Review on Herbal Drug for TB / Ethnopharmacologyof Tuberculosis

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OBJECTIVE :

Aim of this article is to encourage the present and future research scholars to bring out a new drug for Tuberculosis from herbal origin by providing data about the herbs used in the treatment of Tuberculosis and to justify the reasons for the need of new drug. Scientists say that the drug discovery is a long process before it goes for clinical trial. We have to show enthusiastic, rigorous efforts after the anti-TB agent was found in the plant and hoping the possibility of new drug discovery for effective alternatives against TB which is an urgent need for developing nations!

World TB Day – March 24 is a sober reminder that tuberculosis continues to be a major public health concern. Appreciable advances have been made in our understanding about various facets of tuberculosis in recent times. But the drugs, that we use, are more than 40 years old, methods for diagnosing active tuberculosis are more than 60 years old, the vaccine some 80 years old, and the Tuberculin Skin Test (TST) for diagnosing early or latent tuberculosis is 100 years old. Scientific and Medical Journals carry a large number of articles, signaling significant volume of knowledge that is being generated. The scientists and research scholars have been able to give new drugs and tools for the people who need it most.¹

Seidel, from London School of Pharmacy, U.K. stated that a new drug therapy is a global emergency especially an active medicament from plant origin is an urgent need. New drugs are required to reduce the duration and complexity of current therapy, improve treatment of MDR TB and control of latent TB².

The WHO intends to integrate traditional medicine into National Health systems (NHS) globally. This is an opportunity for building safe, affordable and effective NHS especially for Third world countries, rich in both medicinal plant resources and traditional medicine knowledge. It is the time for Governments to found research into holistic health models instead of squandering more billions on 'health genomics', which will increase intervention and iatrogenic damages to health.³

A large number of plant extracts examined have been shown to possess significant activity. In the current popular field of chemotherapy of TB and other mycobacterial diseases, indigenous plants are proving to be of interest. In the view of environment threat to many plant species, screening of such plants for potential therapeutic properties including anti TB activities are urgently required before they lost forever.⁴

HISTORY OF TUBERCULOSIS

The human being appears to be afflicted with more diseases than any other animal species. Among various illness affecting human, respiratory diseases are more prevalence. Tuberculosis is a disease of antiquities which is thought and has evolved sometime between the seventh and sixth millennia B.C. This contagious disease TB was diagnosed in modern medicine at the beginning of 19th century after the identification of mycobacterium by Robert Koch on 24th March, 1882 and the proper treatment was started from the middle of 20th century. But our Indian Systems of Medicine treats this disease from 5000 BC by herbal drugs. TB was diagnosed in Ayurveda and Siddha systems as Rajayakshma and Shayarokam respectively. Pulmonary Tuberculosis named as Ulamanthai in ISM. The symptoms, diagnosis and treatment for these diseases were detailed in our system meanwhile in modern medicine TB was not identified as a single disease until the 1820s, and was not named tuberculosis until 18395.

Phthisis is a Greek word for consumption, an old term for pulmonary tuberculosis around 460 BC; Hippocrates⁵ identified phthisis as the most widespread disease of the times. It was said to involve fever and the coughing up of blood, which was almost always fatal. Epidemiology of TB said that the global spread of this disease exist from time immemorial and treated by traditional system of concerned natives. Skeletal remains show prehistoric humans (4000 BC) had TB, and researchers have found tubercular decay in the spines of Egyptian Mummies dating from 3000–2400 BC.⁶

Ethnomedical information / Global warning of TB

The heart of the TB problem lies in sub Saharan Africa, South East Asia, India and China; roughly 90% of all new cases of TB occur in these areas. In Eastern Europe and Africa, TB deaths are increasing after almost 40 years of decline and biggest burden of TB is in South East Asia. Genetic studies suggest TB was present in the Americas from about the year 100 AD. In USA, approximately 14,000 cases of tuberculosis were reported in 2006, a 3.2% decline from the previous year; however 20 states and District of Columbia had higher rates. (Centers of disease control and prevention-Trends in tuberculosis incidence-2007)

Nearly 1% of world's population is newly infected with TB every year. WHO estimated that between "2002-2020" approximately 1000million people will be newly infected; over 150million people will get sick and 36million will be dying of TB if control is not further strengthened. In India, it is estimated that nearly 2 million people develop active disease every year and almost 0.5 million die from it.⁷

TB is the major Global health problem in this millennium than HIV infection. AIDS patients die mainly because of mycobacterium. All AIDS patients are infected by TB but not all TB patients are infected by HIV. So the control of TB will be very much useful to extend the life span of HIV patients. AIDS can be protected by moral habit and personal chastity of individuals but TB will affect invariably from innocent to immoral personalities.

The latest data released by the World Health Organization (WHO) in November 2010 shows that the number of new cases continues to fall globally and in five of the six WHO regions. The exception is Southeast Asia, where incidence remains stable. In many countries TB prevalence is declining. Worldwide, deaths from TB fell by 35 percent between 1990 and 2009. If current trends continue the world can meet the Millennium Development Goal target for incidence – that new cases should be falling by 2015 – and the Stop TB Partnership target to halve TB mortality by 2015 in comparison with 1990. The Global Fund has helped to accelerate case detection and successful treatment in recent years, with 1.7 million additional cases of TB detected and treated by Global Fund-supported programs in 2010, compared with 1.4 million in 2009 and 1.3 million in 2008. Since the Global Fund's inception in 2002, programs it has financed had supported DOTS for a total of 7.7 million people by December 2010.⁸

Need of new drug from herbal origin

The current therapy for TB consists with third generation antibiotics such as rifambicin, ethombutol, isoniazid and pyracinamide, but the emerge of multiple drug resistant (MDR) and (XDR) strains of mycobacterium is now common in number of patients because of uncontrolled application of these anti TB drugs. However, no single drug or combination therapy was able to control TB completely, because of a factor "cross resistance". Such drug resistance is developed only against purified chemical compound. Any single purified compound will produce resistance in pathogens. The Mycobacteriae are trained themselves to digest the drug by modifying their receptor structure according the chemical structure of the drug. Thus the Mycobacteriae slowly adapt and develop resistance against modern drugs. Herbal drug whether extract or decoction used against any pathogen will not induce drug resistance. Hence an effective and alternative anti tuberculosis drug preferably herbal based drug has to be developed.²

ISM described in detail about the TB and prescribed number of herbal formulations to control and cure TB and also advised the propholytic measures form the period of Rig-Veda. In India, many medicinal plants had been in use since the period of Rigveda. According the Western scholars, the time of Rigveda is BC 4500. We can see the references of 67catogories of herbs in it {Rigveda 1(116):14-16}. Indians worked meticulously to examine and classify the herbs which they came across into groups. The first university in the world was in India where 600 students were studied from 60 countries. Encyclopedia Britannica says, "In the ancient time itself, the medical science of Indians was well developed in two headings like, Saliyatantra and Kaya Chikista (Surgery and General Medicine).

Many herbs and herbal formulations were described in Ayurveda and Siddha system of medicine. For example Glycerrhiza glabra (Athimathuram), Allium sativam (Poondu), Piper longum (Thippili), Zingeber officinalis (Sukku), Ocimum sanctum (thulasi), Solanum surratense (Kantankathiri) etc.,⁹

ANTIMYCOBACTERIAL ACTIVITY OF MEDICINAL PLANTS' EXTRACTS AND COMPOUNDS REPORTED SO FAR SINCE BRITISH INDIA

Records show that 2766 patients of PTB were treated with Ayurvedic drugs in a tertiary care hospital in Kolkata in the year 1933-1947¹⁰. Then up to the middle of 70's herbal research was not supported much by the Government since modern chemotherapy was in trial by authorities.

1946 - Allicin, a compound isolated from Allium sativum was found to posses powerful anti TB activity.¹¹

1950 - The influence of 1-usinic acid on experimental guinea pig TB. Ann. Med. Exp. Biol. Fenniae; 28: 191 - 208.¹²

1954 - The effect of usnic acid on Mycobacterium tuberculosis and other experimental infections¹³.

1968 - The microchemical investigation on some plant acids and their hydrazides of Prunus mume and Schezandra chinensis and reported the anti TB principles. A phenolic compound, Tuberosin was isolated from Purearia tuberose possessed anti TB activity¹⁴.

1975 - Iisolated xanthones from Canscora decussate Schult showing anti TB activity.¹⁵

1976 - Usinic acid is an antibiotic compound obtained from several lichen species like Usnea, Cetraria, Ramalina, Parmelia, Cladonia etc. It is known to be a potent anti TB agent both in vitro and in vivo.¹⁶

1980 - Naturally occurring amides from piper species showed in vivo and in vitro anti TB activity equivalent to $1/5^{th}$ potency of streptomycin.¹⁷

1981 - Found two new amorphous resorcinol derivatives Ardisionol (C20 H32 O2) and Ardisionol-II (C19H30O2) possessed significant antimycobacterial activity.¹⁸

1985 - Anti TB constituents were isolated from Gentianarhodantha. 19

1987 - Three compounds from acidic extract of roots of Euphorbiabracteolata (Euphorbiaceae) showed inhibitory effect against tubercle bacillus²⁰.

1987 - Garlic extract inhibited the growth of four strains of M.tuberculosis in a concentration ranging from 0.98 - 2.94 mg/ml.²¹

1988 - Lavandino essential oil possessed antimycobacterial activity except Mycobacterium tuberculosis²².

1989 - Reported the advantages of herbal adjacent therapy of Japanese traditional formulation that overcome the allergic reactions induced by rifampicin.²³

1991 - Out of 408 plant extracts, the ethanol extracts of 10 plants inhibited the growth of M. tuberculosis H37Rv. Nevadensin isolated from the plant Limnophila conferta showed anti TB activity.²⁴

1992 - The immunological changes were studied in active pulmonary tuberculosis by clinical trial. The Chinese indigenous drug, Fuzheng Guben pill was administered along with usual chemotherapeutic agents. The results revealed that the herbal drug with INH and SM showed immunological modulation.²⁵

1993 - The data on pharmacokinetics and disposition of usinic acid which are required for further clinical evaluation of its anti TB effect have not been reported so for.²⁶

1993 - Report shows that the alcoholic extract of Cressa cretica offered 3 new isoflavones and a new coumarinochromone glycoside which possessed very good antimycobacterial activity.²⁷

1994 - The leaves extracts of Bidens pilosa, Tetrademia riparia and roots extracts of Pentas logiflora showed activity at 1000μ g/ml was observed.²⁸

1995 - Tryptathnrin, an indoloquinazolinone alkaloid has the anti TB activity at $0.5 - 1 \mu g/ml$ MIC. Liposomal asiaticoside a plant glycoside showed anti TB activity.

In clinical trials, the extract of Ottelia alisinoides cured two cases of bilateral tubrculosis of cervical lymph gland within three months²⁹.

1996 - Chemical investigation of the fractions of crude plant extracts of Borrichia frutescens (Asteraceae) was carried out and result the isolation of different triterpenes.³⁰

1997 - Methanol extract of 74 Artemisia species exhibited significant antimycobacterial activity Out of 100 plants 19 methanolic extracts showed antimycobacterial activity. 13 of this were used by first Nations' people to treat TB. Methanol extracts of Heracleum maximum roots. Glehnia littonalis roots and Lomatium dissectum roots completely inhibited the growth of Mycobacterium tuberculosis at a concentration equivalent to 100mg/ disc.³¹ North American medicinal plant known as (Devil's club) Oplopanax horidus (Araliaceae) exhibited significant antimycobacterial activity.32 The roots of Salvia multicaulis yielded 4 aromatic norditerpenoids and a new pimarane diterpenoid. The isolated compounds from these possessed anti TB effect.³³ Water extract of garlic inhibited the growth of M.tuberculosis H37 Rv and M.tuberculosis TRC - C1193 susceptible and resistant to INH respectively. The MIC (Minimum Inhibitory Concentration) was slightly above 80 but less than 160µg/ml and slightly above 100 but less than 200µg/ml for susceptible and resistant strains respectively³⁴.

1998 - Displayed a radiorespirometric bioassay of crude methanol extract of Kenyan shrub Leucas volkensii gurke (Labiatae) against Mycobacterium tuberculosis³⁵. Studied the antitubercular potential of tropical flora of Puerto Rico. Out of 50 plants screened six extracts showed positive result with varying degrees of inhibition.³⁶ Many analogues of the indologuinazolinone alkaloid structure has been synthesized and evaluated for antimycobacterial potencies³⁷. Reported three compounds namely ibogaine, voacangine and texalin were possessed antimycobacterial activity and it was determined by BACTEC 460-TB radiometric methodology.³⁸ An anti TB diterpenoid was isolated from petroleum ether extract of Azorella madreporica, a woody cushion plant grows up to 5cm as an underground rhizome.³⁹ Reported six matricaria esters and two matricaria lactones isolated from the members of tribe Astereae (Asteraceae) were possessed antimycobacterial activity.40 The Eastern

North American medicinal plant, Hydrastis Canadensis (Ranunculaceae) was studied and reported that the berberine is responsible for the activity against MDR-TB. A commercial root sample extract was subjected by BACTEC method.⁴¹

1999 - Reported that the crude methanol extract of Meliavolkensii showed antimycobacterial effect at MIC of 100 μ g/ml. They also carried a bioassay-guided search for antimycobacterial natural products from higher plants, the methanol extract of aerial parts of Ajuga remota Benth (Labiatae) yielded ergosterol-5 and 8 endoperoxide which showed ac tivity at MIC of 1 μ g/ml.⁴²

2000 - Conducted a comparative study of two plants' extracts and reported their anti asthmatic property and stated that their work lead to find an anti-TB herbal drug⁴³. Medicinal plants from Nepal : Evaluation as inhibitors of Luekotriene biosynthesis⁴⁴.

