at different time intervals the beads were removed and excess water was removed by using the filter paper (reported in Table 2 and graphically represented in Fig 2). The swelling rate and the extent of swelling were determined by using the following formula below.

$$\% \text{ Water uptake} = 100 \times \frac{\text{Weight of wet microcapsules–weight of dried microcapsules}}{\text{Weight of dried microcapsules}}$$

Table 2 :

Formulation Code	Weight of dried microcapsules(mg)	Time interval, Weight of swollen microcapsules (mg)		
		45min	90min	135min
F1	100.0mg	190.5mg	200mg	436.3mg
F2		175.5mg	195.5mg	400.5mg
F3		195.5mg	230.0mg	447.5mg
F4		180.5mg	250.0mg	437.5mg

FTIR SPECTROSCOPY

The drug-polymer interaction were studied by FTIR spectroscopy where the spectroscopic studies of Verapamil HCl and the polymers used were done individually first and then compared with the spectra of the formulations (in which the drug was matrixed with different concentration of polymers) as shown in Fig 3.

Determination of drug entrapment efficiency[8]

The microcapsules equivalent to the total amount of drug loaded in the formulations was powdered using a mortar and pestle and taken in a beaker containing phosphate buffer of pH 6.8 and stirred in a magnetic stirrer for 1 hour at 300rpm.The solution was filtered and estimated for Verapamil Hydrochloride content spectrophotometrically at 278nm.The determination for drug entrapment efficiency the following formulas were used:

Actual Drug content (%) = 100 × (Encapsulated protein/ Microcapsule weight)

Theoretical drug content (%) = 100 × (Drug used for encapsulation/Microcapsule weight) Encapsulation efficiency, EE (%) = 100 × (Encapsulated drug/ drug used for encapsulation) = 100 × Actual Drug content / Theoretical drug content

The Encapsulation efficiency for batch F1, F2, F3 and F4 are reported in Table 2.

MICROMERITIC PROPERTIES

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel standing method[10] as given in Table 3.

$\theta = \tan^{-1} H/_{R}$, where θ is the angle of repose, R and H are the radius and height respectively.

BULK DENSITY

Bulk and tapped densities were measured by using 10ml graduated cylinder[10]. The sample poured in the cylinder was tapped mechanically for 100 times, then the tapped volume was noted down and bulk density and tapped density were calculated. Each experiment was done in triplicate manner and reported in Table 3.

Carr's index

Compressibilty index (Ci) or Carr's index value of microcapsules was computed according to the following equation and reported in Table 3.

$$\text{Carr's index (\%)} = \frac{\text{(Tapped Density–Bulk Density)}}{\text{Tapped density}} \times 100$$

Hausner's ratio

Hausner's ratio of microcapsules as reported in Table 3 was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Hausner's ratio} = \frac{\text{Tapped Density}}{\text{Bulk density}}$$

Invitro Dissolution studies

The in vitro dissolution studies were carried out at 37°C±0.5°C at 50rpm using phosphate buffer of pH 6.8(900ml) as the dissolution media in the USP dissolution apparatus II under sink conditions. Accurately weighed quantity of the microcapsules equivalent to the dose of the drug was taken and placed in dissolution apparatus and at pre-set time intervals, 5ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. The samples were analyzed spectrophotometrically at 278nm.The concentration of Verapamil hydrochloride in the test samples was calculated using the regression equation of the calibration curve. The studies were done in triplicate and graphically represented in Fig. 4.

Release kinetics

Data obtained from in vitro release studies were fitted to various kinetic equations[11] to find out the mechanism of drug release from the microcapsules. The kinetic models used were Zero order equation, First order equation.

RESULTS AND DISCUSSIONS

Morphology of the microcapsules

The produced microcapsules from all batches showed very good spherical geometry as shown in Fig 1. The mean diameter was in the range of 71.57-1000μm.

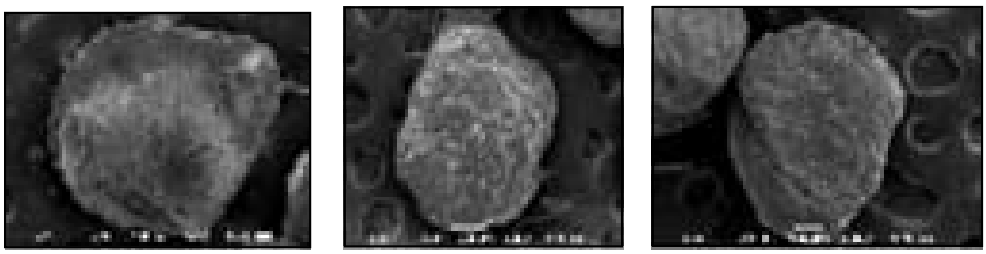


Fig. 1 : *Scanning electron microscopy of F-I, F-II, F-III respectively.*

Swelling Studies

From the swelling studies it was found that the microcapsules using the highest concentration of sodium alginate has the highest ability to uptake water and this may be due to the ability of sodium alginate to take up water with its increased concentration as shown in Fig. 2 and Table 2.

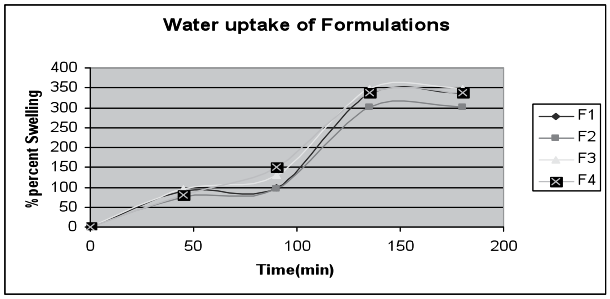


***Fig. 2** : Swelling studies of microcapsule formulations at different time intervals*

MICROMERITIC PROPERTIES

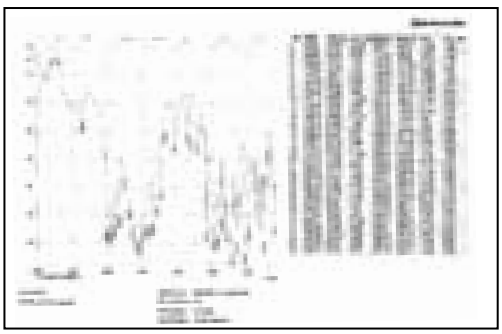
Angle of repose, Hausner's ratio and Carr's index were determined to predict the flowability. A higher Hausner's ratio indicates cohesion between particles while a higher Carr's index is indicative of the tendency to form bridges. The prepared microcapsules showed good flow properties and can be arranged as F3> F2>F4>F1.

Table -3 : Micromeritic Properties

Formulation Code	Angle of repose	Tapped density	Bulk Density	Carr's Index	Hausner's ratio	Encapsulation efficiency
	(θ)	(g/ml)	(g/ml)	Ci (%)		(%)
F1	13.73	0.6024	0.50205	16.65	1.1998	60.7125
F2	12.79	1.11285	0.779	29.99	1.4284	87.6
F3	12.610	0.978	0.746	23.722	1.31099	94.0476
F4	13.071	1.598	1.069	33.103	1.4948	85.22

FTIR SPECTROSCOPY

The spectroscopic studies showed that there was no drug-polymer interaction as the spectroscopy of the drug, polymer and formulation did not show any dissimilar spectra when compared and this is shown in the Fig 3.



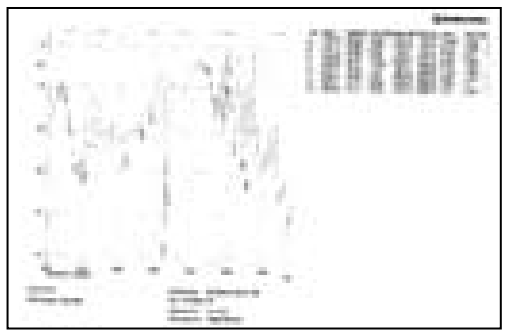
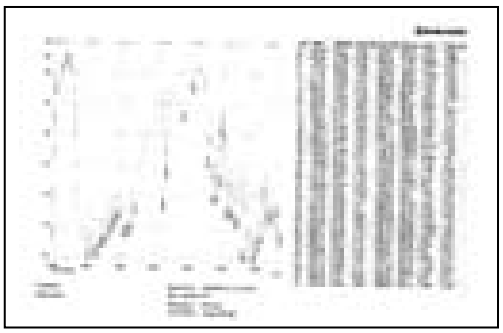
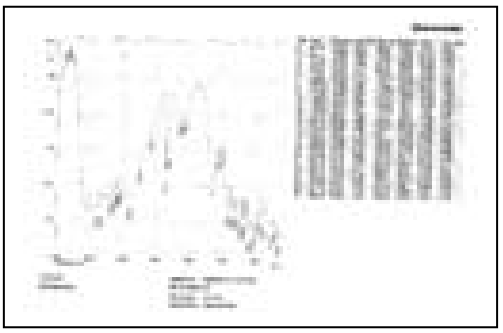
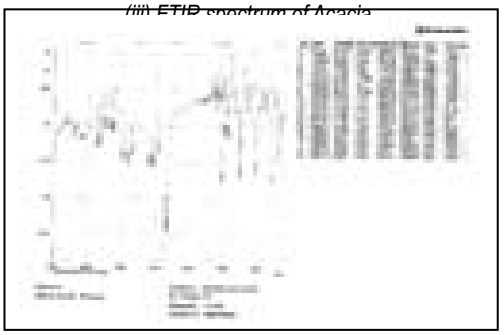
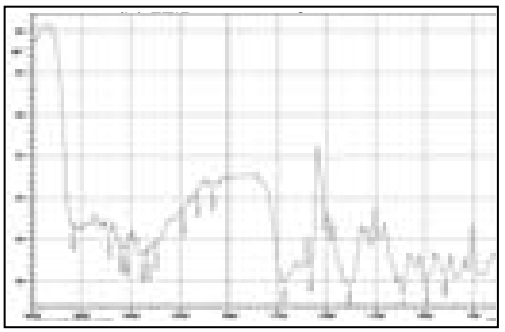


Fig. 3 : *(i) FTIR spectrum of Verapamil HCl* *(ii) FTIR spectrum of Sodium alginate*









(v) FTIR spectrum of Formulation F1

(vi) FTIR spectrum of Tragacanth

INVITRO RELEASE STUDIES

The release profiles of the formulations appear to be slow release with slight burst effect. The burst effect corresponds to the release of the drug located on or near surface of the microcapsules or release of slightly entrapped drug. The rate of release of drug from the microcapsules was slow and found to further decrease with increase in drug to polymer ratio and also polymer to polymer ratio. With the increase in polymer concentration, the rate of drug release from the microcapsules also delayed giving a better controlled release effect. F3 composed of Sodium alginate and Acacia is found to be most satisfactory among all the other formulations, showing a cumulative release of 88.5% at the end of 11.0 hrs. The slow release may be due to the medium being diffused in the polymer matrix and the drug diffusing out of the microcapsules.