2001 - The isolation of (+)-totarol as active compound from Chamaecyparis nootkatensis outer bark was examined for anti TB effect and Investigations on antimycobacterial activity of some Ethiopian medicinal plants were carried out and submitted the first report of tannins exhibiting anti TB activity.⁴⁵

2002 - In Tamil Nadu at TRC (Tuberculosis Research Centre) an attempt has been made to find out the antimycobacterial potential of four medicinal plants that are commonly used to treat respiratory diseases in ISM. The leaves of Adhatoda vasica and Aegle marmelos and the whole plants of Solanum trilobatum and Oldenlandia umbellata were tested and reported that the methanol extract of roots and aerial parts (except leaves) of Oldenlandia umbellate showed significant activity against H37Rv strain at a concentration of 200 μ g/ml. The active constituent Allizarin was identified, purified and isolated.⁴⁶

2003 - In vitro inhibition of drug resistant and drug sensitive strains of mycobacterium tuberculosis by Ethnobotanically selected South African plants extracts showed inhibitory activity at a dose of 0.5mg/ml^{47} . Antimycobacterial activity of diospyrin derivatives and structural analogue of diospyrin were tested against M. tuberculosis in vitro. A group of 22 Mexican medicinal plants were screened for activity against H37Rv strain at concentration from 50-200 µg/ml. Hexane extracts of five and methanol extracts of two of them were possessed activity against MDR-TB organism.⁴⁸

2004 - Antiasthmatic activity of fruits of Solanum surattense and Solanum melongena var. insanum was compared and reported since these plants claims anti TB effects in ISM records.⁴⁹

2005 - An elaborate study on herb-drug interaction was carried out at Dept. of Pharmacy, National University of Singapore with clinical significance. Piperine from black (Piper nigrum) and long (Piper longum) peppers increased the area under plasma concentration of rifampicin in pulmonary $TB^{\rm 50}$

2006 - The pure allicin which arises by conversion of allin in garlic is highly volatile and poorly miscible, has strong antioxidant property. In volunteer studies, patients on garlic extract treatment have generally reported an improvement in their condition after 2&6 weeks, with infections resolving in 3-4 months. The Germen Commission - E, a therapeutic guide to herbal medicines reports no side effects of garlic although some sources suggest that substantial amounts of garlic should not be consumed before surgery, since it can prolong the bleeding time.⁵¹

2008 - Submitted three studies examined herbal preparations for preventing liver damage in TB patients. In two studies from China, the preparations contained 11 different herbs; in one study different combinations of herbs were administered to participants depending on their vital energy (qi) and bodily balance (vin yang). One study from Russia reported the effect of herbal infusions containing on average 25 different herbs tailored to individual patient needs. 51 studies evaluated manufactured herbal products. Extract of milk thistle (Silvmarin) was the most frequently evaluated in the studies we identified (9 studies from China). Oleanic acid (extract of Swertia) and glycyrrhizin (extract of Liquorice) were also common in studies conducted in China. Two studies in India evaluated combinations of many different indigenous herbs and plants; one trial examined Stimuliv tablets, the other a capsule called Optiliv.52

2009 - By this work two medicinal plants were evaluated and confirmed their antimycobacterial activity. The extracts of different solvent system obtained by hot and cold extraction methods were proved their antimycobacterial effect by 1μ g/ml. MIC. All extracts of both species showed activity against standard organism

H37Rv at the concentration of 10μ g/ml and onwards. 7 extracts of SS showed significant bacteriostatic activity at the concentration of 1μ g/ml.⁵³

2011 - A study on "Diagnosis and Treatment of Tuberculosis in HIV co-infected Patients" at National Institute for Research in Tuberculosis (ICMR), Chennai

2012 - Scientists of Bhavnagar-based Central Salt and Marine Chemicals Research Institute (CSMCRI) have discovered anti-TB contents in the roots of salicornia brachiata, a plant that grows naturally in abundance along the Gujarat coast, especially in Saurashtra. This is the same plant which has given world's first vegetable salt. The discovery has come after nearly 13 years of rigorous research initiated under a multi-institutional project titled "Discovery, Development and Commercialization of New Bioactive and Traditional Preparations" started in 1998 by Council of Scientific and Industrial Research.

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Efficacy of 23.4% Hypertonic Saline In the Mannagement of Intracranial Hypertension

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Intracranial pressure (ICP) is the pressure inside the skull and thus in the brain tissue and cerebrospinal fluid (CSF). The body has various mechanisms by which it keeps the ICP stable, with CSF pressures varying by about 1-2 mmHg in normal adults through shifts in production and absorption of CSF. Abrupt changes in intrathoracic pressure during coughing, communication with the vasculature and valsalva has shown to be influencing CSF pressure. ICP is measured in millimeters of mercury (mmHg) and, at rest, is normally 7–15 mmHg for a supine adult, and becomes negative, averaging -10 mmHg in the vertical position, at 20-25 mm Hg, the upper limit of normal, treatment to reduce ICP may be needed. Changes in ICP are attributed to volume changes in one or more of the constituents contained in the cranium¹.

INCREASED ICP

One of the most damaging aspects of brain trauma and other conditions, directly linked with poor outcome, is an elevated ICP. Highly increased ICP is very likely to cause severe harm. Intracranial hypertension (IH) is usually fatal if prolonged, but children can withstand higher pressures for longer periods. An increase in pressure, most commonly due to head injury leading to intracranial hematoma or cerebral edema can crush brain tissue, shift brain structures, cause hydrocephalus, cause brain herniation, and restrict blood flow to the brain. It is a cause of reflex bradycardia².

PATHOPHYSIOLOGY

The cranium and the vertebral canal, along with the relatively inelastic Dura, form a rigid container, such that the increase in any of its contents- brain, blood, or CSF, will lead to increased ICP.

In addition, the Monro-Kellie doctrine relationship clearly states that any increase in one of the components must be at the expense of the other two. Small increases in brain volume do not lead to immediate increase in ICP because the body has various mechanisms by which it keeps the ICP stable. One such mechanism is the ability of the CSF to be displaced into the spinal canal, as well as the slight ability to stretch the falx cerebri between the hemispheres and the tentorium between the hemispheres and the cerebellum. However, failure of intracranial compliance is seen when the ICP reaches around 25 mmHg, small increases in brain volume can lead to marked elevations in ICP³.

Traumatic brain injury (TBI) is a devastating problem which is directly correlated with high subsequent morbidity and high mortality. Injury to the brain occurs both at the time of the initial trauma (the primary injury) and subsequently due to ongoing cerebral ischemia (secondary injury)⁴. The other well recognized causes for secondary injury are cerebral edema, CSF hypertension, circulatory hypotension and hypoxic conditions. IH is generally seen after a severe diffuse brain injury and then leads to cerebral ischemia by compromising cerebral perfusion⁵.

Cerebral perfusion pressure (CPP) is the pressure of blood flowing to the brain and is constant normally due to auto regulation, but for abnormal mean arterial pressure (MAP) or abnormal ICP the CPP is calculated by subtracting the ICP from the MAP: CPP = MAP - ICP. One of the major threats of increased ICP is that it leads to ischemia by decreasing CPP6. Once the ICP approaches the level of the mean systemic pressure, cerebral perfusion falls. The body's response to a fall in CPP is to raise systemic blood pressure and dilate cerebral blood vessels. This results in increased cerebral blood volume, which increases ICP, lowering CPP further and causing a vicious cycle. This results in widespread reduction in cerebral flow and perfusion, eventually leading to ischemia and brain infarction⁷. Increased blood pressure can also make intracranial hemorrhages bleed faster, also increasing ICP⁸.

Severely raised ICP, if caused by a unilateral spaceoccupying lesion (e.g. a hematoma) can result in midline shift, a dangerous sequela in which the brain moves toward one side as a result of massive swelling in the cerebral hemisphere. Midline shift can compress the ventricles and lead to hydrocephalus. Prognosis is much worse in patients with midline shift than in those without it. Another dire consequence of increased ICP combined with a space-occupying process is brain herniation (usually uncal or tonsilar)9. In uncal herniation, the uncus hippocampus becomes compressed against the free edge of the tentorium cerebelli, frequently leading to brainstem compression. If brainstem compression is involved, it may lead to respiratory depression and is potentially fatal. This herniation is often referred to as "coning"¹⁰.

Major causes of morbidity due to raised intracranial pressure are due to global brain infarction as well as decreased respiratory drive due to brain herniation.

CAUSES

- Brain tumor, infarction with edema, contusions, subdural or epidural hematoma, or abscesses all tend to deform the adjacent brain.
- Ischemic-anoxia states, acute liver failure, hypertensive encephalopathy, pseudo tumor cerebri, hypercarbia, and Reye hepatocerebral syndrome are conditions which tend to cause generalized brain swelling and decrease the cerebral perfusion pressure but with minimal tissue shifts.
- Venous sinus thrombosis, heart failure, or obstructions of superior mediastinal or jugular veins are conditions of increased venous pressure.
- Hydrocephalus, extensive meningeal disease (e.g., infection, carcinoma, granuloma, or hemorrhage), or obstruction in cerebral convexities and superior sagittal sinus (decreased absorption) are conditions which tend to obstruct the CSF flow and obstruct the absorption of CSF.

- Meningitis, subarachnoid hemorrhage, or choroid plexus tumor are conditions which tend to increase the production of CSF.
- Idiopathic or unknown cause
- Craniosynostosis

INTRACRANIAL PRESSURE MONITORING

ICP monitoring uses a device, placed inside the head, which senses the pressure inside the skull and sends its measurements to a recording device.



Ventricular drainage system and ICP monitoring

TYPES OF INTRACRANIAL PRESSURE MONITORING

There are three ways to monitor pressure in the skull (ICP):

- A thin, flexible tube threaded into one of the two cavities, called lateral ventricles, of the brain (intraventricular catheter)
- A screw or bolt placed just through the skull in the space between the arachnoid membrane and cerebral cortex (subarachnoid screw or bolt)
- A sensor placed into the epidural space beneath the skull (epidural sensor)

The intraventricular catheter is thought to be the most accurate method, but if immediate access is needed, a subarachnoid bolt is typically used. If no qualified brain surgeon (neurosurgeon) is available to place a bolt, then an epidural sensor will probably be used. To insert an intraventricular catheter, a burr hole is drilled through the skull and the catheter is inserted through the brain matter into the lateral ventricle, which normally contains liquid (CSF) that protects the brain and spinal cord. Not only can the ICP be monitored, but it can be lowered by draining CSF out through the catheter¹¹. This catheter may be difficult to get in place when there is increased ICP, since the ventricles change shape under increased pressure and are often quite small because the brain expands around them from injury and swelling¹².

A subarachnoid screw or bolt is a hollow screw that is inserted through a hole drilled in the skull and through a hole cut in the outermost membrane protecting the brain and spinal cord (Dura mater). The epidural sensor is placed through a burr hole drilled in the skull, just over the epidural covering. Since no hole is made in the epidural lining, this procedure is less invasive than other methods, but it cannot remove excess CSF¹³.

Lidocaine or another local anesthetic will be injected at the site where the incision will be made. A sedative is most likely administered for relaxation. First the area is shaved and cleansed with antiseptic. After the area is dry, an incision is made and the skin is pulled back until the skull is visible. A drill is then used to cut through the bone to expose the epidural tissue. If an epidural sensor is used, it is then inserted between the skull and epidural tissue. If a bolt is used, an incision is made to expose the subarachnoid space and the bolt is screwed into the bone. This allows the sensor to record from the subdural/subarachnoid space. If an intraventricular catheter is used, it is threaded through the brain matter into one of the lateral ventricles¹⁴. This type of catheter is effective and accurate at sensing ICP measurements.

TREATMENT

Hyperosmolar therapy effectiveness is a result of brain shrinkage caused from the shifting of water out of the brain. Mannitol has been accepted as treatment for IH, and is considered a safe and effective osmotic diuretic¹⁵. Considered the drug of choice for IH in Guidelines for the Management of Severe Brain Injury, mannitol also has many drawbacks. Mannitol, an osmotic diuretic, has been associated with volume depletion and hypotension leading to secondary injury to the brain. Injury to the blood brain barrier (BBB), often found in neurological injuries, can be a confounding factor in the management of IH¹⁶. One concern is that solutes may escape from the vascular system to the brain tissue. In the presence of an injured BBB, mannitol may escape the intravascular space and accumulate in already edematous tissue, which may lead to an increase in cerebral edema and IH often referred to as rebound phenomenon¹⁷.

In an attempt to find the "optimal" hyperosmolar agent, the use of other solutions has been explored. An optimal agent should be nontoxic, simple to administer with minimal side effects, have a strong osmotic gradient, and would remain in the intravascular space¹⁸ (reflection coefficient). Reflection coefficient is the measurement of how well a solute crosses a membrane. In regards to treatment of IH, a reflection coefficient of 1 is given to a solute that stays in the intravascular space and is impermeable to an intact BBB¹⁹ (Table 1).

Agent	Reflection Coefficient			
Glycerol	0.56			
Mannitol	0.95			
Hypertonic saline	1.00			
Urea	0.46			

Table 1. Reflection Coefficients

Research has found other agents such as glycerol and urea, with their low reflection coefficients, to be fairly ineffective osmotic agents in the treatment of IH. Hypertonic saline (HS) with its low cost, ease of administration, and titratable osmotic gradient (by adjusting its concentration), and its reflection coefficient of 1 has been recognized as an effective osmotic agent²⁰.

HYPERTONIC SALINE

The use of HS in the treatment of cerebral edema was first proposed in 1919 by Weed and McKibben. Nearly forgotten, more than sixty years later HS reemerged as resuscitation agent for patients in shock²¹. Incidentally found through the resuscitations of patients with multi - organ trauma, use of 23.4% HS in patients with neurotrauma alone became a focus of research. Finding from this early research, in multi - organ trauma, including brain injury, promoted its use in the treatment of IH and herniation syndromes as well^{22, 23}

The proposed mechanism of action of 23.4% HS is similar to that of mannitol. Through osmosis 23.4% HS draws water out of the intracellular space, the edematous brain, and back into the intravascular space. In addition, 23.4% HS has been shown to be effective in resuscitation of patients in shock by the same mechanism²⁴. Unlike mannitol which promotes diuresis, 23.4% HS increases blood volume, decreases viscosity, and increases preload²⁵. All of these characteristics lead to an increase in blood pressure, cardiac output, and CPP, thereby improving perfusion to the brain and decreasing secondary injury. Used as a resuscitation agent, 23.4% HS also has potential to be used as a component of "triple H" therapy (hypervolemia, hemodilution, and hypertension) in the treatment of patients with SAH who are at high risk of severe vasospasm^{26, 27.}

TRAUMATIC BRAIN INJURY (TBI)

Most studies involving TBI, share similar findings that indicate that HS is consistent with a decrease in ICP and an increase in CPP²⁸ (Table 2). Maintaining a physiologic normal CPP is as important as decreasing elevated ICP in preventing secondary injury. By increasing blood volume and preload, HS helps to ensure adequate perfusion and oxygenation to at risk brain tissue. One study compared mannitol boluses versus 23.4% HS boluses²⁹. In that study, Kerwin et al. compared 20% mannitol with 23.4% HS bolus for the treatment of ICP above 20mmHg³⁰. This study demonstrated that patients given 23.4% HS decreased ICP by 50% more than mannitol.³¹.