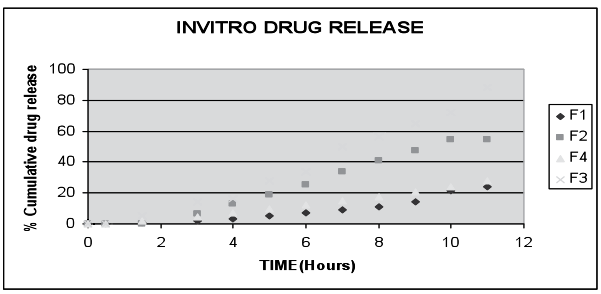


Fig. 4 : *Cumulative percent release of Verapamil HCl from different mucoadhesive microspheres prepared with different drug : polymer ratio*

RELEASE KINETICS

The drug released from the microcapsules was diffusion controlled, as Cumulative percent(%) drug

released Vs Time plots were found to be linear (r^2 nearly 1). It follows the zero order release kinetics.

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Evaluation of Anti-Inflammatory Potential of *Haldina cordifolia* Bark Extracts

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ABSTRACT

Various extracts (pet ether, chloroform, acetone, methanol & aqueous) of ***Haldina cordifolia*** bark at dose of 100 mg/kg orally was tested for anti-inflammatory activity in some acute models viz. carrageenan induced and dextran induced paw oedema. Diclofenac sodium at the dose of 10 mg/kg was used as standard. Methanol extract showed significant activity ($p<0.05$) compared with the control group in both of these models. Thus the present study indicates that the methanol extract of ***Haldina cordifolia*** bark possesses significant anti-inflammatory activity.

INTRODUCTION

Haldina cordifolia (Family – Rubiaceae) is found throughout the India in deciduous and semi-evergreen forests. The roots and bark of ***Haldina cordifolia*** has been used as a traditional medicine in India. The roots are useful in diarrhoea and dysentery. The bark is useful in wounds and ulcers, strangury, skin disease[1]. In the present study an attempt is made to explore anti-inflammatory potential of various bark extracts of ***Haldina cordifolia***.

MATERIALS AND METHODS

Drugs & Chemicals

Carrageenan was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India, dextran was purchased from Sigma, USA & diclofenac sodium was obtained from Ranbaxy Fine chemicals Ltd. The solvents &/or chemicals were of analytical grade & used as received.

Animals

Albino rats of either sex (150-200gm) were procured from institute animal house. They were housed in groups of five animals in polypropylene cages (38x23x10cm) & maintained standard laboratory conditions (temp. 25±2°C) with dark & light cycle (12/12hr) & fed standard diet. The animals were acclimatized to laboratory conditions for seven days before commencements of experiments. Ethical clearance was obtained from institute animal ethical committee for using animals in present study.

Animals were fasted overnight with free access to water prior to each experiment. Five animals were used in treated & controlled groups respectively unless otherwise indicated.

Equipment plethysmograph was used for measuring paw volume of rats in present study.

Collection of plant material

The bark of ***Haldina cordifolia*** (Rubiaceae) collected in Lachiwala, Dehradun district, Uttarakhand state of India and identified by Dr. S.A.S. Biswas, Head, Department of Botany, F.R.I., Dehradun, India. A voucher specimen no.H-16 is deposited at the Department of Pharmaceutical Sciences, S.B.S.P.G.I., Balawala, Dehradun (Uttarakhand), India.

Preparation of the extracts

Fresh bark was shade dried at room temperature, ground into a fine powder, & successively extracted

with the solvents viz petroleum ether, chloroform, acetone, methanol & distilled water using Soxhlet apparatus. During the extraction with acetone some material precipitated out and was found to be insoluble in acetone (Acetone extract ppt.). The solvents were removed under reduced pressure using a thin film evaporator to constant weight. The extracts were stored in clean glass bottles for one week at 4°C & further used for anti-inflammatory activity.

Qualitative analysis

All the bark extracts of the ***Haldina cordifolia*** were screened for their phytoconstituents[2-4].

Toxicity study

Acute toxicity was determined by observing the number of deaths of mice in different groups (n=5) treated with methanol extract in the dose range of 200-2000 mg/kg[5].

Screening for the anti-inflammatory potential of the plant extracts;

Carrageenan induced rat paw oedema model

The rats were divided into ten groups containing five rats in each group (one control, one standard & eight test groups). Acute inflammation was induced according to edema assay[6].

The extracts were suspended in 2.0% tween-80 & administered orally (100mg/kg body wt.) to rats 1hr before carrageenan injection. Diclofenac sodium (10mg/kg body wt.) is given to standard group. Carrageenan was prepared as 1% w/v solution in 0.9% w/v NaCl & inject 0.1 ml underneath the planter region.

Control group : Carrageenan + 2% tween 80 (10 ml/kg body wt.)

Standard group : Carrageenan + Diclofenac sodium (10 mg/kg body wt.)

Test group 1 : Carrageenan + Pet ether extract (100mg/kg body wt.)

Test group 2 : Carrageenan + $CHCl_3$ extract (100mg/kg body wt.)

Test group 3 : Carrageenan + Acetone extract (100mg/kg body wt.)

Test group 4 : Carrageenan + AEP (100mg/kg body wt.)

Test group 5 : Carrageenan + CH_3OH extract (100mg/kg body wt.)

Test group 6 : Carrageenan + H_2O extract (100mg/kg body wt.)

The paw volume was measured at 0 hr & 3 hr after carrageenan injection with the help of plethysmograph. The anti-inflammatory activity was evaluated on the ratio of changes in paw volume in treated & untreated groups as per the formula given below :

% Inhibition = (A-B)/A x 100, Where A denotes mean of increase in paw volume of control & B denotes mean of drug treated animals[7].

Dextran induced rat paw oedema model

The animals were treated by same procedure as done in carrageenan induced model but instead of carrageenan, here 0.1 ml of dextran (1.0% w/v in normal saline) was used as the oedemogen[6]. Since only methanol extract has shown significant activity ($p<0.05$) compared with the control group using carrageenan induced oedema model therefore only this test extract was screened in this model

Control group : Dextran + 2% tween 80 (10 ml/kg body wt.)

Standard group : Dextran + Diclofenac sodium (10 mg/kg body wt.)

Test group 5 : Dextran + CH_3OH extract (100 mg/kg body wt.)

Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. Statistical analysis was carried out using student's t-test and $p<0.05$ was considered significant.

RESULTS

Various leaves extracts were subjected to qualitative analysis for the presence of various phytoconstituents and for anti-inflammatory activity. Among all the extracts, highest as well as significant inhibition ($p<0.05$) was observed in methanol leaves extract 33.33% on Carrageenan induced model and 26.67% on dextran induced model as compared to standard Diclofenac sodium 54.67% and 33.33% respectively. Results are summarized in (Table 1 and Table 2).

Table 1

Phytochemical investigation of bark extracts of *Haldina cordifolia*

Test Performed	Petroleum ether extract extract	Chloroform extract	Acetone extract	Acetone extract ppt.	Methanol extract	DW
1. Test for Alkaloids						
a) Mayer's Test	-	-	-	+	+	+
b) Hager's Test	-	-	-	+	+	+
c) Wagner's Test	-	-	-	+	+	+
d) Dragendroff's Test	-	-	-	+	+	+
2. Test for Protein						
a) Millon's Test	-	-	-	-	-	-
b) Biuret Test	-	-	-	-	-	-
c) Ninhydrin Test	-	-	-	-	-	-
3. Test for Steroids						
a) Salwkowski's Test	+	+	-	-	-	-
b) Libbermann Burchard Test	+	+	-	-	-	-
4. Test for Phenolic compounds Phosphomolybdic acid reagent	-	+	+	+	+	+
5. Test for Saponins	-	+	+	+	+	+
6. Test for Carbohydrates : Molisch's Test	-	-	-	-	+	-
7. Test for Fixed oil : Stain Test	-	-	-	-	-	-

Table 2

Anti-inflammatory activity of various groups on carrageenan induced and dextran induced paw oedema models.

Groups	Carrageenan induced rat paw oedema		Dextran induced rat paw oedema	
	Oedema volume (ml)	% Inhibition	Oedema volume (ml)	% Inhibition
Control group	0.15 ± 0.014	-	0.15 ± 0.024	-
Standard group	0.068 ± 0.022	54.67	0.1 ± 0.02	33.33
Test group I	0.132 ± 0.023	12	-	-
Test group II	0.126 ± 0.018	16	-	-
Test group III	0.12 ± 0.016	20	-	-
Test group IV	0.114 ± 0.03	24	-	-
Test group V	0.1 ± 0.019*	33.33	0.11 ± 0.021*	26.67
Test group VI	0.124 ± 0.026	17.33	-	-

Values are mean ± S.E.M. number of rats=5. *P<0.05 vs. Control group.

DISCUSSION

All extracts were screened for anti-inflammatory activity using carrageenan induced edema models (Table 2). Among all the extracts only methanol extract has shown significant activity ($p<0.05$) compared with the control group therefore to confirm its anti-inflammatory potential this test extract was screened using dextran induced oedema model. Experiment was performed using three doses (50, 100 & 150 mg/kg). No significant activity was observed at the dose level of 50 mg whereas 100 mg & 150 mg showed comparable results therefore 100 mg/kg body wt. is the optimum dose for inhibition of inflammation.

The present study establishes the anti-inflammatory potential of methanol extract of the bark of *H. cordifolia* in both the models used. Carrageenan induced paw oedema is a commonly used model and the inflammation can be divided into two phases: first phase is mediated by the release of histamine and serotonin followed by kinin release and then prostaglandins from the tissue tissue arachidonic acid in the later phage[8]. The extract also showed remarkable activity against dextran induced paw edema model. Dextran induced paw edema is mediated by histamine and serotonin. It also induces fluid accumulation which contains little protein and few neutrophils whereas carrageenan induces protein rich exudation containing large number of neutrophils[9]. Whatever may be the type of exudation, the extract effectively suppressed inflammation in both the models (Table 2). This indicates that probably methanol extract has activity against the inflammatory mediators like histamine and serotonin. However, different and specific models, using these mediators as edemogen should be employed to confirm this notion. Thus from the present study it can be concluded that the methanol extract of ***Haldina cordifolia*** bark possesses significant activity in chemically induced acute inflammation.

ACKNOWLEDGEMENT

The authors are immensely thankful to Management, S.B.S. (PG) Institute of Biomedical Sciences & Research, Balawala, Dehradun, U.K., India, for providing the requisite facilities & financial support and Dr. S. A. S. Biswas, Head, Dept. of Botany, FRI, Dehradun, U.K., India, for authentication of plant.

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Influence of Surface Modification on the Wettability of Bagasse fibre

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ABSTRACT

The aim of this article is to study the influence of chemical modification on morphology, wettability, fine structure of fibres and its impact on the interfacial adhesion of natural fiber-reinforced polymer composites. For that purpose, Bagasse fibres (BF) were oxidized with chlorine dioxide followed by dewaxing of fibres and then, grafting of furfuryl alcohol on fibre surface processed for composite fabrication. Stereo optical and scanning electron microscopy (SEM) micrograph had shown the fibre surface morphology and degradation of fiber due to chemical reaction. Surface properties of fibers before and after modification were determined by means of contact angle determination and fine structural details by Chemiluminescence and Fourier transform infrared-spectroscopy (FT-IR). Grafting of FA resulted in the increase of polarity in fibres, that is contact angle were found decreasing with water and other test liquid. This could be due to the increase in phenolic and secondary alcoholic groups or oxidation of basic structural component, lignin and hemicelluloses after chemical modification as studied by FTIR. Surface morphology and development of polarity onto bagasse fibre after chemical modification resulted in the better fibre/matrix interaction as revealed from SEM micrographs and adhesion force measurement by AFM.

KEY WORDS : Bagasse fibre; Surface modification; Furfuryl alcohol; Surface energy; Adhesion force.

INTRODUCTION

Sugarcane bagasse is a plentiful lignocellulosic waste typically found in tropical countries that process sugarcane, such as Brazil, India, Cuba, and China. It is called bagasse or cane-chuff, and it is obtained as the leftover matter after liquor extraction in a sugar factory. About 54 million tons of bagasse is produced annually throughout the world. In general, sugar factories generate approximately 270 kg of bagasse (50% moisture) per metric ton of sugarcane[1]. The Bagasse Fibre (BF) is unusually coarse and stiff material. It is used either as a fuel for the boilers by the sugar factory or as a raw material for the manufacture of pulp and paper products, various types of building boards, and certain chemicals. It is suitable for making nonwoven products. Disposal of bagasse is critical for both agricultural profitability and environmental protection. Application of agro-industrial residues in industries on the one hand provides alternative substrates, and on the other hand helps in solving pollution problems, which their disposal may otherwise cause. These agricultural residues represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass[2]. This has led to an increasing trend in the society the use of renewable raw materials for production of various products. Bagasse consists of approximately 50% cellulose and 25% each of hemicellulose and lignin[3]. Chemically, bagasse contains about 50% alpha cellulose, 30% pentosan and 2.4% ash[4]. Because of its low ash content bagasse offers numerous advantage in comparison to other crop residues. Sugarcane plants are one of the most efficient convertors of sunlight into chemical energy. Sugarcane juice can be used to prepare molasses which is

fermented to give alcohol (ethanol). Some countries now use alcohol as an additive in petrol since it is a cleaner fuel, which gives rise to only carbon dioxide and water on burning in sufficient air.

There are many literature used bagasse for the production of cellulose, lignin and hemicelluloses. Numerous sugar and alcohol mills in Brazil use bagasse residues as raw materials. Another example is the use of sugarcane in Brazil for production of polyhydroxyalkanoates, which are being explored for medical applications[5]. The recyclable bagasse is also used to produce regenerated cellulose triacetates. This material is used as membranes in osmosis and reverse osmosis as support for the immobilization of enzymes[6]. Due to the high content of xylose it is also used for production of furfural, which in turn, is an important chemical reagent in pharmaceutical sciences[7]. The lignin extracted from bagasse was used as polymeric matrices in composite applications[8]. Hemicelluloses are non-cellulosic hetropolysaccharides consisting of various sugar units i.e. composed of pentosans and hexosans, respectively. The principle sugars in hemicelluloses are D-Xylose, L–arabinose, D–glucose, D-galactose, D–mannose, D–glucuronic acid, 4-O-methyl-D–glucuronic acid, D–galacturonic acid, and to a lesser extent, L–rhamnose, L–fucose, and various O–methylated neutral sugars. The succinoylation of hemicelluloses in bagasse is to produce polysaccharides succinates offer a number of very desirable properties such as high viscosity, low-temperature viscosity stability, high thickening power, low gelatinization temperature, clarity of cooks, and good filming properties. Therefore, succinate derivatives have been recommended as binders and thickening agents in foods, tablet disintegrates in pharmaceutical, surface sizing agents and coating binds in paper[9]. It was reported that bagasse lignin is more reactive towards formaldehyde than other kraft lignin or lignosulfonates[10]. This higher reactivity is attributed to the less substituent at C_3 and C_5 position compared to that of other lignin[11]. The resin prepared by reacting methylolated lignin with resorcinol followed by mixing with phenolic resin, produced cold-setting wood adhesives suitable for structural finger-joints and glulam[12].

Previous research on bagasse has suggested many approaches to convert bagasse into value-added industrial products, such as liquid fuels, feed stocks, enzymes and activated carbon. Use of bagasse fibre for manufacturing material products is another prospective solution. Compared to pure synthetic materials, bagasse fibre-based materials have two advantageous features, light weight and renewability. Several studies have been carried out to understand the structure, properties, and the effect of chemical modification on bagasse fibre. Research and development efforts have been underway to find new applications for bagasse, including utilization of bagasse as reinforcement in polymer composites[13]. An important point to consider is that the reagents used in chemical modification cannot be too expensive, and ideally, the modifications must involve a minimum number of compounds obtained from nonrenewable sources.

The present work describes the development of new valorized application areas for BF and the design of natural composites with superior properties. The fibre surface modification is based on the selective oxidation of guaiacyl and syringyl units of lignin, generating ortho- and para- quinones able to react by Diels-Alder reaction with furfuryl alcohol that is commercially prepared by reduction of furfural, which in turn is obtained from agricultural residues[14, 15]. The goal of the study was to submit BF to softer chemical conditions than those used in the banana, curaua and sisal fibres, aiming to improve fibre/matrix interactions in the composites without excessive degradation of the fibres and, consequently, an increase in interface properties by increasing the polarity of the fibre surfaces.

EXPERIMENTAL

Materials

Sugarcane bagasse was locally collected from agricultural fields of Jharkhand (India) after the sugarcanes had been pressed to separate molasses from filter mud (2008 crops). The bagasse was used for the preparation of sugar and alcoholic products. The residual fibre called "bagasse" or "cane-chuff" were first dried in the sunlight and then further dried in a cabinet oven with air circulation for 16 h at 60°C. Sodium Chlorite, Furfuryl Alcohol, Cyclohexane, Ethanol and other chemicals were obtained as analytical grade of CDH make, India. The test liquids with known surface energy used for contact angle measurement were ultra pure millipore water (Milli-Q Water system, model-Gradient A10, resistivity-18Ω/cm, Millipore), Glycerol (Aldrich, 99%), ethylene glycol (Aldrich, 99%) and α-bromonaphthalene (Aldrich, 98%).

SURFACE MODIFICATION OF BF

Dewaxing of BF

The BF were dewaxed by a soxhlet apparatus with cyclohexane/ethanol, 1:1 v/v, over a period of 48 h, to extract low molecular weight substances [e.g., waxes, therpenes (natural impurities)], and then with water for 24 h, to extract inorganic contaminants. The removal of wax was confirmed by gravimetrically after drying in a circulating air oven (60°C) until constant weight.

Oxidation of BF

The dewaxed fibre was oxidized with a chlorine dioxide-water solution following the equation (1).

$$5ClO_2^- + 4H^+ \rightarrow 4ClO_2 + 2H_2O + Cl^- \qquad (1)$$

In the experimental procedure, the molar solution of sodium chlorite and acetic acid were prepared in distilled water and mixed them in close-system to generate chlorine dioxide (ClO_2). Here acetic acid adding as a catalyst, oxidizing sodium chlorite to produce chlorine dioxide gas act as a good oxidants for guaiacyl and syringyl units of lignin polymer.

Grafting of furfuryl alcohol (FA) on oxidized bagasse fibre (BF_{ox})

The BFox impregnated with furfuryl alcohol were heated at 100°C for 4 h in a specially designed reaction vessel with an outlet and inlet for passage of N_2 gas. The fibres were dried for about 24 h at 50°C, and weight gains due to reaction were determined on the basis of the original and final oven-dried fibre weights. The fibre was named as FA grafted bagasse fibre ($BF_{Ox\text{-}FA}$).

Fluorescence measurements

Fluorescence spectra were obtained by spectrofluorophotometer, with set wavelength at 220-600 nm, and the excitation wavelength was 335 nm. Firstly, the oven-dried unmodified and oxidized BF were ground into fine powder of particle size 2μm or less and were dissolved in 1,4-dioxan-water (9:1, v/v) mixture having concentration $1x10^{-5}$ moles/litre. The solvent mixture dissolves the lignin portions of fibre, but limits the wavelength to above 240 nm, due to absorption of energy by dioxan's functional groups. The fluorescence spectrum was measured before and after oxidation of BF.