Principle author	Design	Sample	Osmotic agent	Amount administered	Frequency/ Triggers	Soc	Results
Kerwin 2009	Pilot study prospective	n=22 e=210	23.4%HS 20% mannitol	Varying doses	ICP>20	Х	HS↓ICP by 50% more than mannitol
Ware 2005	Retrospec- tive	n=13 e=22	23.4% HS	30ml bolus	One time		HS: longer duration of effect than mannitol

n=number of participants, e=number of IH events NS=normal saline PbO2=brain tissue oxygenation SOC=standard of care including mechanical ventilation for GCS<S, vasopressors to maintain cerebral perfusion, elevated head of bed, and sedation and analgesia

Table 2. Summary of Study Findings for Traumatic Brain Injury

MIXED NEUROLOGICAL INSULTS

Samples with mixed neurological injuries were examined in two studies (Table 3). Koenig et al. studied the effect of 23.4 % HS bolus in patients with signs of impending herniation syndromes. Concentrated HS was successful at reversing herniation syndromes in 57 of the 76 events³².

Principle author	Design	Sample	Osmotic agent	Amount administered	Frequency / Triggers	Soc	Results
Koenig 2008	Retrospective	n=68 e=76	23.4% HS	30ml or 60ml	Clinical hernia- tion syndrome	х	Herniation reversal occurred 57/76
Suarez 1998	Retrospective	n=8 e=20	23.4% HS	300cc over 15-20min	One time		↓ICP by 50% ↑CPP

n=number of participants, e=number of IH events SOC=standard of care including mechanical ventilation for GCS<S, vasopressors to maintain cerebral perfusion, elevated head of bed, and sedation and analgesia

Table 3. Summary of Study Findings for MixedNeurological Injuries

SUBARACHNOID HEMORRHAGE (SAH)

Only two studies were found that evaluated the use of 23.4% HS in the SAH population (Table 4). Tseng et al. studies patients with SAH in two separate groups. One study was performed in patients with elevated

ICP33. In the other study the same concentration and dose was given to patients with stable ICP between 10-20mmHg. These studies used 23.4% NaCl boluses^{34, 35}. The goal of both studies by Tseng et al. was not focused on ICP reduction but on increased cerebral blood flow. By increasing cerebral blood flow, arteries may be "splinted" open during periods of vasospam thereby preventing ischemic events. In both studies, cerebral blood flow increased, with reduction in ICP, and an increase in CPP after administration of 23.4% HS was seen³⁶.

Principle author	Design	Sample	Osmotic agent	Amount administered	Frequency / Triggers	Soc	Results
Tseng 2007	Prospective	SAH n=10 e=17	23.4% HS	2ml/kg	↓CBF by TCD or Xenon CT		↑CPP ↑MCA velocity
Tseng 2007	Prospective	SAH n=35 e=50	23.4% HS	2ml/kg	↑ICP		↓ICP by 93% ↑CPP by 21%

n=number of participants, e=number of IH events NS=normal saline CBF=cerebral blood flow, TCD=transcranial Doppler, MCA=middle cerebral artery SOC=standard of care including mechanical ventilation for GCS<S, vasopressors to maintain cerebral perfusion, elevated head of bed, and sedation and analgesia

Table4.Summary ofStudyFindingsforNon - Traumatic Injuries

MONITORING

Repeated dose of 23.4% HS or treatment with 23.4% HS in patients with IH must be carried out after the monitoring of the following important criteria, at least for every 4 hours until the ICP reduction is recorded and thereafter every 6 hours.

- Serum sodium level 150-155 mEq/L
- If the serum sodium level >155 mEq/L, then check serum sodium level every 4 hours and further dose of 23.4% HS must be avoided until the sodium level stabilizes.
- Increase in serum sodium level should be generally

limited to 12-24 mEq/L over 24 hours to avoid central pontine myelinolysis.

- 23.4% HS must be avoided in patients with subacute or chronic hyponatremia (Na < 130 mEq/L) to avoid central pontine myelinolysis and osmotic demyelination syndrome.
- Other electrolytes level must also be monitored for preventing hypernatremia, hypokalemia, hyperchloremia and hyperosmolarity.
- Serum osmolarity 320-340 mOsm/L
- If the serum osmolarity >340 mOsm/L, then check serum osmolarity level every 4 hours and further dose of 23.4% HS must be avoided until the osmolarity level stabilizes.
- HS has a diuretic effect, hence volume status of patients should be carefully monitored to prevent intravascular volume depletion and rebound ICP elevation.
- ICP monitoring for patients must be done to assess and guide response to therapy, before the administration of 23.4% HS or immediately after it.

- Additional doses of 23.4% HS may be administered as needed on the basis of initial ICP response to therapy.
- Other monitoring criteria are vital signs, continuous pulse oximetry, daily weights.
- After the discontinuation of therapy, serum electrolyte level must be checked for every 12 hours for at least 24 hours to assess possible complications such as hypokalemia, hyperchloremia and metabolic acidosis.
- 23.4% HS should be avoided in patients with congestive heart failure, pulmonary edema and renal insufficiency.

RESEARCH IMPLICATIONS

The above studies provide a base for future research. Further research is needed to strengthen the evidence for a standard therapy. Research is needed in designing a standard therapy with 23.4% HS for patients with SAH with vasospasm. Studies are needed in which 23.4% HS is used as a treatment of cerebral edema prior to other treatments. Finally, studies that focus on the use of 23.4% HS in other than severe traumatic brain injury should be considered for future research. Further studies should also be done in assessing the potential adverse effects of using 23.4% HS as a treatment for IH.

CONCLUSION

23.4% HS offers promising results as a treatment for IH. Current studies and research seem to support its effectiveness in reducing ICP. 23.4% HS appears to be used as an initial treatment for elevated ICP or as a rescue therapy for refractory IH.

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Low Dose Methotrexate on Long Term Use In Rheumatoid Arthritis Leads to Liver Fibrosis - A Review

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ABSTRACT

Methotrexate the gold standard drug for chronic auto immune disease rheumatoid arthritis pose certain safety issues especially hepatic insult event if the dose used is much lesser than for its major indication cancer chemotherapy. Methotrexates' mechanism of action in Rheumatoid Arthritis is different from that in cancer chemotherapy and hence side effects like cytopenia are much less with low dose Methotrexate. Patients with risk factors like hyperlipidemia,ob esity,nonalcoholic fatty liver disease,alchoholism or concomitant administration of hepatotoxic drugs should be closely monitored for hepatotoxicity As with other hepatic insults routine liver function test which is recommended prior to initiation and during treatment is not always reliable in predicting hepatic fibrosis induced by this drug and demands liver biopsy in some cases. Liver biopsy being an invasive procedure may invite related morbidity and mortality. Newer non invasive tests to measure hepatic fibrosis like Fibro Test that combines the quantitative results of five serum biochemical markers Alpha 2 Macroglobulin, Haptoglobin, Apolipoprotein A1, Gamma Glutamyl Transpeptidase and total bilirubin with patient's age and gender should be considered as predictive markers of hepatic fibrosis induced by Methotrexate .

Key words : Methotrexate, hepatotoxicty, hepatic markers.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic auto immune disorder characterized by a chronic polyarticular synovial inflammation that may lead to irreversible joint damage with disability and deformity. Diseasemodifying anti-rheumatic drugs (DMARDs) in particular methotrexate(MTX) and corticosteroids together forms the conventional treatment for Rheumatoid Arthritis. MTX is recommended as a first-line drug by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) in the management of early and established RA.1 Weekly low dose MTX therapy is the most commonly used treatment option for rheumatic diseases.MTX acts primarily as an anti-inflammatory drug, specifically through the release of adenosine, rather than as an anti-metabolite drug as in cancer.² It is currently considered to be the most widely used DMARD.

MTX is widely used in dermatology as it is found to be very effective for the treatment of psoriasis (PsA). Though

the dosage used in rheumatology and dermatology is much lesser than that in oncology, toxicity has been a major issue which demands patient monitoring. There is a marked difference in the toxicity profile of MTX given weekly at a low dose in rheumatoid arthritis(7.5–25 mg) than that of the drug given at a high dose (100–1000 mg/m2 of body surface area per cycle), as in cancer chemotherapy.³

MTX toxicity remains one of the major reasons for discontinuation of treatment. The toxicity profile of Methotrexate has been well defined and patients are being monitored for gastrointestinal, hepatic and pulmonary toxicity, bone marrow suppression and stomatitis. Gastrointestinal toxicity and hepatotoxicity associated elevation of liver enzymes are the most frequent adverse events seen with Methotrexate. A rise in liver enzymes, in particular the Aspartate and Alanine transaminases, occurs frequently during MTX treatment. Hypersensitivity pneumonitis may occur rarely with Methotrexate therapy. Overall toxicity scores, including both hepatotoxicity and GI side effects, have been reduced by means of folic acid supplementation.^{3,4}

Hepatotoxicity is marked by an elevation of any one of the liver enzymes like Aspartate and Alanine transaminases (values >60) which may lead to treatment cessation. A large variety of histological liver lesions including dystrophic nuclei, macrovesicular steatosis, cell necrosis, cholestasis, cell hyperplasia, portal inflammation, liver fibrosis and even cirrhosis has been described and the severity of these lesions has been associated with the duration of treatment.²

Mechanism of Action and Kinetics of Methotrexate

MTX was developed to be a highly selective competitive Dihydrofolate Reductase enzyme inhibitor. MTX inhibits the enzyme dihydrofolate reductase, thereby depleting the pool of reduced folates, which act as donors of 1-carbon moieties in the formation of metabolic intermediates. including purines, deoxythymidylate monophosphate and methionine, and producing a state of effective folate deficiency. In cancer chemotherapy, at high doses, MTX acts as a cytotoxic drug by interfering with purine and pyrimidine synthesis in tissues with a high rate of cellular turnover. It has a hepatic metabolism and a renal excretion.MTX normally enters the cell through an active transport mechanism. Once inside the cell, the enzyme Folypolyglutamate synthase (FPGS) converts it into the polyglutamate form. The same process can be reversed by the enzyme folylpolyglutamate hydrolase. The polyglutamate form of MTX retains MTX within the cell for long periods, inhibits DHFR which mediates the conversion of dihydrofolate to tetrahydrofolate (THF)^{1,5,6}

Increase in the cyclic AMP levels leads to immunosuppression. MTX effects via adenosine induced immunosuppression. MTX typically blocks tetrahydrofolate dependent steps in cell metabolism which are involved in purine biosynthesis and hence several consequences can appear which result in adenosine overproduction. All in all Methotrexate exerts its action by inhibiting cytokine production and purine biosynthesis, and by stimulating the release of adenosine, all of which contributes to its anti-inflammatory properties.^{7,8}

Risk Factors for Hepatotoxicity

Alcohol consumption, Lack of folate supplementation, Obesity, Duration of therapy and Cumulative dose of MTX consumed, Presence of Diabetes Mellitus, and late age at first use of MTX were found to be the risk factors for hepatic fibrosis in patients with RA taking MTX. Risk for transaminase elevation was found in patients having lack of folate supplementation, untreated hyperlipidemia, and elevated BMI. In patients with hyperlipidemia and obesity, nonalcoholic fatty liver disease could be the underlying risk factor for transaminase elevation. Modifiable risk factors include Hyperlipidemia, Obesity, and lack of folate supplementation.⁹ Pre-treatment hepatic abnormalities, diabetes, obesity, current and previous heavy alcohol use etc are the risk factors associated with MTX hepatotoxicity in rheumatoid diseases. Non-alcoholic steatohepatitis (NASH) or nonalcoholic fatty liver disease (NAFLD) is very common and can lead to cirrhosis particularly when present with another hepatic insult such as alcohol or MTX and can result in abnormal liver function tests (LFT) values.¹⁰

Guidelines for Monitoring Hepatotoxicity

In 1994, guidelines were published by the American College of Rheumatology for monitoring the development of hepatotoxicity related to MTX. These recommendations included measuring aspartate aminotransferase(AST), alanine aminotransferase (ALT) and albumin every 4–8 weeks. In addition, a complete blood count and serum creatinine concentration are evaluated at baseline and repeated at intervals by most rheumatologists. The guidelines have been revised and current follow-up recommendations consist of performing a liver biopsy after a cumulative dose of 1–1.5 g of MTX has been administered to low-risk patients, and close to initiation of therapy (within 2–4 months) in patients with risk factors.³

A study conducted by M Hoekstra et al indicated that the absence of folate supplementation and high BMI were the responsible reasons for hepatotoxicity leading to MTX withdrawal. Severe hepatotoxicity occurred in 36/137 (26%) of the placebo group, and 11/274 (4%) of the folate group. Hepatotoxicity in patients with a BMI of 20–25 were reported to be 16/190 (8%) and in patients with a BMI of 30–35 this occurred in 10/51(20%). The mean BMI in this study was 25 (range 20-40).⁴ Dermatology guidelines for MTX monitoring in psoriasis suggest that a baseline liver biopsy should be performed at 2-4 months if there are recognized risk factors for hepatic fibrosis and thereafter, at a cumulative dose of 1-1.5 g of MTX . Rheumatologists have developed guidelines separately by monitoring the LFT only in RA based on long-term safety data.

Supplementation of oral folic acid with MTX protects from liver function abnormalities to some extend and may have a protective effect against serious liver diseases.^{10, 11}

The histological features of MTX-associated hepatotoxicity are non-specific and resemble those of non-alcoholic steatohepatitis (NASH), which is a common form of liver disease; It is more frequently associated with diabetes and obesity with the propensity to develop into cirrhosis. A meta-analysis of studies of long - term MTX treatment in RA, psoriasis and Psoriatic Arthritis suggest that there may be three times greater risk of hepatic fibrosis in psoriatic disease. Major factors responsible for the apparent difference between liver fibrosis in psoriasis and rheumatoid disease include increased alcohol consumption in psoriasis, anti-inflammatory medications suppressing hepatic inflammation in RA and better availability of long-term safety data in RA. The liver biopsy therefore remains the gold standard for investigating suspected hepatic fibrosis. Reports from case studies having routine serial liver biopsies for psoriasis treated with long-term MTX have an incidence of hepatic fibrosis of 13-34% and cirrhosis of 0-20%.^{10,12} Another study by Roenigk HH Jr, Auerbach R on hepatotoxicity after long-term MTX therapy in patients with PsA found that the risk of developing cirrhosis may be as high as 25%. However, similar studies among RA patients by Phillips CA and Cera PJ reported substantially lower rates of liver cirrhosis of <2% as well as a low risk of mild liver fibrosis and abnormal liver function tests.^{13,14}

Different studies report different incidence of hepatic damage during MTX therapy. A study of 33 RA patients by Coleiro B, Mallia C showed a high rate of liver function test abnormalities in 57% of the patients. Though Liver biopsy has been associated with various risks and complications; the most reliable information about organ damage is obtained by performing this procedure. The combined sensitivity of AST, ALT and bilirubin for detecting an abnormal liver biopsy has been rated at 0.86, whereas the negative predictive value of these test results was estimated at 0.93. Both RA and psoriasis patients were found to have hepatic injury. Females were affected more often than males, regardless of primary disease or age. The risk for hepatic damage rises moderately in correlation to the cumulative dose of MTX.^{3, 15}

Among patients with psoriasis, daily MTX therapy resulted in the development of liver fibrosis and cirrhosis with increasing cumulative doses in up to 24% of patients. In addition to daily dosing, risk factors for liver toxicity include high alcohol consumption, obesity, and diabetes. Weekly dosing was found to be better tolerated than daily dosing. In the long-term treatment with MTX awareness of hepatotoxic side effects are necessary, although it has not been a substantial problem with weekly low-dose MTX in patients with RA. There are proposals to change the guidelines for monitoring liver function tests. A study conducted by WEINBLATT ME showed transient slight elevations in liver enzymes in up to 48% of patients and in 53% in a more recent study by VAN EDE AE, LAAN RF. Normalisation of these elevated levels occurred after dose reduction, a change in the concurrent NSAID therapy, folic acid supplementation or even with the unchanged continuation of treatment. Structural liver abnormalities were indicated by the frequent elevations of aminotransferases and were correlated significantly with liver biopsy grades. Galactose elimination capacity and the aminopyrine breath test declined significantly during MTX treatment over 3.8 years. A high prevalence of minor histologic changes in the liver of Rheumatoid Arthritis patients are seen, which may be classified as mild reactive hepatitis in one third of patients.^{16, 17, 18}

A small study of 14 patients by Suzuki Y et al suggested a reduction in liver function test abnormalities in patients with a sustained elevation of serum ALT (alanine transaminase) while taking MTX, in whom the administration of folic acid caused ALT to decrease in all patients within 3 months.¹⁹

Methotrexate should be stopped if there is a confirmed increase in ALT/AST greater than three times the upper limit of normal(ULN), but may be reinstituted at a lower dose following normalisation. The dose of methotrexate should be adjusted; If the ALT/AST

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levels are persistently elevated up to three times the upper limit of normal. In case of persistent elevated ALT/AST more than three times the upper limit of normal after discontinuation, then diagnostic procedures should be considered.²⁰ Leflunomide being a newer disease modifying anti rheumatic drug has shown to be a safe and effective therapy for the treatment of RA. Leflunomide is a prodrug that is actively converted in the GI tract and this active metabolite inhibits dihydroorotate dehydrogenase (DHODH) and denovo pyrimidine biosynthesis. Elevation of transaminases has been observed as the most common adverse event in patients who took both MTX and Leflunomide in combination.²¹

Fibro Test has been carried out by the Gastroenterology departments to discriminate between insignificant and significant fibrosis in order to avoid liver biopsy. Liver biopsy being regarded as the gold standard for the staging of liver disease, it has several limitations, including the sampling error resulting from heterogeneous distribution of pathological changes. It is only about 80% accurate in staging fibrosis and it might miss advanced fibrosis in 30% of patients. Taking into account the invasive nature of the liver biopsy with its morbidity and mortality rates and its sampling error, a number of non-invasive methods have been developed for the estimation of liver fibrosis and most of them are based on combinations of blood parameters or using transient elastography (TE). Transient Elastography measures the stiffness or ability of a tissue not to undergo deformation when mechanical stress is applied to it. TE in comparison with other non-invasive markers of fibrosis offers some advantages like the procedure being easy to perform, is relatively operator independent, is easy to learn, and provides immediate results.Two categories of non-invasive serum markers are in use to predict liver fibrosis: direct markers, which reflect extra cellular matrix turnover, and indirect markers, which reflect alteration in hepatic function. Indirect markers may predict liver fibrosis either as individual markers: serum alaninaminotransferase (ALT) levels, ALT/AST (aspartate aminotransferase) ratio, platelet count, prothrombin index or as a multicomponent indirect fibrosis test: APRI. Fibro Test is a non-invasive blood test that combines the quantitative results of five serum biochemical markers (Alpha 2 Macroglobulin, Haptoglobin, Apolipoprotein A1, Gamma Glutamyl Transpeptidase and total bilirubin) with the patient's age and gender. $^{22,\ 23,\ 24}$

Conclusion

MTX is relatively safe when used in lower doses for long-term, for the treatment of chronic inflammatory diseases and remains as the gold standard drug for Rheumatoid Arthritis. Hepatotoxicity may develop in a large percentage of patients who are treated with this drug and is marked by an elevation of any one of the liver enzymes like transaminases in most cases. Elevation of Liver enzymes alone cannot be used as a predictive factor for hepatotoxicity as it is variable with negative predictive value of 0.93. As liver biopsy is an invasive procedure and has been associated with higher risk, in order to diagnose hepatic fibrosis, non invasive measures such as Fibro tests and Fibro meter has to be considered in Rheumatoid arthritis patients on MTX. Female gender and cumulative MTX dosages were found to be the predictive factors for the higher risk of liver failure. Oral folic acid supplementation with MTX seems to provide protection against gastrointestinal side effects and LFT abnormalities to some extent

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Protective Effect of *Uvaria narum* (B.L.) Leaves on Paracetamol Intoxication in Rats.

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ABSTRACT

Oral pre-treatment with ethanolic extract of the leaves of *Uvaria narum* (200 and 400 mg/kg, p.o.) showed significant dose dependent hepatoprotective activity against paracetamol induced hepatotoxicity by decreasing the activities of serum marker enzymes, bilirubin, triglycerides cholesterol, urea and increase in total protein, albumin & glucose. Paracetamol treatment depleted stores of antioxidant parameters like GSH, SOD & CAT and enhanced LPO, the condition was reversed in extract treated animals. Further hepatoprotective activity was supported by histopathological examinations. All the results were comparable to reference std Silymarin (100mg/kg). Data also showed that *U.narum* leaves extract possessed antioxidant activity, which may probably lead to the promising hepatoprotective activity of plant leaves.

Key words : serum marker enzymes, antioxidant, hepatoprotective activity, paracetamol

INTRODUCTION

Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These radicals, which react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer.^[1] A major defence mechanism is the antioxidant enzymes, antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by the interruption of ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules.^[2]

Liver is the vital organ responsible for drug metabolism and appears to be a sensitive target site for substances modulating biotransformation.^[3] Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation & other oxidative stress in the liver.^[4] Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders.^[5] Medicinal plants used for the hepatoprotection, in traditional medicine have been shown to possess promising hepatoprotective activities in animal models. This reveals that evaluation of herbal drugs is still needed. However there is dearth of information on leaves extract being use form the plants for liver diseases, therefore the baseline information on the plant helped us to investigate hepatoprotective activity against Paracetamol.

MATERIALS & METHODS

Plant material

The fresh leaves of *U.narum* Blume used for the present studies were collected from local areas of Mangalore. It was authenticated by Mr. Dinesh Nayak Advisor (Green belt), Mangalore SEZ Limited. The leaves were shade dried, pulverized into coarse powder and were extracted using ethanol as a solvent by using Soxhlet apparatus, until colourless solvent appeared in siphon tube. Further extract was dried & kept in desiccator for further study.

ANIMALS

The study was carried out on either sex of Wistar rats (150-200g). The rats were procured from Srinivas College of pharmacy. Rats were fed with a standard pellet and water ad libitum and were kept in standard environmental conditions (temperature 25-28°C and 12h light/12h dark cycle). The Institutional Animal Ethics Committee approved the Experimental protocol.

DRUGS & CHEMICALS

All chemicals and solvents used in the study were of analytical grade. Paracetamol (Yarrow chem, Mumbai) and other chemicals like Nitroblue tetrazolium. Phenazine methasulphate. NADH. Thiobarbituric (Himedia suppliers) & all estimation kits were obtained from Agapee distributers.

ACUTE TOXICITY STUDIES

The acute oral toxicity study of was performed as per the OECD guideline No. 425. Limit test was performed at dose 2000 mg/kg. Animals were observed after dosing individually at least once during the 30 minutes for 4 hrs, periodically during the first 48 hours and daily there after for 14 days for signs of toxicity and mortality, if any.^[6]

PARACETAMOL INDUCED HEPATOTOXICITY

Treatment protocol

Wistar rats of either sex weighing between 150-200g were divided into five groups of six animals each. For the first nine days of study Group I & II were fed with normal feed & water. Group III animals were treated with Silymarin 100mg/kg and group IV & V were treated with U.narum leaf extract (UNLE) 200mg/kg and 400mg/kg (extract was suspended in 1% Gum tragacanth) respectively for 9 days. All the treatment was done post orally. On 9th day, all the animals except Group I were intoxicated by the administration of Paracetamol (PCM) (1g/kg in 40% sucrose solution p.o.). After 48hrs of intoxication by paracetamol administration, blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Animals were sacrificed using ether anesthesia and liver was dissected out and used for histopathological studies.^[7]

ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY

Biochemical parameters & tissue antioxidants

The collected blood was used for estimation of serum biochemical parameters like SGOT, SGPT, ALP, total (BILT) & direct bilirubin (BILD), total proteins(TOT albumin(ALB), total cholesterol(CHO), triglycerides(TG), urea & glucose contents were

estimated by using commercially available reagents kits (AGAPPE) according to manufactures instruction. Liver tissue was estimated for Lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT) & superoxide dismutase (SOD) were assayed according the methods described by previous workers and were expressed in Absorbance & % increase or decrease.^{[8],} [9], [10], [11]

Relative organ weight analysis

PRO).

On the 9th day the animals were sacrificed, liver, spleen, left lung, heart, Kidney were removed, washed with ice cold saline and were immediately weighed and liver volume was also measured ,all the weights of organs were expressed as g/100g B.W.^[12]

Histopathological studies

For histopathological study, the fresh liver tissues were collected and immediately fixed in 10% formalin followed by dehydration in ethanol (50-100% v/v), cleared in xylene and embedded in paraffin. Sections (4-5 µm) were prepared and then stained with hematoxylin-eosin dve for photo microscopic observations.

STATISTICAL ANALYSIS

All the results are expressed as Mean ± SEM, the results were analysed for statistical significance by oneway ANOVA followed by Dunnett's test. P<0.05 was considered as statistical significant. Graph pad prism 5, software was used for statistics.

RESULTS AND DISCUSSION

Acute toxicity studies revealed that the UNLE was safe at a dose of 2000mg/kg hence one tenth (200mg/kg) of maximum & one fifth (400mg/kg) of maximum was selected for present study.

Paracetamol is a widely used as antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses.[14] Bioactivation of paracetamol by hepatic cytochrome P-450 leads to formation of a highly reactive and toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is normally detoxified by conjugation with glutathione (GSH) to form mercaptopuric acid is excreted in urine. Toxic overdose of PCM depletes hepatic reduced GSH content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then causes acylation or oxidation of cytosolic & membrane proteins & generation of reactive oxygen species. This leads to further oxidation of protein thiols, lipid peroxidation and DNA fragmentation.^[15]

Elevated levels of serum biomarkers like SGPT, SGOT and ALP which indicates cellular leakage & loss of functional integrity of hepatic cell membranes implying hepatocellular damage. Functional status is revealed by decrease in serum proteins, elevation in urea & bilirubin levels.^[16] Elevated levels of Cholesterol & triglycerides are due to impaired lipid metabolism due to hepatic damage, where was observed in experimental animals intoxicated with paracetamol. Experimental results revealed that prophylactic treatment of animals with UNLE - 200 & UNLE - 400 showed significant dose dependent decrease in the elevated serum biomarkers, bilirubin, urea and lipid contents and elevated serum Total proteins, glucose and albumin levels which were comparable to the standard. (Table No : 1 & 2) The extracts also ameliorate the harmful effect of PCM on organ weights of Rats (Table No : 3).

Table 1 : Effect of Silymarin and UNLE on Serum SGPT, SGOT, ALP, BILT and BILD inPCM induced liver toxicity.

Groups	Treatment	SGPT (U/l)	SGOT (U/l)	ALP (U/l)	BILT (mg/dl)	BILD (mg/dl)
N. e. marca 1		72.07	127.7	384.7	0.42	0.12
Normal	Saline	±	±	±	±	±
control		4.28	4.70	27.03	0.03	0.01
т ·	DCM	142.5	165.3	827.7	1.19	0.35
I OXIC	PCM	±	±	±	±	±
control	1g/kg	18.23	7.05	72.58	0.16	0.04
	Silymarin 100mg/kg	88.98	123.8	524.3	0.47	0.10
Std		±	±	±	±	±
		6.88**	8.50**	75.58**	0.07***	0.01***
т		98.44	132.2	541.8	0.52	0.17
Low	UNLE 200 /	±	±	±	±	±
dose	200mg/kg	4.14*	8.56*	34.71**	0.06***	0.05**
Iliah		91.14	126.7	535.3	0.49	0.12
High	UNLE 100ma /las	±	±	±	±	±
aose	400mg/kg	5.98**	7.14**	34.48**	0.06***	0.03***
All the values	are Mean ± SEN	1, n=6 ns-p>0.05 Dunnett's te	5, *p<0.05, **p<0 st compared to t).01, ***p<0.001 oxic control.	, One way ANO	VA followed by

Croups	Treatmont	TOT pro	ALB	TG	СНО	Urea	Glucose
Groups	ITeatment	(g/dl)	(g/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
Normal		8.10	4.36	95.33	96.33	37.50	129.7
Normal	Saline	±a	±	±	±	±	±
Control		0.22	0.36	4.06	5.00	2.11	13.96
T	DCM	4.66	2.50	171.8	190.5	67.50	91.17
loxic	PCM	±	±	±	±	±	±
control	Ig/Kg	0.38	0.25	6.76	10.57	4.51	3.89
	0.1	7.85	3.98	119.8	87.13	34.17	153.8
Std	Silymarin 100mg/kg	±	±	±	±	±	±
		0.28***	0.23**	3.40***	2.42***	1.06***	7.61***
T		7.56	3.28	147.0	99.00	38.50	127.8
Low	UNLE 200ma /las	±	±	±	±	±	±
aose	200mg/kg	0.27***	0.20 ^{ns}	5.77*	3.58***	2.47***	10.8*
		7.75	3.68	139.3	92.13	36.33	145.1
High dose	UNLE	±	±	±	±	±	±
	400mg/kg	0.28***	0.30*	8.73**	1.19***	2.57***	10.5***
	ll the velues or	o Moon + SEN	$I_n = 6 n \epsilon n > 0$	05 *n<0.05 *:	*n<0.01 ***n	-0.001 One w	

Table 2 : Effect of Silymarin and UNLE on Serum TOT Protein, ALB, TG, CHO,Urea, and Glucose in PCM induced liver toxicity.

All the values are Mean ± SEM, n=6 ns p>0.05, *p<0.05, **p<0.01, ***p<0.001, One way ANOVA followed by Dunnett's test compared to Toxic control.