METHODS AND CHARACTERIZATION

Fluorescence spectroscopy

The fluorescence measurements were obtained with a chemiluminescence spectrofluorophotometer, Schimadzu Corporation, Japan (model – RF 5301 PC). The instrument is used to measure fluorescence wavelength maximum of unmodified and oxidized BF under excitation at fixed wavelength. The wavelength scan range was 220-900 nm with zero order light having accuracy ±1.5 nm. The experiment was done in fast mode of scan speed and high sensitivity using a xenon lamp as a light source.

Fourier Transform Infra Red Spectroscopy (FTIR)

The Fourier Transform Infrared Spectrometric study was carried out from Schimadzu Corporation, Japan (model-FTIR Prestige-21). The spectra were taken at a resolution of 4 cm^{-1} and 20 scans were carried for each specimen. The scanning range was 4000 to 400 cm^{-1} in transmittance mode. FTIR spectra in the form of KBr pellets of unmodified, oxidized and FA grafted BF were recorded.

Stereo optical microscopy

The image analysis of unmodified, oxidized and FA grafted BF were measured by Leica Optical Stereo Microscope (Model-Leica FW4000) by taking photographs with the help of digital camera (Leica stereo zone X3.2).

Dynamic Contact Angle Measurements

Dynamic Contact Angle Tensiometer (DCAT-21), Data Physics, Germany was employed for analyzing dynamic contact angle (DCA) by using Wilhelmy plate technique. The bunch of fibres hanged vertically to the surface of test liquids. The fibre bundles are dipped into the test liquids up to a depth of 5 mm with a speed of 50 μms^{-1}.

Atomic Force Microscopy (AFM)

AFM measurements were done on Solver Pro-4, NT-MDT, Russia in contact mode. All measurements were made in ambient conditions in a room with climate control. The force constant of the cantilevers was determined as a function of the Z-scanner piezotube extension i.e. DFL (height) signal with respect to stiffness constant of the cantilever. After contacting the surface, a scan of the topography was made and subsequently force curves were measured on the same area, collecting two force curves on 10 areas spaced at 770nm intervals for a total of 20 force curves per samples. The data were manually collected at different location of deflection curve and a relationship was set up with dynamic contact angles and surface energy of fibre samples.

Scanning Electron Microscopy (SEM)

The SEM studies were conducted using JSM 6390 LV, Jeol, Japan with the following specifications: accelerating voltage, 20 KV; image mode, secondary electron image; working distance, 20mm. The test samples were coated with gold metal to avoid electrical charging during examination. The test samples were scanned into 1000 times of magnification.

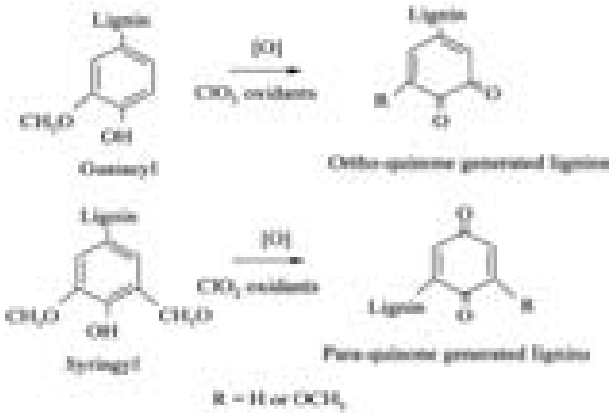
RESULTS AND DISCUSSION

Surface modification

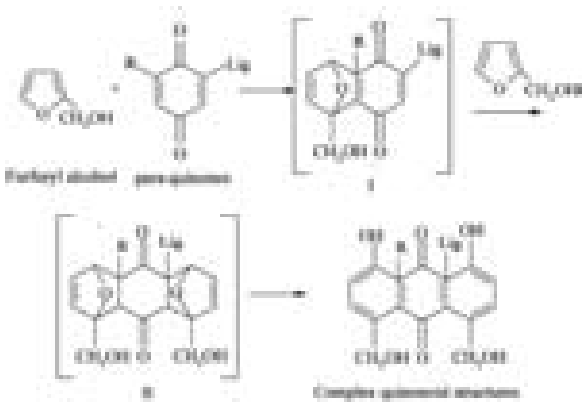
The fibre dewaxing allows removal of waxes from fibre surfaces, preventing weakening of the interactions at the interface of fibre-matrix. As a consequence of cyclohexane/ethanol and then water

extraction, a loss of weight was observed near 2%. The dewaxed fibre was treated with chlorine dioxide-water solution generating ortho- and para- quinones on its surface. By visual inspection, it was observed that the fibres had turned yellow-red after reaction. It was then washed with water until neutrality. The chemical reactions involved during oxidation were shown in Scheme 1 and the formation of adducts between lignin generated para– quinones and FA were shown in Scheme 2. The reaction with ClO_2 and FA was studied and percent graft yield for BF was found to be 17%[16]. The percent graft yield was calculated using the following equation :

$$\% \text{ Graft Yield} = \frac{\text{Dry weight grafted BF - Dry weight unmodified BF}}{\text{Dry weight unmodified BF}} \qquad (2)$$



Scheme 1 *The oxidation reactions of guaiacyl and syringyl units of lignin macromolecule in unmodified BF*



Scheme 2 *The adducts of lignin generated para-quinones and furfuryl alcohol*

Fluorescence study

The emission spectra of aromatic ring substitution in the lignin moieties of fibre sample were shown in Figure 1. It was observed from analysis that the peaks at 410 and 455 nm were in accordance with fluorescence properties of unmodified and oxidized BF, respectively. The peak at 410 nm is attributable to the natural fluorescence of lignin moieties of fibre sample in dioxan solution. The oxidized BF

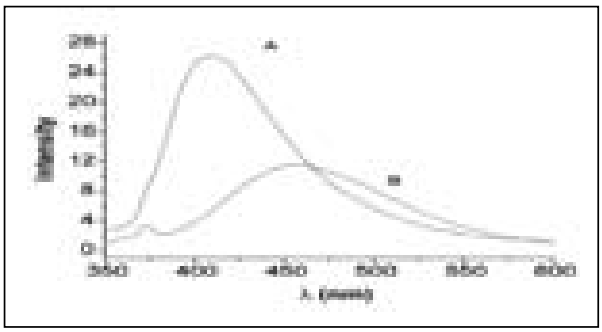


Figure 1 *: The overlay of fluorescence spectra of (A) unmodified and (B) oxidized BF*

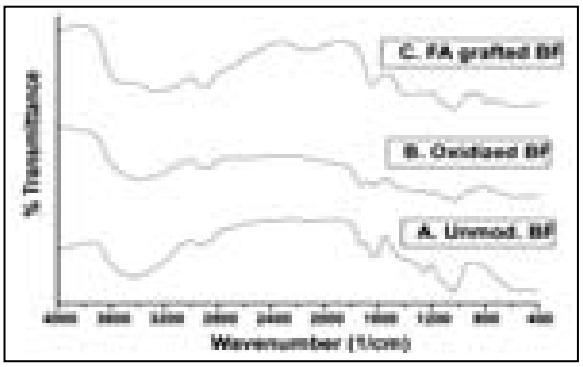


Figure 2 *: The join visible spectra of (A) unmodified, (B) oxidized, and (C) FA grafted BF*

showed an intense peak at 455 nm, supporting the formation of complex quinonoid structures, as expected from Diels-Alder reactions between para-quinones and conjugated dienes[17].

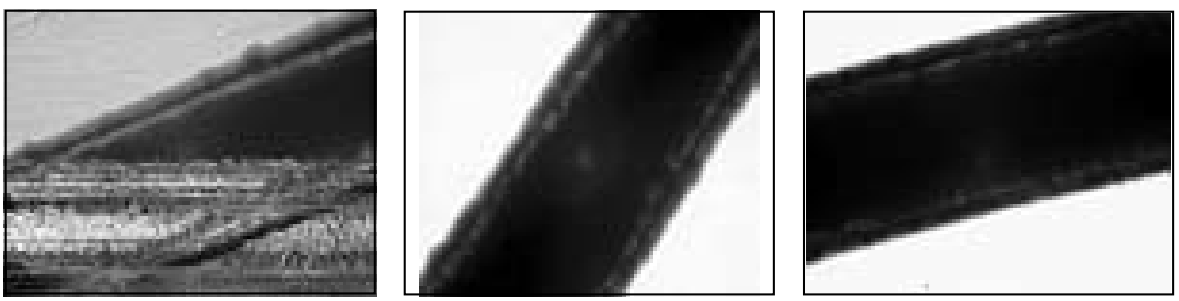
Spectral analysis

The FTIR spectra of unmodified, oxidized and FA grafted BF were shown in Figure 2. It was observed that the absorption bands and peak intensities after chemical modification has changed significantly. All the spectra present typical vibration bands of the components mainly corresponding to cellulose, hemicellulose and lignin.

When comparing Fig. 2B with Fig. 2A, we find that a decrease in intensity is observed for aromatic bands at 1629, 1480 and 1035 cm^{-1} and for the para-hydroxy phenyl propane band at 890 cm^{-1}. Degradation of the aromatic polymer after ClO_2 oxidation seems to be important. The increase in intensity observed for the band at 1720-1740 cm^{-1} corresponding to carbonyl stretching, is indicative of the formation of muconic acid, quinones and ester derivatives[18]. After modification with furfuryl alcohol, the infrared absorption spectra revealed a significant change, thereby confirming the occurrence of grafting. From Fig. 2C it is observed that FA grafted BF show the presence of signals at 735, 1016, 1150, 1510, 2125 and 3150 cm^{-1} that are attributed to furan rings[19]. Due to opening of some furan rings, carbonylic structures are formed and characterized by the absorption band at 1711 cm^{-1}. A similar type of observation has been reported by Rout et al[20].

Optical image analysis

The optical view of surface morphology for unmodified, oxidized and FA grafted BF were shown in Fig. 3(A through C). The physical appearance of the fibres before and after surface modification gives an idea about wettability of fibre with resin. After oxidation, the fibre surface becomes smooth and



A. Unmodified BF *B. Oxidized BF* *C. FA grafted BF*

Figure 3 *: The optical micrographs of (A) unmodified, (B) oxidized, (C) FA grafted BF*

uniform reduces its diameter. In Fig. 3C, clearly there were seen the grafting of FA around the fibre surface extending the capabilities of fibre with resin. It may be due to loss of natural protecting wax layer, lignin and hemicellulose.