Table 3 •	Effect of Silvm	arin and UNI	E on Relative	organ weight	t (σ/100σ R	W) in PC	M induced live	r toxicity
Table 5.	Effect of Shym		L'UII KCIAUV	organ weight	l (g/ I vug D	• • • • • • • • • • • • • • • • • • •	vi muuccu nve	i toxicity.

Groups	Treatment	Liver wt	Liver vol	Spleen	Kidnev wt	Left	Heart wt	
				wt		Lung wt		
Normal		3.46	3.59	0.32	0.61	0.24	0.37	
Control	Saline	±	±	±	±	±	±	
Control		0.31	0.31	0.03	0.05	0.01	0.01	
Taxia	DCM	5.81	5.49	0.55	1.14	0.44	0.51	
		±	±	±	±	±	±	
control	1g/kg	0.55	0.42	0.04	0.13	0.03	0.03	
	Gilverania	3.25	3.28	0.36	0.63	0.27	0.36	
Std	Silymarin 100mg/kg	±	±	±	±	±	±	
		0.23**	0.24***	0.02***	0.09***	0.01***	0.01***	
		3.90	3.61	0.40	0.78	0.31	0.41	
Low dose	UNLE 200mme /lea	±	±	±	±	±	±	
	200mg/kg	0.31*	0.31**	0.02**	0.04*	0.01**	0.01*	
		3.55	3.36	0.38	0.71	0.30	0.38	
High dose	UNLE 100m a /la a	±	±	±	±	±	±	
	400mg/kg	0.25**	0.30***	0.03**	0.05**	0.01**	0.06***	
						0.001.0		

All the values are Mean ± SEM, n=6 ns p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunnett's test compared to Toxic control.

Enzymatic antioxidant plays an important role in elimination of free radicals (ROS). Lipid peroxidation has been postulated to the destructive process of liver injury due to acetaminophen administration. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. The non-enzymatic antioxidant, glutathione is one of the most abundant tripeptide present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate of glutathione peroxidase and GST.^[17] Tissue activities of superoxide dismutase (SOD) and catalase (CAT) are the most sensitive enzymatic index in liver injury caused by ROS and oxidative stress. SOD is one of the most abundant intracellular antioxidant enzymes present in all aerobic cells and it has an antitoxic effect against ROS. Cat is a haemoprotein it protects the cell form the accumulation of H202 by dismutating it to form H20 and 02.^[18]. Experimental results showed elevation in Lipid peroxidation & decrease in GSH, CAT & SOD in animals intoxicated with paracetamol. But condition was reverted in animals pretreated with Std Silymarin (100mg/kg) and UNLE - 200 and UNLE - 400 in dose dependent manner (**Table No: 4**).

Groups	Treatment	GSH Abs at 412nm	LPO Abs at 535 nm	SOD Abs at 560 nm	CAT Abs at 620 nm
Normal control	Saline	0.59 ± 0.04	0.03 ± 0.01	0.81 ± 0.04	0.58 ± 0.04
Toxic control	PCM 1g/kg	0.28 ± 0.04	0.22 ± 0.02	0.07 ± 0.01	0.20 ± 0.02
Std	Silymarin 100mg/kg	0.51 ± 0.02*** (+76.87)	$0.11 \\ \pm \\ 0.01^{***} \\ (-60.92)$	0.88 ± 0.02*** (+88.57)	0.38 ± 0.03*** (+86.49)
Low dose	UNLE 200mg/kg	$0.44 \\ \pm \\ 0.01^{**} \\ (+52.60)$	0.19 ± 0.03 ^{ns} (-29.67)	0.54 ± 0.06*** (+81.34)	$0.31 \\ \pm \\ 0.02* \\ (+54.51)$
High dose	UNLE 400mg/kg	$0.47 \\ \pm \\ 0.02^{**} \\ (+64.16)$	$0.16 \\ \pm \\ 0.02^{**} \\ (-40.54)$	$0.62 \\ \pm \\ 0.03^{***} \\ (+83.82)$	$0.33 \\ \pm \\ 0.02^{***} \\ (+65.09)$

All the values are in absorbance Mean \pm SEM, % increase (+) or decrease (-) shown in parentheses, n=6 ns -p>0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunnett's test compared to Toxic control. Our observation in the histological examination of hepatic tissues further validates the result of the biochemical studies. The mild to moderate necrosis of liver tissues of rats pretreated with UNLE-200 and

UNLE-400 followed by the administration of PCM 1g/ kg is suggestive of the hepatoprotective nature of the extract. This result was comparable to that administered with a known reference Std Silymarin. (Fig: 1)

Fig 1 : Haematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 40X



Hepatocytes of the normal control group showed a normal lobular architecture of the liver (I). In the Paracetamol treated group the liver section showed severe focal necrosis. (II) Silymarin pretreated group showed mild necrosis compared to paracetamol treated group. (III) Whereas UNLE-200 showed moderate necrosis (IV) & UNLE-400 treated rats showed mild to moderate necrosis the results were comparable to Silymarin(100mg/kg) treated animals.

CONCLUSION

Present investigation indicates that ethanolic extract of *U.narum* leaf exert significant dose dependent protection against paracetamol induced liver toxicity, normalizing biochemical & hepatic biomarkers in animals plausibly by modifying lipid peroxidation and endogenous antioxidants. The activity was comparable to standard silymarin. As both doses were effective in relative organ weight analysis, *U.narum* extract might possess organ protective activity against Paracetamol toxicity.

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Formulation and Evaluation of Midazolam Mucoadhesive Buccal Patches for Acute Seizures

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ABSTRACT

Midazolam is a Benzodiazepine(BZD) group of drug used for the treatment of acute seizures, moderate severe insomnia and amnesia.Because of poor bioavailability of Midazolam by oral route, there is a need to increase its bioavailability by formulating it into buccal dosage form. Hence, Midazolam is a suitable drug for buccal dosage form and may provide a bettertherapeutic profile than oral route. In the present research work,Midazolambuccal patches wereprepared by using hydrophilic and hydrophobic polymers. Buccal patches were characterized for parameters like physical appearance and surface texture, weight uniformity, folding endurance, swelling index, surface pH, drug content uniformity,drug–excipientsinteraction study and in vitro drug release study. All the patches were uniform and translucent, having goodstrengthand smooth surface. Folding endurance of all prepared patches was > 200. The result of swelling index was between the range of 9.99-64.41% and the surface pH was in the pHrangeof buccal region. The results of drug content were in the prescribed limit. In vitro release studies conducted for Midazolam loaded patches exhibited drug release in the range of 54.59-99.26 % in 24 hrs.FT-IR studies revealed that, there was no interaction between drug and excipients used.Release of Midazolam from all patches followed zero order kinetics and mechanism was diffusion rate limited. Finally it can be concluded that F1 was found to be the best formulation.

Keywords: MIDAZOLAM, CMC,SCMC,HPMC,HPC-L,HPC-M.

INTRODUCTION

The interest in novel route of drug administration occurs from their ability to enhance the bioavailability of the drugs impaired by narrow absorption window in the gastrointestinal tract. Drug delivery via the buccal route using bioadhesivedosage form offer such a novel route of drug administration. This route has been used successfully for the systemic delivery of number of drug candidates. Problems such as high first pass metabolism and drug degradation in the gastrointestinal tract can be circumvented by administering the drug through buccalroute.Moreoverbuccal drug delivery offers safe and easy method of drug utilization, because drug absorption can be promptly terminated in case of toxicity by removing buccal dosage form from buccal cavity. The buccal region offers an attractive route of administration for systemic drug delivery. The mucosa has a rich blood supply and provides rapid absorption for drugs than oral route. The oral route has been the preferred route of administration for many drugs. Pharmaceutical aspects

of mucoadhesion have been the subject of great interest during recent years because it provides the possibility of avoiding either destruction by gastrointestinal contents or hepatic first-pass inactivation of drug. Various studies have been conducted on buccal delivery of drugs using mucoadhesive polymers. Attempts have been made to formulate various mucoadhesive devices including tablets,pill,patches, strips and gels.

The objective of the present work is to develop mucoadhesive patches of Midazolam using solvent casting technique, by various film formers such as CMC,SCMC,HPMC,HPC-L,HPC-M. The prepared patches will be evaluated for parameters related to buccal drug delivery system like weight uniformity, folding endurance, swelling index, surface pH, drug content estimation, in vitro release study and drug polymer interaction.

MATERIALS AND METHODS

Midazolam base was obtained as gift sample from Centaur Pharmaceuticals Pvt. Ltd. (Mumbai, India). HPC-L and HPC-M were obtained as gift sample from Arihant trade and Co. HPMC, CMC, SCMC and propylene glycol were provided from our laboratory.

Preparation of mucoadhesivebuccal patches

Buccal patches of Midazolam were prepared by solvent casting technique. The mucoadhesive patches were prepared using polymers like CMC,SCMC,HPMC, HPC-L,HPC-M. Propylene glycolwas used as plasticizer.

Buccal films of Midazolam were prepared by solvent casting method. 750mg of polymer was weighed and soaked with 5 ml of distilled water over night. 93mg of Midazolam was taken and dissolved by using 2ml of 0.1M hydrochloric acid. The drug solution was mixed with polymeric solution and an excess of 8 ml of distilled water and 2 drops of propylene glycol was added as plasticizer. The solution was mixed thoroughly using magnetic stirrer for one hour. The polymeric solution was poured in flat petridish and dried in hot air oven. After complete evaporation of solvent, the film was removed from the petridish and cut into 1cm2size.Composition of circular cast patches of various formulations are mentioned in Table - 1.

CODE INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
CMC (mg)	750	-	-	-	-	-	375	375	-	375	_	
SCMC (mg)	-	750	-	375	375	-	375	-	-	-	-	-
HPC-L (mg)	-	-	375	375	-	-	-	375	750	-	-	-
HPMC (mg)	-	-	375	-	375	750	-	-	-	375	-	375
HPC-M (mg)	-	-	-	-	-	-	-	-	-	-	750	375
PROPYLENE GLYCOL(ml)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
SOLVENT (ml)	15	15	15	15	15	15	15	15	15	15	15	15

Table 1.Composition of mucoadhesivebuccal patches.

CMC-Carboxy Methyl Cellulose SCMC-Sodium Carboxy Methyl Cellulose HPMC-Hydroxy Propyl Methyl Cellulose HPC-L-Low viscosity Hydroxy Propyl Cellulose HPC-M-Medium viscosity Hydroxy Propyl Cellulose

Uniformity of weight :

Five films of 1cm2from each formulation were taken and weighed individually on a digital balance. The results were analyzed for mean and standard deviation.

Drug content uniformity :

The films were tested for drug content uniformity by UV-Spectrophotometric method. Films of 1cm2diameter were cut from three different places from the casted films. Each film was placed in 100 ml volumetric flask and diluted with phosphate buffer pH 6.6. The absorbance of the solution was measured at 219 nm. The percentage drug content was determined using standard graph and the same procedure was repeated for three films of each formulation.

Percentage moisture absorbance :

Percentage moisture absorbance

The percentage moisture absorbance test is carried out to check the physical stability of the buccal film. Three 1cm2 films were cut out and weighed accurately and then placed in desiccators containing saturated solution of sodium chloride. After three days the films were removed and weighed. The percentage moisture absorbance was calculated and the results were analyzed for mean and standard deviation.

(Final weight-Initial weight) ×100

Initial weight

Percentage moisture loss :

The Percentage moisture loss is used to check the integrity of the buccal film in dry condition. Three 1cm2 films were cut out and weighed accurately and then placed in desiccators containing fused anhydrous calcium chloride. After three days the films were removed and weighed.

Percentage moisture absorbance was calculated and the results were analyzed for mean and standard deviation.

Percentage moisture loss =
$$\frac{\text{(Initial weight-Final weight)} \times 100}{\text{Initial weight}}$$

Swelling index of films :

The pre weighed (w1) three 1cm2 films of each formulation were placed in Petri dish containing 2% agar gel. After one hour interval, (up to3 hrs.) the patches were removed and excess water on their surface was carefully removed using filter paper. The swollen patches were weighed (w2) accurately. The percentage Swelling index was calculated using the formula,

Percentage Swelling index = $(w2 - w1) / w1 \times 100$

Folding endurance :

The Folding endurance of the film was determined by repeatedly folding one film at the same place till it broke or folded up to 200 times manually, which was considered satisfactory to reveal good film properties. The number of times the films could be folded at the same place without breaking gives the value of folding endurance. The same procedure was repeated for other formulations.

Surface pH :

Three films of each formulation were kept in contact with 1ml of distilled water for 1hr at room temperature. The surface pH was measured using pH paper. The mean of the readings was recorded.

In - vitro diffusion study :

The diffusion study of the films was done by using openend tube. One end of the tube covered with cellophane membrane acts as a semi permeable membrane [the membrane was previously treated with glycerin and water mixture (1:4). The film was placed on the inner side of the tube and the tube was immersed in a donor compartment .The whole assembly was placed on a magnetic stirrer and periodically 5 ml of sample was withdrawn and same volume of fresh medium was replaced.The concentration of drug content was analyzed by using UV - Spectrophotometrically at 219 nm.

RESULTS AND DISCUSSION :

Mucoadhesive patches of Midazolam were prepared using mucoadhesive polymers like CMC, SCMC, HPMC, HPC - L, HPC - M. The drug delivery system was designed as a matrix. All the patches showed smooth surface and elegant texture. The physical characteristics of various patches are given in Table 2. The weight of 1cm 2 patch were in the range of 0.0518 to 0.0171g. Surface pH of patch was 6. The folding endurance was measured and found to be greater than 200. Fig 1 shows the results of percent swelling index. The drug content range from 93.11 to 99.24% of drug in the three different formulations. The percentage moisture absorbed range between 1.8331 and 3.681 % w/w. The percentage moisture loss range between 0.606 and 0.167% w/w. The swelling index range between 9.999 and 70.837 % w/w. Sodium carboxy methyl cellulose (SCMC), Carboxy methyl cellulose (CMC) and its other combined films shows high proportion of swelling. The order of swelling is F2 > F1 > F7 > F5 > F10 > F4 >F8 > F6 > F12 > F3 > F11 > F9. Cumulative percentage release is shown in Table 3. The IR spectra as shown in Fig 7 and Fig 8 indicates that the drug is compatible with the polymer.



Fig 1. Percentage - Swelling Index of Different Formulations.

Formulation code	Weight Uniformity (mg) ± SD, (n=3)	%Drug content ± SD, (n=3)	Surface pH (n=3) Folding Endurance (n=3)		% Swelling Index ± SD, (n=3)	%Moisture Absorbance ± SD, (n=3)	% Moisture Loss ± SD, (n=3)
F1	0.0161 ±0.000141	93.111±0.2842	6	>200	64.417 ±1.0639	1.1848±0.010	1.0378±0.288
F2	0.0159 ±0.000209	93.413±0.0191	6	>200	70.837 ±0.8390	2.489±0.0194	1.0537±0.306
F3	0.0166 ±0.000183	98.775±0.0249	6	>200	27.950 ±0.2523	3.420±0.1651	1.376±0.2911
F4	0.0158 ±0.000109	95.594±0.0436	6	>200	44.672 ±0.2529	3.1056±0.015	1.896±0.5199
F5	0.0168 ±0.000214	92.929±0.0211	6	>200	55.012 ±0.4156	3.629±0.0272	2.1947±0.304
F6	0.0163 ±0.000161	99.831±0.0441	6	>200	39.918 ±0.3173	2.454±0.0122	1.0164±0.287
F7	0.0169 ±0.00018	95.414±0.1101	6	>200	61.923 ±0.4949	3.642±0.0432	1.7754±0.491
F8	0.0168 ±0.00018	97.059±0.0669	6	>200	41.683 ±0.1035	3.6813±0.036	2.1793±0.255
F9	0.0165 ±0.00019	99.241±0.0441	6	>200	9.999 ±0.1749	2.4242±0.011	1.205±0.085
F10	0.0161 ±0.00027	95.790±0.5396	6	>200	49.796 ±0.1440	3.092±0.0241	1.4318±0.273
F11	0.0165 ±0.00016	98.776±0.0254	6	>200	11.894 ±0.2701	1.1331±0.015	0.606±0.0006
F12	0.0171 ±0.0001	98.185±0.1688	6	>200	31.835 ±0.2440	3.3566±0.234	1.6773±0.506

Table 2. Physical Evaluation of Mucoadhesive Buccal Patches of Midazolam .

Note : Values in parenthesis are standard deviation (±SD).

Table 3. Physical Evaluation of Mucoadhesive Buccal patches of Midazolam .

Time	Cumulative Percentage release											
(min)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
10	17.7	0.92	13.13	10.9	12.6	14.9	10.46	12	15.2	14.5	8.62	7.81
20	18.8	1.58	20.59	15.1	20.3	28	16.92	19.6	22.4	20.2	16.5	16.3
30	20.4	2.32	28.73	19.8	26.2	39.8	23.61	28	27.4	29.9	21.8	21.6
40	23.3	3.57	35.73	30.2	35.2	46	29.63	33.9	35.4	34.8	30.2	29.8
50	24.9	3.79	39.94	35	40.4	56.3	36.77	42.4	42.6	40.4	35.8	35.4
60	28.8	6.3	46.53	41	49	63.9	44.07	47.3	49.5	43.3	43.3	43.3
120	34.7	8.69	54.79	50	61.6	74.5	50.22	59.3	59.1	56.9	50.4	50.2
180	40.3	10.5	65.46	56.8	68.6	83.2	59.49	72.7	66.3	67.7	57.7	57.8
240	43.7	15.6	75.11	67.6	78.4	95	65.08	87.4	77.4	77.8	62.7	62.5
300	50.7	16.9	83.43	78	85.1	98.7	74.95	92.3	86.1	85.2	73.8	75.2
360	80.8	27.3	95.15	92.1	91.2	99.9	83.51	98.9	92.5	94.5	80.1	84.3
400	86.4	54.6	96.77	93.4	94.1	104	91.04	99.8	98.4	99.3	91.8	91.6



Fig2.Zero order Release kinetics



Fig3.First order Release kinetics



Fig 4.Higuchi kinetics



Fig 5.Korsmeyer-peppas equation

Table 4 : Regression coefficient value

Kinetic model	R value	Equation of straight line
Zero order release kinetic	0.9993	Y=2.948X+14.428
First order release kinetic	0.9062	Y=-0.031X+1.961
Higuchi release kinetic	0.9125	Y=16.551X+0.6363
Hixon Crowell release kinetic	0.8907	Y=3.977X+0.121
KorsemeyerPeppas release kinetic	0.9643	Y=0.4208X+1.2709



Fig 6.Hixson and Crowell erosion equation

The kinetic parameters revealed that data of formula F1 showed r2 values of 0.9993 which is close to 1, indicating that release of drug follows zero order kinetics and release is independent of concentration. The release kinetics are shown in Fig 2 - Fig 6 and the r2 values are furnished in Table 4.

COMPATIBILITY STUDIESOF DRUG AND POLYMERS :

TITLE : MIDAZOLAM BP



TITLE : CMC - Midazolam (F1)



CONCLUSION:

In vitro diffusion studies demonstrate the suitability of the developed formulation for the release of Midazolam. The results were analyzed using different release kinetics models. The formulation F1 follows zero order kinetics and it releases the drug in controlled manner. The kinetic parameters revealed that data of formula F1 showed r2 values of 0.9993 which is close to 1, indicating that release of drug follows zero order kinetics and release is independent of concentration. The IR spectra indicatesthat the drug is compatible with the polymer. Thus Midazolam can be convenientlyused for the formulation of buccal patch.

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Clinical Pharmacist's ward round Experience of Antimicrobial utilization: Need for a Sustainable Stewardship program for a University Tertiary Care Hospital

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ABSTRACT

Bacterial resistance to antimicrobials is increasingly threatening to be an adverse healthcare misgiving. It is reported that antimicrobial resistance is directly proportional to the consumption of antimicrobials in various countries. Antimicrobials prescribed in developing countries are reported to be in-appropriate to an extent of 44-97% in hospitalized patients which is deemed as unnecessary. The present study was conducted to identify areas for quality improvement and devise a strategy for the implementation of a management system in a tertiary care hospital. An unstructured survey questionnaire was designed with literature support to notify the existing practices and possible areas for quality improvement of antimicrobial management. The survey questions were administered to five practitioners of particular medicine department by a clinical pharmacist during ward rounds to elucidate the responses and to obtain the perception. Survey results could reveal the status of the antimicrobial management system and how it impacted routinely. Even though the hospital had an antimicrobial policy, its implementation was a concern and its adherence by physicians was unknown in routine practice. Hence, through practitioners'suggestions we could arrive at a consensus to strategize for improving the existing antimicrobialmanagementsystem.

Keywords : Antimicrobials, guidelines, policies, inappropriate, prescribing, stewardship, India

INTRODUCTION

Bacterial resistance to even the newest antimicrobials is increasingly threatening to be an adverse healthcare misgiving. Due to this fact, it is widely considered to be a global concern - the World Health Organization (WHO) had proclaimed this to be the central focus of World Health Day 2011.¹⁻³ While the use of antimicrobials is an important factor determining antibiotic-resistant bacteria,^{4,5} it is well established in medical practice that the large proportion of antimicrobials are prescribed without proper indication.⁶⁻¹¹ It is reported that antimicrobial resistance is directly proportional to the consumption of antimicrobials in various countries.12 Countries encountered with high prevalence of antimicrobial resistance, is unfortunately not restricted to the host country alone rather may spread across borders too. Thus, it can potentially snowball into an alarming public health disaster and could create a serious impact on other sectors as well.

Antimicrobials prescribed in developing countries are reported to be in-appropriate to an extent of 44-97% in hospitalized patients which is deemed unnecessary.13-17 Thisis attributed to factors like socio-economic and behavioral factors18 and the reasons contributing to this has been multifactorial which is termed complex and interconnected. Some of the them are redundantprescribing of antimicrobials, self - prescribing by the patients, lesser quality of available antimicrobials, poor infection control protocol and implementation, inefficient routine susceptibility testing and surveillance.¹⁹ In addition to this social-economic parameters like lack of funds together with low literacy rate, ignorance and no proper availability and accessibility to health and diagnostic facilities has been found to be an area of concern.²⁰

According to WHO, globallythe burden of infectious disease is found to be highest which translates to the fact that antimicrobials utilization is expected to play a defining role in preventing disease progression, complications and death across the spectrum of patients.²¹ Data retrieved from the database of 679 studies in 97 countries between 1990 and 2006, created by the WHO to study and understand the usage patterns in transitional and developing countries revealed that antimicrobials were inappropriately prescribed for upper respiratory and diarrhea spanning over a period of time.²²⁻²³

Indian data on antimicrobial utilization in various healthcare settings are scanty. One study which was done in out-patients pointed out that overprescribing and overuse were seen in all healthcare settings: public and private hospitals and community clinics and pharmacies. The studies reported that antimicrobials were prescribed often irrationally and inappropriately like incorrect dose, frequency, or duration, are redundant, and as having drug interaction potential with concomitant medications. Decoding the exact reasons for inappropriate prescriptions across Indiais similar to other parts of the world. Uncertainty in initial diagnoses, addressing the co-morbidities, physician's lack of training in treating the suspected or confirmed target infection, absence or lack of knowledge of local resistance or other related epidemiological data, incorrect interpretation of the microbiologicaldata.26

Empirical and definitive therapy is always a challenge where consequences of inappropriate prescribing areadverse health outcomes, rising healthcare costs,greater incidences of Clostridium difficile infection, healthcare associated infections following invasive procedures, catheter usage for intravenous administration of antimicrobials and other nosocomial infections all of which as lead to prolonged hospital stay and also the inevitable death.²⁷⁻³⁴ In addition to these, inappropriate antimicrobial usealso have known to result in therapeutic failure associated with narrow coverage while super-infection and multi-drug resistance organisms are a major concern with broad spectrum coverage.35

There is a need to understand antimicrobial use and issues associated with it from institutional perspectiveas policies and situations vary from institution to institution. The present work was planned to study the antimicrobial management system in a tertiary care teaching hospital in terms of antimicrobial management, infection control committee, antimicrobial policy and related issues in a tertiary care teaching hospital.

Methodology

The study was a prospective observational study, where the situational analysis was performed by a clinical pharmacist during the course of 4 months during daily rounds with the medical team. Survey of the antibiotic usage in various wards and Intensive care unit (ICU) of the hospital was performed. Assessment of practitioner'sopinion on antimicrobial management was performed with the help of a questionnaire to identify the existing practices and possible areas for quality improvement. The questions were chosen and was antimicrobial management was prepared based on the available literature.³⁶ Based on Infectious Disease Society of America guidelines for implementation of an antimicrobial stewardship program and the roles of the stewardship team and with essentials were used to formulate specific for our institution ³⁷ and with the past experience in a tertiary care oncology hospital.³⁸ The survey questionnaire covered the areas likecurrent scenario and issuesfaced in antimicrobial usagebased on worldwide practice specifically focusing on he status existing in Indiawere performed. The survey questions were administered to five practitioners of medicine department and administrators to elucidate the responses and the responses were summarized to get an overall picture.

Results

The present study could find areas where an effective intervention was desired which to yield better outcomes in antimicrobial utilization. The survey of clinicians to identify the aspects influencing antimicrobials use in

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Questions	Responses		
1. Does your hospital have a local multidisciplinary Drugs and Therapeutics Committee (DTC)?	Yes		

2. Does your hospital have a multidisciplinary Local Antimicrobial Committee? If yes, how often does your antimicrobial committee meet per year?	Yes/Every quarter		
3. Has the hospital carried out training/educational program of healthcare staff on ABC (antibiotic consumption)? If yes, how often these educational pro grams take place?	No		
 4. Does the hospital have one or several antibiotic specialist consultants? If yes, who is (are) this (these) antibiotic specialist consultant(s)? Medical microbiologist Other physicians 	Yes/ Medical microbiologist/ Professors and Associate Professors in General Medicine		
5. Does the hospital have a written antibiotic formulary including a list of restricted use antibiotics? (Antibiotic formulary ¼ a list of antibiotic routine ly stocked in the hospital; restricted antibiotic ¼ an antibiotic that cannot be prescribed without an additional authorization) How often is the antibiotic formulary published?	Yes/First list published/there are no preauthorization rules.		
6. Is there a system for the controlled dispensation of some antibiotics in your hospital? Which antibiotics are concerned with the controlled dispensation?	No		
 7. Which are the prescription characteristics for these antibiotics ? a. The duration of the delivery is limited in the time b. The duration of treatment has to be provided c. Detailed dosage has to be provided 	ANoBNoCNo		
 8. Which are the dispensation conditions for these antibiotics in first intention? i. The dispensation of certain antibiotics is limited to certain indications ii. A request/the agreement of an antibiotic specialist for these antibiotics is necessary iii. A written justification, based on clinical or microbiological evidence, is necessary 	i No ii No iii No		
9. Does the hospital have implemented a systematic reassessment of antibiotic treatments after 72 h?	No		
10. Does the hospital have computerized antibiotic prescribing?	No		
11. Does the hospital have computerized antibiotic dispensing?	No		
12. Does the hospital have a standard prescription protocol on the use of antibiotics by clinical diagnosis?	No		
13. Does the hospital have a written guideline on good ABC?	No		

thehospital was conducted. The questionnaire used for the interview of clinicians provided details of practitioners' responses. The responses are summarized in the table.1.

Survey results threw light on the antimicrobial policy and its impact in routine practice. Like in many other hospitals the antimicrobial policy was prepared by a group of members representing the hospital infection control committee. The institution antimicrobial policy is uploaded into the hospital intranet in a portable document format for physicians' and surgeons' online access. Data presented to the physicians were limited to antimicrobials and their choices in sepsis. The contents of the policy were antimicrobials to be indicated for various infections and other septic conditions with first choice and alternative choices. The policy also consists of the likely organisms for the suspected infectious conditions.

At the end of survey, physicians were requested to give various suggestions based on their perception on the issues, which will improve the antimicrobial management. The suggestions and the justifications are summarized in Table.2.

S. No	Suggestions	Justification
1	To conduct a study on the utilization pat- terns of antimicrobials.	To understand the physicians prescription practices of anti- microbials under various circumstances and settings within the hospital. To provide the adherence patterns to the institutionguideline.
2	To study the attitude towards following the antibiotic policy.	The possible factors which is inherent towards conformance to the set guidelines.
3	To perform a questionnaire based study with the physicians and surgeons on the factors that influence.	Influences that dominate antimicrobial prescribing.
4	Physician's perception towards antimicro- bials prescribing.	Factors contributing to its choices and outcome beliefs.
5	Feedback about the contents of the policy.	To device an effective tool aiming to achieve short-term and long-term goals.
6	The strategies to be implemented to opti- mize antimicrobial usage.	An ideal institution specific approach in resolving the prob- lems and overcoming the challenges faced.

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Discussion

Antimicrobial policy forms the key structure of antimicrobial management in any health care system. In the present study setting the policy was available at the institutional intranet but the clinicians use it less often than necessary pointing the need for improving the accessibility and acceptability to the practitioners.