Fibre surface energy

BF present comparable contact angles with each test liquid. During the test, it is possible to obtain the advancing and receding angles. The advancing angle has been employed due to its reproducibility. The measurement of dynamic contact angles of unmodified and FA grafted BF using by different test liquids were given in Table 1.

Table 1 : Advancing contact angles of unmodified and FA grafted BF

Fibre	Water	Glycerol	EG	Bromonaphthalene
(A) Unmodified BF	76.2	55.4	52.8	38.3
(B) FA grafted BF	72.4	50.3	49.1	35.7

The BF presents a similar chemical distribution of their polar and disperse components in their surfaces. The contact angles observed with millipore water and other test liquids show that these fibre samples get both polar and disperse components. Total surface energy (γ^T) can be decomposed into polar (γ^P) and disperse (γ^d) components as shown in Eq. (3)[21]. The polar component arises due to dipolar interactions, while the disperse component arises from Van der Waals forces between the molecules of the material[22]. With progress of time, various theories developed for calculating the surface energy of solid samples. The Young-Dupres Eq. (4), described for perfectly smooth, chemically homogeneous surfaces, relates the contact angle (θ) of a probe liquid on a fibre (solid) substrate to the energies of the liquid surface (γ_L), fibre surface (γ_S), and the interface between the fibre and liquid (γ_{SL}).

$$\gamma_L = \gamma^P + \gamma^d \quad (3)$$

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta \quad (4)$$

The surface energy (SE) of the fibre can be determined by measuring the contact angle of a series of well-characterized polar and non-polar liquids on the material surface. Together with Eq. (4) the surface energy of the fibre (solid) can be estimated using the semi-empirical equation developed by Owens and Wendt, applicable for a wide range of surface energies and materials[23].

$$\gamma_{SL} = \gamma_S + \gamma_L + 2[(\gamma_L^d \gamma_S^d)^{1/2} + (\gamma_L^P \gamma_S^P)^{1/2}] \quad (5)$$

In present paper, the polar and disperse components of the fibre SE were estimated from the contact angle measurements using the method of Owens-Wendt-Rabel-Kaelbe (OWRK) which allows for a linearization of the contact angles of the various liquids using Eq. (6), so that the polar and disperse components of the solid (fibre) surface energy can be obtained from the slope and intercept of the plot of transformed variables as indicated by Eq. (6)[24].

$$\frac{-(1+\cos\theta)\gamma_L}{2\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^P}\sqrt{\frac{\gamma_L^P}{\gamma_L^d}} + \sqrt{\gamma_S^d} \quad (6)$$

$$Y = \frac{-(1+\cos\theta)\gamma_L}{2\sqrt{\gamma_L^d}}, X = \sqrt{\frac{\gamma_L^P}{\gamma_L^d}}$$

The SE of test liquids must be different to evaluate the polar and disperse components of the total SE of fibre samples by the OWRK method. The total surface energies, and its polar, and disperse, components obtained from dynamic contact angle measurements to unmodified and FA grafted BF are gathered in Table 2.

Table 2 : Surface energies calculated by OWRK method for unmodified and FA grafted BF as derived from contact angle measurements using different test liquids with known SE

Fibre	γ^d (mJ/m^2)	γ^P (mJ/m^2)	γ_T (mJ/m^2)	χ_p
(A) Unmodified BF	24.80	10.61	35.41	0.2996
(B) FA grafted BF	26.62	15.80	42.42	0.3724

The analysis of data shows that the total SE is increased after chemical modification. This increment is mainly due to the polar component, which can be easily explained by the increase in polar units of furfuryl alcohol grafted on BF[25]. From analysis it was interesting to calculate the polarity, χ_p, defined as the ratio[26]. The calculated values given in Table 2 compared to the one of unmodified fibre show a decrease of the polarity with FA grafted BF. This confirms the introduction of aromatic hydrophobic ring onto FA grafted BF. The polarity is significantly modified, taking into account the increase of polar groups on BF surface.

The AFM study of adhesion force

The AFM recorded the amount of force felt by the cantilever as the probe tip is brought close to- and even indented into- the sample surface and then pulled away. This technique can be used to measure the adhesion force between the probe tip and the sample surface elucidating local chemical and mechanical properties like adhesion and elasticity. The adhesion force typically show the deflection of the free end of the AFM cantilever as the fixed end of cantilever is brought vertically towards and then away from the sample surface. Experimentally, this is done by applying a triangle – wave voltage pattern to the electrodes for the Z-axis scanner. The deflection of the free end of the cantilever is measured and plotted at many points as the Z-axis scanner extends the cantilever towards the surface and then retracts it again. By controlling the amplitude and frequency of the triangle – wave voltage pattern with varying the distance and speed, the AFM cantilever tip travels during the force measurements[27, 28].

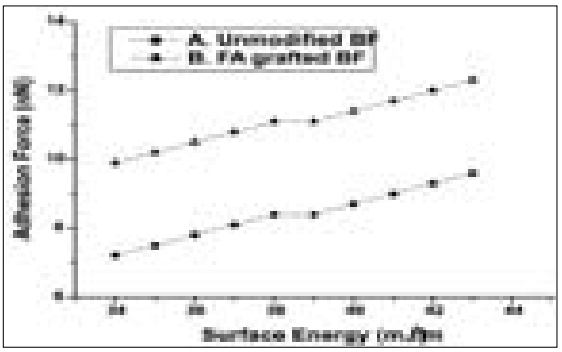


Figure 4 *: The relationship between AFM adhesion force and dynamic contact angle of BF*

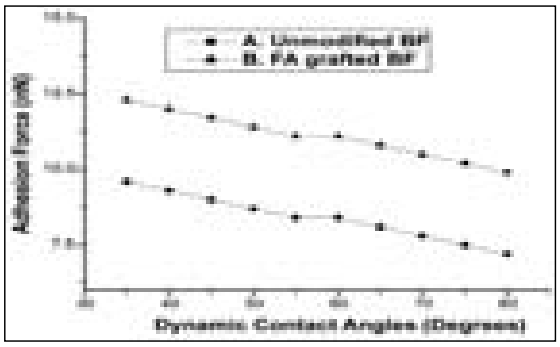


Figure 5 *: The relationship between AFM adhesion force and surface energy of BF*

A B C

Figure 6 *: The SEM micrographs of (A) unmodified, (B) Oxidized, and (C) FA grafted BF*

Relationship between surface energy and AFM adhesion force

Dynamic contact angles and surface energies for unmodified and FA grafted BF are listed in Table 1 and 2, respectively. The relationship between the AFM adhesion force (Fadh) and the fibre samples is shown plotted against the DCA in Fig. 4 and against the fibre SE in Fig. 5. These results are averages of adhesion force measurements made on fibre samples using AFM cantilever tips. From Figs. 4 and 5, it is clear that a relationship does exist between the adhesion force and the fibre SE over this wide range of samples under investigation. For more hydrophobic samples, with DCAs above 60° (surface energies less than 40 mJ/m2), a decrease in adhesion force occurs. The adhesion on more hydrophilic samples (contact angles less than 60° or surface energies greater than 40 mJ/m2) dramatically increases adhesion force.

SEM Analysis

SEM provides an excellent technique for examination of fibre surface morphology. Examinations were carried out on the unmodified and chemically modified BF to study the morphological changes that occurred after modification of the fibre samples. The SEM micrographs of longitudinal surface of unmodified, oxidized and FA grafted BF were shown in Fig. 6A-C (micrographs A through C), respectively. It can be seen in Fig. 6A that unit cells run longitudinally with more or less parallel orientations. Surfaces contain a large number of pores covered by pithy and waxy materials. Waxes and oils provide a protective layer to the surface of the fibres. The intercellular gaps, in the form of shallow longitudinal cavities, can be clearly distinguished, as the unit cells are partially exposed. The intercellular space is filled up by the binder lignin and by fatty substances that hold the unit cells firmly in the fibre[29]. Fig. 6B shows a larger number of surfaces cracks and separation of fibre bundle, compared to Fig. 6A. These features might result from the partial removal of wax and fatty substances during treatment with the dewaxing solvent mixture. The individual ultimate fibres also show a slight separation, which was not obvious in the unmodified BF. Very interestingly, the parallel unit cells look partially split due to the removal of fatty materials. A micrograph of FA-grafted BF, shown in Fig. 6C, revealed that the intercellular gaps were reduced, and FA bounded onto the fibre surface. FA grafts took place more on the surface of unit cells of fibre[20].

CONCLUSIONS

A recently developed process was set to utilize the chemically modify BF in an aqueous medium, using raw materials from renewable resources such as nonwood fibre and furfuryl alcohol. This was

in agreement with the current tendency to prioritize developing processes under environmentally friendly conditions. My objective is to make a utilization of some agro-based waste material as good reinforcing agents for polymer composites. The first stage of the chemical modification, consisting in oxidation of lignin macromolecule with chlorine dioxide creating quinones or muconic derivatives followed by dewaxing of fibres, reveals that the aromatic and aliphatic hydroxyl units are degraded. In second stage, the para-quinones were reacted with FA introducing a number of adhesion points on BF surface. This polymeric thin coating of FA can decrease the intrinsic hygroscopicity of BF and increase the interactions fibre/matrix (phenolics, epoxy etc.) at the interface for BF reinforced composites. The chemistry involved on a molecular basis, chemiluminescence spectroscopy has pointed out the formation of para-quinones, whereas FTIR spectroscopy have shown the appearance of FA on BF surfaces. This can be also observed by visual inspections of optical images. Measurements of surface energies can lead to a prediction of the compatibility of fibre/matrix. Wettability of BF with some polar matrices e.g. epoxy phenolics etc can be improved by chemical modification due to increment of the polar components of their surface energies. AFM adhesion force measurements on BF are found to be highest on more polar samples such as FA grafted BF and lowest on less polar samples such as unmodified BF. Based on the above studies, it is expected that these grafted fibre may be used as good reinforcing materials for developing fibre reinforced polymer composites. Further research work needs to be carried out in the development of natural fibre composites. This is important if new improved materials are to be developed for safe usage against crack growth and environmental pollution.