The policy though available seemed less frequently accessed as the format available was too passive. Voluntaryphysician adherence to guidelines invariably is not known to be appreciable as evident from studies reported earlier which obviously warrants better active strategies in order to achieve the target goals. ^{39, 40} Due to the lack of a dedicated antimicrobial stewardship team updating of the guideline were not feasible frequently.There wasthe need for auditing andto detect adherence to the guidelines which was due to a lack of a stewardship team or its activities. Once the guideline is made available, physician orientation and other healthcare professionals on the different aspects of antimicrobialmanagement by an infectious disease specialist or a clinical pharmacist followed by antimicrobial order reviews, getting feedbacks from the prescribers have shown to be effective ways of containing inappropriate usage. ^{41,42} Except for the last edition of the institutional guidelines (prepared January 2013), the previous issues were not containing the list of restricted antimicrobials and that may require pre-authorization. Formulary restriction is one of methods in controlling physicians from freely prescribing those antimicrobials which might be needed only after expert consultation (infectious disease physician/microbiologist) or criterion-based. The goal of a restricted formulary may be institution cost containment on pharmacy budgets, to reduce in over-prescribing of reserved class or broad spectrum antimicrobials.^{43, 44}

A number of studies have shown the value of clinical pharmacists in optimizing the rational use of medicines within hospitals.⁴⁵ They are considered to be the key for a successful antimicrobial stewardship program⁴⁶ because of their expertise in reviewing medication orders and their pivotal role in the institutional formulary development. Apart from this they can also render their services towardsoptimizing the use of antimicrobials by early switching of intravenous to oral agents and close liaison with the microbiology.41The role of clinical pharmacist as part of antimicrobial stewardship is well recognized by national and international healthcare accreditation bodies like the Joint Commission of the United States and National Board of Accreditation of Hospitals in India. The information on antimicrobial, microbiology and other patient related factors were not available to the physician at the time of delivering patient care or disease management. This could be possibly achievedby expert computer systems which have been reported to deliver better patient outcomes.^{47, 48}

The study institution, a tertiary-care teaching hospital; standard protocols are in place for infection-control which also occupies the antibiotic management program. Due to various factors and time constraints of teaching faculties and resident staffs a self-sustaining antimicrobials stewardship and management program could not be effectively implemented. Simultaneous implementation of various antimicrobial stewardship strategies has been reported to yield good outcomes but at the same time can be difficult to measure the efficacy of each strategy and the single most efficacious one.⁴⁹The study highlighted the need an antimicrobial management program practice and its management with an effective, cost-effective and a self-sustaining stewardship strategy as it can impact the clinical, microbiological, economic and other possible ecological aspects which could translate to be animportant contributing factor towards providing a quality healthcare ecosystem.⁵⁰

Conclusion

Significant obstacles which threaten to confront stewardship goals and its implementation are a concern even in advanced countries. From our initial situational analysis by obtaining physician'sinclinations towards implementing such evidenced antimicrobial management programs were appreciative. It is noteworthy to mention and from a clinical pharmacists' observations through our observational study that antimicrobial stewardship activities with a dedicated team islike an unborn child waiting to be born under a watchful eyes of an obstetrician than saying is still in infancy in adeveloping country like India. The study identified and assessed the current scenario and could notify the components of existing antimicrobial management system. The results of our study havehighlighted the need for the administrators and other stakeholders todevelop and formulate a comprehensive antimicrobial management system for the hospital.

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The Effect of an Active Ward Round Participation of Clinical Pharmacist in Cardiac Care - A Prospective Study

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ABSTRACT

Drug Related Problems (DRP) arises at all stages of the medication process from prescription to follow-up of treatment and most problems are centered on prescribing, administration, dispensing and the patient's use of a medicinal product. Intervention is defined as an action by a clinical pharmacist, which results in a change in the patients' therapeutic management. Pharmacists by working closely with the medical team can facilitate improved prescribing and management of medicines. To assess the contribution pharmacists make to patient care by determining the clinical outcomes associated with pharmacist interventions. A 2-month prospective randomized intervention study was conducted at Multi specialty hospital. During the study, clinical pharmacists documented intervention episodes, patient demographical details, and the drug(s) involved. Clinical outcomes resulting from pharmacist-initiated changes to drug therapy and patient management were assessed. Interventions were made on Incorrect Dose, Incorrect Duration , Incorrect Frequency , Side effects , Adverse drug reactions (ADR'S), Interactions, Drug therapy omission, Self administering medications inappropriately , Stopped medicine. Out of total interventions made, around 82.25% of interventions were accepted by the physicians and necessary alterations were made on the prescription. Clinical pharmacists are in an optimal position to provide effective interventions aimed at decreasing risk factors for cardiovascular disease. Hence a clinical pharmacist service during inpatient care may improve quality of prescribing and patients Health Related Quality Of Life

Keywords : Intervention, Administration, Interaction, Clinical pharmacist

INTRODUCTION

Drug-related problems (DRPs) can be defined as any event or circumstance involving the drug treatment, which interferes or potentially interferes with the patient, achieving an optimum outcome of medical care. Drug related problems may result in reduced quality of life, morbidity and mortality and are even frequent¹.

Drug-related problems include medication errors (an error in the process of prescribing, dispensing, or administering a drug, whether there are adverse consequences or not) and adverse drug reactions (any response to a drug which is noxious and unintended; occurs at doses normally used in humans for prophylaxis, diagnosis or therapy of disease or for modification of physiological function)². Furthermore, adverse drug events can be defined as an injury whether or not causally related to the use of a drug³. These problems arises at all stages of the medication process from prescription to follow-up of treatment and most problems are centered on administration, dispensing and the patient's use of a medicinal product. Lack of follow-up and reassessment of medical treatment is also a major problem. Also problems regarding prescription could entail serious consequences. Unnecessary drug expenses, uncomfortable symptoms, adverse drug reactions and a poorer state of health are other consequences for patient and society⁴.

The risk of patient for iatrogenic adverse drug events in hospitals is potentially increased by increased use of medication and availability of new drug therapies. Iatrogenic adverse events are considered important because it cannot only prolong hospital stay but also increase expenditure of patient health care. Therefore, it is important that all drug related problems are evaluated to assess whether improvement in the healthcare delivery system can reduce the like hood of similar events occurring in the future^{5, 6.}

Pharmaceutical services can reduce the number of adverse drug reactions and length of hospital stays; Drug-related morbidity and mortality are often preventable⁷. Thus participation of pharmacists at the stage of ordering and prescribing in ward rounds where all types of drug related problems should be discussed. Therefore participation and intervention of clinical pharmacists in health care positively influence clinical practice ⁸.

Intervention is defined as an action by a clinical pharmacist, which results in a change in the patients' therapeutic management. Though significant change has been seen in pharmacy practice in recent years; it continues to evolve towards the provision of better pharmaceutical care⁹. Pharmacists by working closely with the medical team can facilitate improved prescribing and management of medicines. This model provides a safer system, better resource utilization and improvements in pharmaceutical care. Therefore clinical pharmacist's intervention has a positive impact on reducing drug related errors in overall patient care¹⁰.

Clinical pharmacists provide comprehensive drug management to patients and providers (includes physicians and additional members of the health care team) and are uniquely trained in therapeutics. Outcomes of pharmacist intervention include health related quality of life, patient satisfaction, medication appropriateness and adverse drug reactions. The role of clinical pharmacist in the care of hospitalized patients has evolved over time, with emphasis on patient interaction and collaborative care. The addition of clinical pharmacist services in the care of inpatients results in improved care, with no evidence of harm.

The core business of clinical pharmacists is safe and effective medicine use. Comprehensive and accountable clinical pharmacy services are an essential component of contemporary healthcare practice with the focus on individual patients. By working to ensure that medicine therapy is optimum, safe and cost-effective, the provision of clinical pharmacy services serves the interests of individual patients and also the wider community¹¹.

MATERIALS AND METHODS

The study was conducted from February 2010-Aptil 2010, in a 900 bedded multi- specialty Hospital. It was a Prospective Randomized. Observational & Interventional study which was conducted in cardiology department. The aim of the study was to identify drug related problem, Medication use, enhance continuity of care and encourage patients to avail themselves of healthcare services to prevent future adverse outcomes by patient counseling. After getting ethical committee approval for this study the subject were included as per the following inclusion and exclusion criteria. Eligible patients were - Cardiology Inpatient's, both male and female, Age group > 30 .We excluded Patients who has undergone Pacemaker surgery or Heart transplantation, Pregnant women and the observations has been obtained from Patient's medical records, Medication charts, Patient's past history records, laboratory investigations and diagnosis reports. Intervention's has been analyzed from the patients Dose, duration, frequency, side effects, Adverse drug reactions, Interactions, Adherence, Drug omission, Drug addition, Medication errors. The intent of intervention is to optimize the drug related problems.

The males are the age group of 41-50 yrs are having cardiac problem compare to other group age groups. 54.54% of study population were alchololic .Excessive alcohol consumption has long been associated with cardiovascular disorders, including cardiomyopathy, hypertension, coronary artery disease, and stroke. In our study population 81.81% were non vegetarian which showing that non vegetarian have a 32% lower risk of hospitalization or death from cardiovascular disease than people who consume meat¹². In this study population we observed diabetes and Hypertension both may contribute to cardiovascular disease.

RESULTS

Table.1 : Demographic detail of the study populations

Gender	Number (n)	Percentage
Male	72	63.63%
Female	41	36.37%
Age Group /yrs	Number	Percentage
31-40	21	18.18%
41- 50	51	45.45%
51-60	10	9.10%
61-70	31	27.27%
Life style habits	Number	Percentage
Smokers	33	29.2%
Non smokers	80	80.8%
Alcoholics	62	54.54%
Dietary habits	Number	Percentage
Vegetarians	21	18.19%
Non vegetarians.	92	81.81%
Co morbidities	Number	Percentage
Diabetes mellitus	61	54.54%
Hypertension	51	45.45%
Asthma	10	9.10%
Urinary Tract Infection	21	18.18%

Interventions

Medical errors

Various medical errors related to Prescribing, Adherence and Monitoring were analyzed in patients from Cardiology ward which has been shown in the table : 3

Medical errors	Error	Intervention		
Prescribing errors				
Incorrect Dose Ramipril	2.5 mg dose alone was given for 6 days.	Ramipril 2.5 mg dose twice daily has to be administered for 2 days then the dose has to be increased to 5mg.		
Monitoring				
Insufficient Lab Ordering	Aspirin may cause prolonged bleeding time	Prothrombin time should be monitored routinely.		
-	Gemer was found to produce leucopenia, Agranulocytosis Thrombocytopenia, Aplastic anemia.	Complete blood count should be monitored.		
	Diabetes Mellitus Patient	Patient has diabetes mellitus but blood glucose level was not monitored frequently. Suggested for monitoring blood glucose.ess of clinical		

Table. 3 : Various Medical Errors in Cardiology Ward

Prescribing error	Percentage		
Incorrect dose	27.27		
Incorrect duration	6.2		
Drug - drug interaction	80.2		
Drug - food interaction	19.8		
Treatment duplication	2.65		
Contra indication	0.88		
Non Adherence	Percentage		
Stopped medicine inappropriately	9.09		
Monitoring	Percentage		
Insufficient lab ordering	36.36		
Inadequate lab follow up	18.18		

Table. 4 : Percentage of patients with Medicalerrors in cardiology ward

Drug interactions

Drug interactions are a major source of clinical problems. It is estimated that 6-30% drug interaction leads to adverse drug reactions. The drug interactions alter the pharmacokinetic and pharmacodynamic properties of the drug which will affect the better outcome. A large number of studies needed to assess the possibility of drug interactions. Here we analyzed the most common drug classes involved in interactions were antiplatelets and anticoagulants. Aspirin were the most common drug responsible for drug-drug interaction. These DDIs were classified as Severe (13.2%) followed by moderate (32%) and mild case (54.8%).Interactions which are clinically not significant were neglected by physician.

Table	5	:	Percentage	of	intervention	and	outcome
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Type of Intervention	(%) Outcome		
Patient education	100		
Dose adjustment	45.45		
Discontinue medication	9.09		
Add medication	9.09		
Change prescription directions to persons Native language	45.45		
Others	9.09		

Drugs	Intervention
Recommended Alternative Treatment	
Atorvastatin	Atorvastatin 20mg was prescribed which will reduce LDL but patient has low HDL hence fibrates to be prescribed to improve the HDL level.
Dose Adjustment	
Aspirin Vs Ramipril	Aspirin reduces the effect of Ramipril when co-administered hence Aspirin dose is reduced.
Aspirin Vs Metoprolol	The dose of Metoprolol was increased from 25mg to 50mg since Aspirin slightly reduces the effect of Metoprolol.
Aspirin Vs Ramipril	The dose of Ramipril was increased from 2.5mg to 5mg since Aspirin reduces the effect of Ramipril.
Digoxin Vs Lasix	Dose of lasix was reduced from 40mg to 20mg since Digoxin has its toxicity increased by furosemide.
Digoxin Vs Amiodarone	Dose of Digoxin is reduced to half since Digoxin has its effect markedly increased by Amiodarone.
Discontinued Medications	
Aspirin Vs Enoxaparin	Aspirin severely interacts with Enoxaparin and the risk of bleeding is increased when co-administered. Hence Aspirin was stopped and Enoxaparin was continued.

 Table. 6 : Various Medical Errors in Cardiology Ward

From our observations given in Table.5 we found that we have provided 100% patient education in cardiology wards. Pharmacists are in an ideal position to provide patient education and optimize patient care. Greater understanding about the illness and a change of attitude and practice would in turn results in a better therapeutic outcome.

We have done dose adjustment with 45.45%. Although pharmacotherapy can be beneficial, it can also lead to drug - related problems (DRPs), including untreated indications, drug use without an indication, improper drug selection, sub therapeutic dosage, over dosage, medication error, medication non-adherence, drug interactions, adverse drug reactions, adverse drug withdrawal events, and therapeutic failure.

As a clinical pharmacist we intervened on discontinued medications which we found to be 9.09 %. We have added medications to prevent drug interactions and side effects which were found to be 9.09%. From our data we found 70% of the patients were illiterate and have come from rural areas hence most of the prescriptions have been changed to their native languages which is around 45.45%.

Physician Acceptance

Out of total interventions made, around 82.25% of interventions were accepted by the physicians and necessary alterations were made on the prescription. Acceptance of the pharmacist's recommendation was significantly associated with the strongest predictor of the patient achieving the desired clinical outcome. Pharmacist - Physician co - management is effective for better patient care.

DISCUSSIONS

Our study supports the concept of rational drug use for patients during their hospital stay.

This randomized prospective observational & interventional study demonstrated а high rate of beneficial outcomes achieved by pharmacist interventions. Participation of a clinical pharmacist in the daily multidisciplinary team rounds in our study settings significantly reduces unfavorable morbidities and enhances therapeutic outcomes.

A clinical pharmacist can promote optimal medication therapy in inpatients by working with physicians and other health care professionals. We identified large numbers of drug related problems and made clinically significant recommendations. Implementation of recommendations was predicted to improve the outcome of patient care. Clinical pharmacist interventions were effective in reducing the number of drug interactions per patient, the risk of discrepancies and medical errors.