ACKNOWLEDGEMENT

I thank Birla Institute of Technology, Mesra, Ranchi (India) for financial support.

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❑ ❑ ❑

Rapid and Sensitive RP-Ultra Performance Liquid Chromatography (UPLC) Method for Estimation and Validation of Paclitaxel in Bulk and its Injection Dosage Form

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KEY WORDS : Paclitaxel, UPLC, Method development and validation.

ABSTRACT

A sensitive, specific and very fast reverse phase ultra performance liquid chromatographic (UPLC) method has been developed and validated for the quantitative determination of paclitaxel in bulk and its injection dosage form. The UPLC system consists of a waters BEH Phenyl, 50 × 2.1mm, 1.7 μm particle size column was used with mobile phase comprising of water and acetonitrile (65: 35, v/v). The analytical column was thermostated at 40° and flow rate set at 0.6 ml per min. Detection is performed by using photo diode array (PDA) detector at 227 nm. The paclitaxel was eluted at about 7.0 min. and no interfering peaks were observed. The method was validated in terms of linearity, accuracy, precision and specificity. Linear relationships ($r2 > 0.995$) were obtained between the peak height ratios and the corresponding sample concentrations over the range of 100 to 500 μg/ml, respectively. The limit of detection and limit of quantification were obtained 0.1522 and 0.4613 μg/ml. Percentage recoveries were obtained in the range of 98.35 % and 99.25 %. The proposed method is precise, accurate, selective and reproducible. The UPLC assay procedure, which proved superior because of its greater sensitivity and relatively shorter (10 min) run time than ordinary HPLC procedure. It is an important tool for future speedy analysis of paclitaxel in bulk and its injection dosage form.

INTRODUCTION

Paclitaxel is chemically known as 5β, 20-epoxy-1,2α, 4,7 β,10 β, −13 α-hexahydrotax-11-en-9-one4,10-diacetate2-benzoate13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine[1]. It has an empirical formula C47H51NO14, molecular weight 853.9 and chemical structure given in Figure1[2]. Paclitaxel is the most effective antitumor agent developed in the past three decades. It has been used for effective treatment of a variety of cancers including refractory ovarian cancer, breast cancer, non-small cell lung cancer, AIDS-related Kaposi's sarcoma, head and neck carcinoma and other cancers[3-8]. Paclitaxel, a parenteral antineoplastic agent is a diterpenoid taxane derivative initially obtained from the needles and branches of the Himalayan yew tree Taxus baccata. The drug is presently obtained via a semi-synthetic process from Taxus baccata. It represents first agent from novel class of taxoids[9].

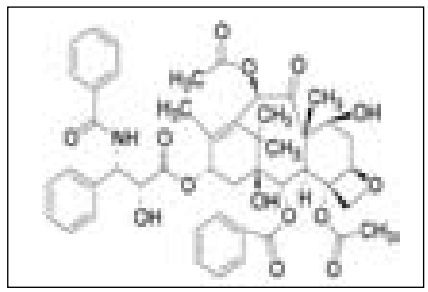

***Fig.1** : Chemical structure of paclitaxel*

Paclitaxel and its injection is official only in Indian Pharmacopoeia[2]. Literature survey reveals that several analytical methods have been reported for the determination of paclitaxel by high performance liquid chromatography[10, 11], liquid chromatography-mass spectrometry[12] and UPLC-MS-MS[13]. No UPLC

method for the quantification of paclitaxel in bulk and its injection dosage form has been reported so far. In the present investigation efforts has been made to develop a very fast, accurate and precise method for the analysis of paclitaxel in bulk and its injection dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade paclitaxel working standard (Lot No. 8LQ008, Purity 98.2 %) and Paclitaxel for injection concentrate 30 mg / 5 ml with brand name Intaxel (Batch No. 7A0004) were provided by Dabur Pharma Ltd., Sahibabad, (UP). Acetonitrile of HPLC grade was purchased from E. Merck, Mumbai. Other chemicals and reagents used were of analytical grade. The water used was of Milli-Q grade purified by a Milli-Q UV purification system (Millipore, Bedford, MA, USA).

Instrument

The instrument used for the study was a Waters, Acquity UPLC (Waters, Milford, MA, USA) equipped with a binary solvent manager, sample manager, inline degasser, and 2996 photo diode array (PDA) detector. Empower-2 Software was used for instrument control along with data acquisition and data processing.

Chromatographic conditions

Column	:	Waters BEH-Phenyl (Bridge Ethylene Hybrid-Phenyl), (50 × 2.1)mm x 1.7 μm particle size
Column oven temperature	:	40°
Mobile Phase	:	Water and Acetonitrile (65: 35,v/v)
Flow rate	:	0.6 ml/min.
Detection wavelength	:	227 nm
Injection volume	:	1 μl
Pump mode	:	Isocratic
Run Time	:	10 minutes
Retention Time	:	7.003

CAUTION

Paclitaxel is potentially cytotoxic. Great care has been taken in handling the powder and preparation of solutions. The drug is light and heat sensitive,hence the assay was carried out in subdued light and the samples and standard kept at temperature between 2-8°.

Preparation of standard solution

About 10 mg of paclitaxel working standard was weighed accurately into a 20 ml volumetric flask, dissolved and diluted to volume with acetonitrile to obtain a solution of 500 μg/ml. Further 6.0 ml of this solution was diluted to 10 ml with acetonitrile to obtain a concentration of 300 μg/ml.

Preparation of sample solution

About 10 mg of bulk was weighed accurately into a 20 ml volumetric flask, dissolved and diluted to volume with acetonitrile (500 μg/ml). Further 6.0 ml of this solution was diluted to 10 ml with acetonitrile (300 μg/ml).

For injection, an accurate volume 1.0 ml of paclitaxel injection (30 mg /5 ml) was taken and transferred to a 20 ml volumetric flask, dissolved and diluted to volume with the acetonitrile to obtain a concentration of 300 μg/ml.

Procedure for assay

1 μl of standard solution of paclitaxel was injected on UPLC system. The system suitability was checked by injecting replicate injections and results obtained are within the range. The relative standard deviation on five replicate injections was obtained 0.4 %, tailing factor 1.03, and the column efficiency 15147 theoretical plates.

1 μl of standard and sample solutions was separately injected. The assay of the bulk and injection was calculated and found 99.89 % and 102.0 % of the labeled claim, respectively.

RESULTS AND DISCUSSION

The developed method was validated for the assay of paclitaxel as per ICH guidelines14.

Specificity and Selectivity

Specificity and selectivity were studied for the examination of the presence of interfering endogenous components. Paclitaxel standard solution of 300 μg/ml was injected and none of the impurities were interfering in its assay. The retention time obtained by UPLC for paclitaxel is about 7.0 min. (Figure 2).

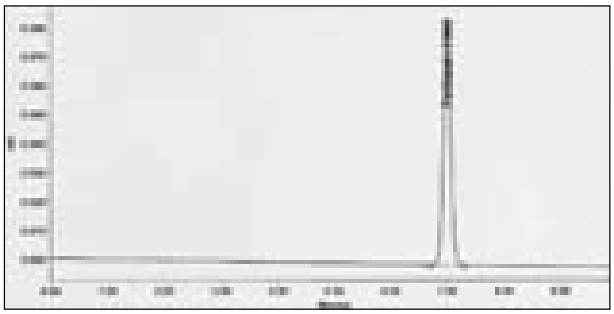

***Fig. 2** : A typical UPLC chromatogram of paclitaxel standard*

Linearity

Linearity was studied by preparing standard solutions of paclitaxel at different concentration levels. The linearity ranges were found in the range of 100-500 μg/ml. The standard calibration curve was generated using regression analysis with Microsoft excel. The assay was judged to be linear as the correlation coefficient was greater than 0.995 by the least-square method.

Accuracy

Recovery studies of the drug were carried out for the accuracy parameter. These studies were carried out at three different concentration levels i.e. multiple level recovery studies. A known amount of paclitaxel standard was added into pre-analysed injection and subject them to the proposed UPLC method. Percentage recovery was found to be within the limits as listed in Table 1.

Table 1 : Recovery studies of paclitaxel in injection

Label Claim (mg/ml)	Amount Amount %	Total Amount Added (mg)	Amount Recovered (mg)	(%) Recovery*
Paclitaxel 6.0	80	4.7	10.6	99.07
	100	6.1	11.9	98.35
	120	7.3	13.2	99.25

*Average of three determinations

Precision

Precision was studied to find out intra and inter day variations in the test methods of paclitaxel in the concentration range of 100-500 μg/ml for three times on the same day and inter day. Precision was determined by analysing corresponding standard daily for a period of three days. The inter-day and intra-day precision obtained was % RSD (< 2) indicates that the proposed method is quite precise and reproducible.

Table 2 : Validation parameter of UPLC method for paclitaxel

Validation parameter	Paclitaxel
Range (μg/ml)	100-500
Regression equation	y = 2455x + 3581
Correlation Coefficient (r2)	0.998
L. O. D. (μg/ml)	0.1522
L. O. Q (μg/ml)	0.4613

Limit of detection and limit of quantification

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) was calculated by the method based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) is shown in Table 2.

Robustness

Robustness was done by small changing in the chromatographic conditions and found to be unaffected by small changing like ± 2% change in volume of organic solvents of mobile phase.

Stability

Stability of each component in solution was assessed by determining the % RSD of replicate injection of same solution over a period of 72 hrs and was found to be stable during the procedure.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amount of solvents and change of set of conditions requires a short time. Many samples can be simultaneously and suitably employed for the routine quality control analysis of paclitaxel in bulk and injection dosage form. It does not suffer from any interference due to common excipients present in the pharmaceutical preparation and can be conveniently adopted for quality control analysis. We have developed a sensitive and fast UPLC method for the determination of paclitaxel using small volumes of sample (1 μl). The developed method proved superior and economic because of its greater sensitivity and relatively shorter (10 min) run time than ordinary HPLC method. It is an important tool for future speedy analysis of paclitaxel and its injection dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Dabur Pharma Ltd., Ghaziabad for providing standard and samples of paclitaxel and Waters India Ltd., Noida, for providing excellent facilities for carrying out this research work.

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Personalized Medicine – Future of Medical Genetics

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ABSTRACT

The foundation of personalized medicine is Pharmacogenomics-the study of how a person's genetic inheritance affects the body's response to drugs. Patients frequently react differently to drugs, independent of other factors such as age or weight. An effective treatment for one person may have no impact on someone else, or may even cause adverse reactions. The main cause of this varying effect is the diversity of humans' genetic make-up. Understanding the individual differences in the human genome thus helps physicians select and tailor treatments for their patients. Personalized medicine tantalizes the public with its promise of providing the right medicine for the right patient at the right dose, saving lives and preventing dangerous side effects. Delivering this promise, in turn, is predicated on the development and availability of genetic tests that accurately and reliably predict a patient's response to a drug.

KEY WORDS : Personalized medicine, pharmacogenomics, genetic test.

INTRODUCTION

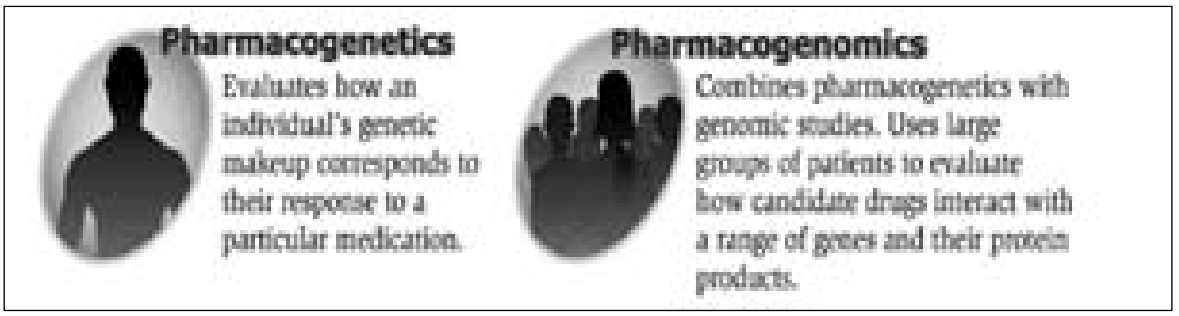
Genes can be considered as the instructions, stored within every living cell, that are required to make and maintain a living organism. Genetic information is encoded by the structure of deoxyribonucleic acid (DNA). A gene is a specific length of DNA that encodes the information to make functional proteins, or parts of them; some proteins are encoded by more than one gene[1]. Proteins are the macromolecules that perform most cellular functions. The properties and functions of a cell are determined almost entirely by the proteins which it is able to make.

The sum total of the genetic information for any organism is called its genome and the study of the genome is termed genomics. Genes are carried on structures called chromosomes; humans have 23 pairs of chromosomes, one chromosome inherited from each parent. The specific site of a gene on a chromosome is termed a locus[2].

Genes may exist in alternative forms, which are called alleles. Specific sets of alleles forming the genome of an individual are called its genotype; the visible appearance or behaviour of an individual is termed the phenotype[3].

DNA is a double stranded molecule, organized as a double helix. The helix consists of a sugar–phosphate backbone with chemical bases that extend from the backbone like the rungs of a ladder. There are four different bases in DNA: adenine (A), thymine (T), guanine (G) and cytosine (C), termed nucleotides. The bases on one strand can only pair with a specific base on the other strand. A always pairs with T, and G always pairs with C. The order of these bases determines the structure, and therefore the function, of the protein made by the DNA[4-7].

Personalized medicine – the newest application of testing technology that permits an assessment of whether and how a patient will respond to a particular drug or dose of a drug based on genetic variations in his or her DNA – has captured the public's imagination and has raised expectations for



therapies that are safer, more effective, and targeted to individual health needs. For example, molecular diagnostics enables the rapid and accurate identification of exact type of breast cancer in a particular woman – and thus, the most effective treatment. The key to personalized medicine is the close connection between therapeutics and diagnostics, so called "theranostics"[8-11].

Personalized medicine tantalizes the public with its promise of providing the right medicine for the right patient at the right dose, saving lives and preventing dangerous side effects. Delivering this promise, in turn, is predicated on the development and availability of genetic tests that accurately and reliably predict a patient's response to a drug.

Today, genetic tests are available clinically for more than 900 diseases. Genetic testing has evolved from a pursuit primarily of academic laboratories studying rare diseases to part of mainstream medicine. Genetic testing encompasses a wide net that includes carrier screening to predict one's risk of having a child with a genetic disease, prenatal diagnosis to assess fetal risk of genetic disease, pre-implantation genetic diagnosis to select embryos with which to start a pregnancy, and pre-dispositional testing to assess an individual's risk for developing disease in the future[12-13].

Instead of adopting a trial-and-error approach, physicians can choose the most effective medication with the fewest side effects from the start.

MEDICAL GENETICS AS A NEW FIELD OF MEDICINE

Genetics was established as a major discipline at the end of the nineteenth and the first half of the twentieth century's, though there was very little interest in the medical aspects of genetics at the time. The situation changed dramatically in the late 1950s, when genetics became an increasingly important part of medical research and practice. Initially, the field focused on diseases due to a single defective gene that could be traced through families in a way that followed Mendel's laws of inheritance , or disorders due to defects in the structure or number of chromosomes. Remarkable progress was made in protein chemistry and biochemistry, making it possible to define the underlying cause of the disease in at least a few cases, although it remained a mystery as to how this was mediated at the level of the gene. However, these advances led to major improvements in the diagnosis of genetic disease.

BEGINNINGS OF PHARMACOGENETICS

Pharmacogenetics was born during the period of intense interest in clinical genetics in the 1950s, after three quite independent discoveries. First, studies of the red blood cells of African–American soldiers who had developed severe anaemia after taking the anti-malarial drug primaquine were found to be deficient in the enzyme glucose-6-phosphatedehydrogenase. This inherited error of metabolism was later found to affect 400 million people worldwide. Second, it was found that individuals who

received the drug isoniazid for the treatment of tuberculosis could be clearly divided into slow and rapid metabolisers of the drug, and that this rate was genetically determined. Third, it was found that patients who had prolonged effects of the anaesthetic agent succinycholine, had an atypical enzyme, in this case a cholinesterase that was inherited. In 1957, based on these and related discoveries, the American geneticist Arno Motulsky wrote an article outlining the basic concepts of pharmacogenetics, but the word pharmacogenetics was not used until 1959 by the German geneticist Friedrich Vogel[5-6].

During the 1960s and 1970s numerous other examples of unusual drug responses due to inherited enzyme defects were discovered. An important advance in understanding severe side effects was initiated by work directed at two drugs: debrisoquine, an agent used for treating hypertension, and sparteine, used for treating abnormal cardiac rhythm. Both drugs are metabolised in the liver by the same enzyme, a cytochrome P450 mono oxygenase, later designated CYP2D6. This enzyme is involved in the metabolism of a wide range of other drugs, including antidepressants and opioids such as morphine, hydromorphine and codeine.

Studies during the 1970s depended on identifying variable responses to drugs followed by an analysis of the enzymes responsible for their metabolism. But towards the end of the 1970s methods were becoming available for cloning and sequencing human genes and so pharmacogenetics, like the rest of human genetics, moved from the protein to the DNA era[7, 14].

MOLECULAR PHARMACOGENETICS

From the 1980s onwards it was possible to isolate, clone and sequence many of the genes that had been found to be responsible for variation in drug metabolism. For example, numerous additional alleles (alternative forms of the gene) of the CYP2D6 system were discovered; currently, nearly 80 distinct genetic variants of this metabolic system have been defined. Hundreds of variants of the glucose-6-phosphatedehydrogenase gene have been found in different ethnic groups with a deficiency of this enzyme. Forty years after the discovery that variation of the metabolism of the anti-tuberculous agent isoniazid is under genetic control, the gene involved was isolated and, again, numerous different mutations were found. In addition it has been discovered that structural changes of genes that encode drug transporters and drug targets may also be involved in varying response to therapeutic agents.

POST-GENOMICS

It was soon clear that post-genomic technology might make it possible to obtain detailed profiles of the genes involved in drug action, and that this would ultimately lead to an understanding of individual variation in response to a wide range of therapeutic agents, or to the promise of 'personalized medicine'. The pharmaceutical industry also recognised that this technology offered a promising way of defining variable response to drugs at an early stage of their development and that this might greatly improve the efficiency of drug trials. Furthermore, genome searches for genes involved in complex multigenic diseases like heart disease, stroke and diabetes might well yield promising targets for drug development. Because many of the mutated oncogenes are potential drug targets, it was recognised that the concept of personalized medicine might also be applied to the cancer field.

PATTERNS OF INHERITANCE

There are two main patterns of inheritance relevant to pharmacogenetics.

1. Monogenic inheritance : Monogenic inheritance implies that a trait, or disease, is due to the action of a single variant gene.
2. Multigenic (or polygenic) inheritance: Multigenic (or polygenic) inheritance implies that a trait or disease requires the action of several variant genes.

DNA VARIATION

Pharmacogenetics aims to identify genes that may be involved in the mode of action of drugs, and how variations in the structure and function of these genes between individuals is related to differences in the response of patients. Although human DNA sequences are 99.9% identical to each other, the remaining 0.1% of variation is of great interest. When a variation in DNA between individuals is found sufficiently frequently in normal populations, it is referred to as a polymorphism. Examples of polymorphisms include single nucleotide polymorphisms (SNPs), insertions and deletions of nucleotides, and repetitive sequences (microsatellites). SNPs may occur in linked groups called haplotypes, defined as a combination of alleles from closely linked loci found on a particular chromosome. Sometimes particular linked alleles occur together more than would be expected by chance, a phenomenon called linkage disequilibrium, reflecting selection of the combination.

GENE HUNTING

The identification of a gene that is involved in the actions of a drug can be discovered in two ways :

a. By making an educated guess and examining a gene which has a high probability of being involved (a candidate gene), or

b. By examining the whole genome.

COMMONLY USED TECHNIQUES IN PHARMACOGENETICS

DNA Cloning : The copying of any specific part of a DNA (or RNA) sequence to be produced in unlimited amounts.

Polymerase Chain Reaction (PCR) : A method used to make multiple copies of DNA.

DNA Sequencing : The determination of the order of the base pairs in a segment of DNA.

Fluorescence In Situ Hybridization (FISH) : A technique that uses fluorescent molecules to locate the position of a DNA sequence along the chromosome.

PROSPECT FOR PHARMACOGENETICS

There is a clear prospect for pharmacogenetics to explain and predict some of the variation in drug efficacy. Currently there are 22 approved drugs where reference to genetic testing is made in the drug labelling or package insert as a guide to how the drug should be used. Some important examples are described in Table 1.

BENEFITS OF PERSONALIZED MEDICINE

- Diagnosing disease or predicting risk of disease.
- Determining whether a treatment is working or not.
- Monitoring healthy people to detect early signs of disease.

Table 1 : Examples of drugs for which the target patient population may be determined by predictive pharmacogenetic testing

Drug/Manufacturer	Disease	Biomarker	Label description
Somatotropin (several)	Prader Willi Syndrome	Chromosome 15 aberration	Use of drug is indicated for patients with the presence of the biomarker
Retinoid (Vesanoid) Roche	Acute promyelocytic leukaemia	PML/RAR gene	Use of drug is indicated for patients with the presence of the biomarker
Cetuximab (Erbitux) Imclone/BMS	Colorectal cancer	EGFR	Use of drug is indicated for patients with the presence of the biomarker
Trastuzumab (Herceptin) Roche/Genentech	Breast cancer	HER 2 protein	Use of drug is indicated for patients over-expressing the biomarker
Alpha1-proteinase inhibitor (Prolastin) Bayer	Congenital alpha1-proteinase inhibitor deficiency	PiMS or PiMZ alpha1-antitrypsin deficiency phenotypes	Use of drug is not indicated for patients with these phenotypes
Imatinib (Gleevec/ Glivec) Novartis	Chronic myeloid leukaemia Gastrointestinal stromal tumours	Philadelphia Chromosome positive CD117 (c-kit) positive	Use of drug is indicated for patients with the presence of the biomarker

- Producing safer drugs by predicting the potential for adverse effects earlier.
- Targeting specific groups of people most likely to benefit from a drug, while keeping its use from those who may be harmed by it.
- Providing researchers the opportunity to get a global view of the events that are always changing within a cell.
- Producing new classes of structural materials that are expected to bring about lighter, stronger, smarter, cheaper, cleaner, and more precise medical products.

CONCLUSION

The promises of personalized medicine will remain unfulfilled promises unless scientists, clinicians, policy makers, and industry leaders can overcome challenges that include :

- Educating doctors and patients about the goals and limitations of personalized medicine,
- Reorienting the pharmaceutical industry to develop and market drugs for relatively small populations,
- Designing clinical trials to maximize the possibilities offered by personalized medicine,
- Developing diagnostic tests with sufficient sensitivity and specificity and adopting appropriate regulatory mechanisms to ensure the tests' clinical validity,
- Protecting the privacy of individuals when genetic tests indicate an elevated risk for future disease,
- Addressing health care disparities, and
- Encouraging health insurance companies to cover new treatments based on personalized medicine.

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Study of Ovarian and Uterine Ascorbic Acid Level After Intravaginal Neem Oil Treatment in Albino Rat

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ABSTRACT

0.1 ml crude neem oil when administered intravaginaly to cyclic female albino rats group for different duration, a highly significant ($P<0.001$) decreased level of ovarian ascorbic acid was observed in weekly one month and again a highly significant ($P<0.01$) decreased in fortnightly three months treated group was observed in comparison to control group of rat.

Where as uterine ascorbic acid level showed a highly significant increased at the extent of $P<0.01$ to $P<0.001$ value in comparison to control group of rat.

These research findings clearly established the possible role of neem oil as intravaginaly contraceptive in albino rat at biochemical level.

KEY WORDS : Neem oil, Uterus, Ovary, Estrous cycle, Ascorbic acid.

INTRODUCTION

Neem-a green treasure is the most versatile multifarious trees of tropical regions with immense biological activity[1]. Its non wood product is neem oil which contains a number of chemical compounds. Azadirachtin is one of them having a mixture of seven structurally related isomers of tetranortriterpenoid which may use as spermicidal and contraceptive[2, 3]. Numerous studies indicated that ascorbic acid is an antioxidant and having demonstrated to be effective in various reproductive processes[4] reported that ascorbic acid deficiency showed endogen deprived effect in male rat. It was reported that ascorbic acid protect against LPS-induced intrauterine fetal death and intrauterine growth retardation in mice[5].

Therefore in the present research investigation, the effect of intravaginal neem oil treatment on ovarian and uterine ascorbic acid level was studied to know the contraceptive effect of neem oil at biochemical level.

MATERIALS AND METHODS

Female albino rats of Charls Foster strain of 120 -130 gm body weight were employed in this investigation. Rats were divided into 7 groups each consisting of 6 rats. Their regular estrous cycle was studied by vaginal smear technique[6]. One group of 6 rats was considered as control group and other were considered as experimental groups.

All the experimental as well as control rats were maintained at uniform animal husbandry conditions of food, water, light and temperature throughout the period of experimentation.

Neem oil was procured from Khadi and Village Industries Commission, Patna. Different experimental groups on the basis of treatment duration were divided into six groups as shown in Table-1.

For treatment of neem oil, experimental rats were held in the head down position. A fine Polyethylene tube filled to syringe with the help of needle was passed gently into the vagina as deep as possible.

Table 1 : Showing the details of material employed during experimentation

S.No.	Status of rats	Group No.	Body weight of rats(gm)	Amount of Neem oil (in ml)	Frequency of Neem oil administration	Total duration of experimentation
1	Control	I	120 – 130	–	–	–
2	Exp.	II	"	0.1	Weekly	1 month
3	Exp.	III	"	0.1	Fortnightly	1month
4	Exp.	IV	"	0.1	Monthly	1 month
5	Exp.	V	"	0.1	Weekly	3months
6	Exp.	VI	"	0.1	Fortnightly	3 months
7	Exp.	VII	"	0.1	Monthly	3 months

The animal was allowed to stay in same position for one to two minutes for proper dispersion of oil into the vagina. In the similar way control rats were treated as with glass distilled water by following the same time, frequency and duration. After two days of the last treatment the rats were scarified and uterus and ovary were taken out for biochemical estimation. 20mg uterine and ovarian tissues were macerated separately and 2ml distilled water was added to each to prepare homogenate. 0.1 ml from each was taken for the estimation of ascorbic acid[7].

RESULTS

As indicated in table 2. at the level of ovarian ascorbic acid, a highly significant ($P<0.001$) decreased was observed in weekly one month treated group and a highly significant ($P<0.01$) decreased in fortnightly three months treated group were observed in comparison to control group of rat.

Whereas at the level of uterine ascorbic acid, a highly significant ($P<0.001$) increased was observed in weekly one and three months and again a highly significant ($P<0.01$) increased in fortnightly and monthly three months in comparison to control group of rat were observed.

Table 2 : Ovarian and Uterine Ascorbic Acid level after different duration intravaginal treatment of neem oil

S.N.	Group No. (each) contains 6 rats)	Frequency of Treatment	Ascorbic acid level in (in μg/ mg) Mean±SE	
			Ovarian tissue	uterine tissue
1	I	Control	0.74± 0.03	0.19± 0.02
2	II	Weekly for one month	0.44± 0.02	0.52± 0.02
3	III	Fortnightly for one month	0.68± 0.07	0.29± 0.01
4	IV	Monthly for one month	0.73± 0.02	0.24± 0.02
5	V	Weekly for 3 months	0.62± 0.08	0.45± 0.02
6	VI	Fortnightly for 3 months	0.50± 0.03	0.36± 0.03
7	VII	Monthly for 3 months	0.72± 0.03	0.33± 0.02

P –Value of

Ovarian Ascorbic Acid	Uterine Ascorbic Acid
In between	In between
I and II P < 0.001 (HS)	I and II P < 0.001 (HS)
I and VI P < 0.01 (HS)	I and III P < 0.01 (HS)
	I and V P < 0.001 (HS)
S – Significant, HS – Highly Significant	I and VI P < 0.01 (HS)
	I and VII P < 0.01 (HS)

DISCUSSION

As indicated in Table no.2 the results obtained showed that a highly significant decreased level of ovarian ascorbic acid, this significant decreased level of ascorbic acid during different duration treatment were marked. Dixit finding indicates that biologically active botanical substances or fertility regulating agents of plant origin interfere with the natural process of reproduction[8]. The decreased level of ovarian ascorbic acid in ovarian tissue might be an indication of disturbed pituitary ovarian axis by different duration intravaginal treatment of neem oil. It was also reported that ovarian ascorbic acid act an indicator of ovarian LH[9]. Our finding concludes that the seed extract of neem significantly reduced the number of normal follicles in the ovaries of rats[10].

The result of present finding indicates increase level of uterine ascorbic acid after different duration, intravaginal neem oil treatment. This increase level might be an indication of stressed and disturbed histoarchitecture of uterus. In the reproductive process uterus undergoes a series of morphological and biochemical changes which render this organ receptive to blastosis. Earlier reports of Deb and Chattarjee[11] and Chinoy et al.[4] can be correlated if the present finding that ascorbic acid has possible role in normal functioning of uterus for implantation of blastosist and their in enhance level were observed during invasion of toxin, foreign material and in stress condition of organ. The earlier findings of Prakash et al.[12] also support our finding the neem oil denatured the endometrial lining of the uterus and keep it in stressed condition.

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PHARMBIT

ISSN : 0973-6204

Scientific Journal of Pharmaceutical Society

Vol. XX. No. 2, Jul-Dec, 2009

Indexing in "Chemical Abstract & Natural Science Database"

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Single copy INR 250

Printed & Published by :
Dr. R.N. Gupta, *Editor*
on behalf of Pharmaceutical Society
Department of Pharmaceutical Sciences
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Printed at Kailash Paper Conversion Pvt. Ltd.
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