These results demonstrate the high value of clinical pharmacist involvement in disease management and provide evidence that involvement of clinical pharmacists in therapeutic management results in better drug therapy selection that is more consistent with evidence based guidelines.

Additionally, this study demonstrates the need for changes in health care systems that prevent patients from being lost to long term follow-up. Clinical pharmacy services provided by pharmacist on an internal medicine ward contribute to rationalization of drug therapy and are therefore likely to increase medication safety. A clinical pharmacist service during inpatient care may improve quality of prescribing and patients' Health Related Quality Of Life (HRQOL).

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Cardioprotective Activity of *Pseudarthria Viscida(L.)* Wight & Arnott Roots In Isoproterenol Induced Myocardial Necrosis In Rats

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ABSTRACT

The Present study was performed to evaluate Cardioprotective activity of ethanolic extract of *Pseudarthria viscida* (*L.*) Wight & Arnott roots in Isoproterenol induced myocardial necrosis in rats. Animals were divided into different groups. Two doses 200 mg/Kg and 400 mg/Kg b.wp.o of the PVRE (Pseudarthria viscida roots extract) were subjected for the evaluation of Cardioprotective activity against Isoproterenol (ISO) induced myocardial necrosis in rats. Propranolol (10 mg/kg b.w p.o) was used as a standard drug. The influence of prophylactic treatment was analysed by quantification of biomarkers, antioxidants and histopathological observations. Both PVRE-200 and PVRE-400 showed significant reduction in CK-MB, CK-NAC, LDH in the extract treated rats when compared with positive control. Both extracts showed increase in SOD and CAT levels. Cardioprotective effect was also confirmed by Histopathology of Hearts which showed less necrosis in extract treated rats when compared to untreated rats of toxic control group. The results obtained were comparable with that of the standard. The present study concluded that *Pseudarthria viscida* (*L.*) Wight & Arnott. roots were found to be effective against Isoproterenol induced myocardial necrosis (ISO).

Keywords : Pseudarthria viscida (P. viscida), Isoproterenol, Propranolol, Cardioprotective

INTRODUCTION

The heart is an organ of paramount interest which is responsible for pumping oxygenated blood to the different parts of the body and collects the deoxygenated blood through blood vessels for purification by repeated, rhythmic contractions1. According to the findings of the National Vital Statistics Report (2008) and the Morbidity and Mortality Weekly Report (2007) of the Centers for Disease Control and Prevention (CDC), cardiovascular diseases including myocardial infarction (MI) and the resultant complications in cardiac function represent the leading cause of morbidity and mortality in developed countries2,3.Moreover, with advanced life style in developing countries, such as India, particularly in metropolitan cities, MI is making increasingly important contribution to mortality statistics4. Acute condition of myocardial necrosis occurs as a result of imbalance between coronary blood supply and myocardial demand5,6.Because of high incidence of morbidity, various drugs and regimes have been advocated for the control of CVS diseases. Recently attention has been

focused towards herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases such as Terminalia arjuna (arjun), Trigonella foenum-graecum (fenugreek), Curcuma longa (turmeric), Garcinia indica (kokum) and Vitis vinifera(grapes)7. Roots of Pseudarthria viscida has been proved for its anti diabetic, antioxidant, anti-diarrheal and neuroprotective activity. Pseudarthia viscida consists of phytochemical constituents like alkaloids, glycosides, flavonoids, tannins, phenolic compounds and saponins. Alkaloids, glycosides, saponins and flavonoids were reported to show cardioprotective activity8.Hence, the plant is selected for the present study.

MATERIAL AND METHODS:

Materials

All chemicals and solvents used in the study were of analytical grade. The DL - Isoproterenol hydrochloride (Sigma Aldrich, Germany), other chemicals like Nitroblue tetrazolium, Phenazine methasulphate, NADH (Himedia) used for the present study & all estimation kits (Agapee) were obtained from their respective distributers. Instruments like U.V (Shimadzu), Micro centrifuge (REMI), rotary flash evaporator (Superfit, Rotavap), Semiautoanalyser (Mispa-plus) and Microscope (Magnus), Perfusion pump (Shanbhag & Co, Mumbai), Force transducer (INCO, India), Physiograph (INCO, India), Students Physiograph (INCO, India) were used for the present study.

Plant material:

The roots of *Pseudarthria viscida*(L.) Wight & Arnott used for the present studies were collected from Local Ayurvedic Pharmacy, Mangalore, in August, 2012. The roots were dried under shade. The dried roots were pulverized separately into coarse powder by a mechanical grinder and were used for extraction.

Preparation of extract:

The powdered material was subjected to batch extraction in Soxhlet apparatus by using ethanol as solvent. The powdered material (1250g) of *P.viscida* roots were packed in Soxhlet extractor and extracted using ethanol as solvent for 36 hours. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated by using rotary flash evaporator. The concentrated extract was then air dried at room temperature, weighed and percentage yield was calculated. The colour and consistency of the extract were noted.

Animals :

Healthy Wistar albino rats (175-200 g) of either sex were used for the experiment. They were maintained under standard conditions (temperature 22 ± 20 C, relative humidity $60\pm5\%$ and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Dose :

Two doses of PVRE were selected9.

Low dose : 200 mg/kg of PVRE

High dose : 400 mg/kg of PVRE

Isoproterenol induced myocardial necrosis in rats10:

Wistar rat of either sex weighing between 150-200g were divide into 5 groups of 6 animals each. Propranolol was administered to group III animals one week before administration of Isoproterenol. All the animals in group IV & V were treated once daily post orally for 4 weeks. On 29th and 30th day after the treatment, myocardial infarction was induced in group II - group V animals by sub cutaneous injection of Isoproterenol.

- Group I normal control
- Group II ISO control (Isoproterenol (ISO): 85 mg/Kg, s.c.)
- Group III Reference standard (Propranolol: 10mg/ Kg, p.o.)
- Group IV *Pseudarthria viscida* roots extract (low dose: 200 mg/Kg, p.o)
- Group V *Pseudarthria viscida* roots extract (high dose: 400 mg/Kg, p.o)

48 hrs following ISO administration, blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Animals were sacrificed using ether anesthesia and hearts were dissected out and used for histopathological studies.

Statistical analysis :

All data were expressed as Mean±SEM. The statistical Significance between groups was compared using one way ANOVA, followed by Dunnett's (multiple comparison test).

RESULTS :

Results of the preliminary phytochemical investigation of ethanolic extract of *P.viscida* roots showed presence of Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Steroids, Tannins, and Proteins.

Serum levels of cardio specific enzymes like CK - MB, CK - NAC & LDH were elevated in Isoproterenoltreated animalswhen compared to normal control. The prophylactic treatment with standard (Propranolol) showed extremely significant (P <0.001) reduction in marker enzyme CK-MB. The animals pre-treated with PVRE-200 showed less significant (P <0.05) reduction in CK-MB level where as PVRE-400 treated animalsshowed extremely significant (P<0.001) reduction in marker enzyme CK-MB level as compared to Positive control group.

The prophylactic treatment with standard (Propranolol) showed extremely significant (P <0.001) reduction in marker enzymes CK-NAC level. The animals pre-treated with PVRE-200 showed moderately significant (P <0.01) in CK-NAC level where as

PVRE-400 treated animalsshowed extremely significant (P<0.001) reduction in marker enzyme CK-NAC level as compared to Positive control group.

The prophylactic treatment with standard (Propranolol) showed extremely significant (P <0.001) reduction in marker enzyme LDH levels. The animals pre-treated with PVRE-200 showed extremely significant (P <0.001) reduction in marker enzyme LDH level where as PVRE-400 treated animalsshowed extremely significant (P<0.001) reduction in marker enzyme LDH levels as compared to Positive control group. The results are summarized in **Table 1**.

Table 1 : Effect of Propranolol and PVRE on Serum CK - MB, CK - NAC, LDH inISO induced myocardial necrosis

Groups	Treatment	CK-MB (U/l)	CK-NAC (U/l)	LDH (U/l)
Normal control	Saline	65.30±7.633	172.00±16.09	283.20±22.07
ISO control	ISO 85 mg/kg , s.c	240.60±30.37	343.50±17.43	727.9±34.73
Standard	Propranolol 10mg/kg , p.o	97.65±10.27***	197.2±15.24***	329.7±18.41***
Low dose	PVRE 200mg/kg, p.o	210.7±12.19*	314.00±9.218**	552.0±21.80***
High dose	PVRE 400mg/kg, p.o	119.30±10.87***	241.9±14.79***	435.2±17.94***

All the values are Mean±SEM, n=6 ns p>0.05, *p<0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunette's test compared to positive control.

Animals in Isoproterenol treated group developed a myocardial damage observed as decrease in CAT and SOD when compared to normal control. Animals treated with Standard (Propranolol) showed extremely significant (P <0.001) increase in SOD. Pretreated with PVRE-200 showed moderately significant (p<0.01) increase in SOD. Whereas PVRE-400 showed extremely significant P<0.001 increase in SOD as compared to positive control

Animals treated with Standard (Propranolol) showed significant (P <0.001) increase in CAT. Pretreated with PVRE-200 showed moderately significant (p<0.01) increase in CAT. Whereas PVRE-400 showed extremely significant (P<0.001) increase in CAT as compared to positive control. Results are summarized in **Table 2**

Groups	Treatment	SOD (Abs at 560 nm)	CAT (Abs at 620 nm)
Normal control	Saline	0.81 ± 0.02	0.46 ± 0.02
ISO control	ISO, 85mg/kg, s.c.	0.14 ± 0.06	0.13 ± 0.02
Standard	Propranolol 10mg/kg, p.o	$0.62 \pm 0.02^{***}$ (+87.66)	0.34 ± 0.01*** (+87.76)
Low dose	PVRE 200mg/kg, p.o	0.26± 0.03** (+81.33)	0.21 ± 0.02 ** (+52.84)
High dose	PVRE 400mg/kg, p.o	0.56 ± 0.03*** (+85.82)	$0.39 \pm 0.02^{***}$ (+73.53)
All the values are in absorbance Mean ± SEM, % inhibition shown in parentheses, n=5 ns p>0.05, **p<0.01, ***p<0.001 One way ANOVA followed by Dunette's test compared to positive control			

Table 2 : Effect on antioxidant Parameters like SOD and CAT in Isoproterenol induced myocardial necrosis.

Histopathological observations showed normal texture of heart tissue in Normal control group, Severe tissue degeneration & necrosis in Positive control (ISO) group, Mild tissue degeneration & necrosis in Standard (Propranolol) treated group, Moderate heart tissue degeneration & necrosis in low dose (PVRE-200) treated group & Mild to moderate tissue degeneration & necrosis in high dose (PVRE-400) treated group.Histopathological observations are showed in **Figure 1**



A) Normal control: Normal texture of heart tissue.
B) Positive control (ISO treated): Severe tissue degeneration & necrosis. C) Std (Propranolol): Mild tissue degeneration & necrosis. D) Low dose (PVRE-200): Moderate tissue degeneration & necrosis.
E) High dose (PVRE-400): Mild to moderate tissue degeneration & necrosis

DISCUSSION :

The current research was designed to evaluate the cardioprotective activity of Pseudarthria viscida (L.) Wight & Arnott roots (PVRE) against the Isoproterenol induced myocardial damage in Albino Whister Rats. The findings of the investigations witnessed the beneficial role of ethanolic extract of P.viscida when treated in conditions of anticipated cardiac injury. Isoproterenol (ISO), a synthetic catecholamine and β-adrenoceptor stimulant, is known to cause myocardial damage at higher concentrations11. ISO is also known to generate free radical and to stimulate lipid peroxidation, which probably leads to the irreversible damage of the myocardial membrane. Isoproterenol administration results in increase in calcium uptake and energy consumption leading to cell death12. It is well established that the biological markers like endogenous enzyme are organ specific and leak from the damaged organ during necrosis. Different cardiac specific biomarker enzymes such as Creatinine kinase-MB (CK-MB), Lactate dehydrogenase (LDH) and kinase-NAC Creatinine (CK-NAC) elevation in serum is due to the leakage from the heart as a result of Isoproterenol-induced necrosis13. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the mvocardial membrane. This alters membrane permeability, leading to the loss of function and integrity of myocardial membranes. Hence leakage of endogenous biological markers and free radical formation are attributed for myocardial distress in post Isoproterenol administration14. The activity of PVRE was observed in dose dependent manners reference myocardium protection with to by scavenging oxidative free radicals and diminishing the permeability of these endogenous biomarkers to surrounding cardiac regions. Propranolol blocks beta-adrenergic receptors and prevents Isoproterenolmediated hyper stimulation of sympathetic system and was used as a standard drug. Excessive activation of sympathetic system by Isoproterenol accompanied by vagal hypo activity produces severe myocardial damage12. The histopathological studies of the heart tissue evident in myocardial edema and separation of fibers with loss of striation as mark of myocardial injury in ISO control. The myocardial edema and 55

separation of fibers were ameliorated with treatment of PVRE-400. The potential of protective effect may be due to the rich source of bioflavonoid present as a chief chemical constituent but the exact mechanism is still not clear.

CONCLUSION:

The present investigation showed a dose dependent increase in cardio protective efficacy of PVRE during Isoproterenol induced and Ischemia reperfusion induced myocardial damage. The cardioprotective activity was found to be more significant in high dose (PVRE-400mg/kg) when compared to low dose (PVRE-200mg/ kg) in animal model. PVRE showed cardioprotective effect possibly by reverting back the level of cardiac biomarkers & biochemical parameters and by modulating tissue lipid peroxidation and augmenting endogenous antioxidants (CAT, SOD) defense mechanisms. There is direct correlation between in-vivo antioxidant and cardioprotective activity. The cardioprotective effect of PVRE in experimental model may be due to presence of flavonoids, which are attributed for the antioxidant activity. The cardioprotective effect of PVRE may be attributed to the individual or combined action of phytoconstituents present in it. Histopathological observation revealed that treatment with PVRE has reversed the cardiac damage effectively in ISO induced damaged. The exact mechanism for the cardioprotective activity of PVRE is still not clear and further studies are needed to isolate, characterize the active principles and to find out the mechanism responsible for its cardioprotective activity.

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Development and Validation of Spectrophotometric Methods for Quantification of Etoricoxib In Tablets

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ABSTRACT

Two simple and precise spectrophotometric methods (A and B) were developed for the estimation of etoricoxib (ETX) in bulk drug as well as in pharmaceutical dosage form (tablets). Method A is Area Under Curve (AUC) method which involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths. Method B is First derivative spectroscopy method. The First derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength (dA/d λ versus λ). It is characterized by a maximum, minimum and a cross-over point at the λ max of the absorption band. Beer's law was obeyed in the concentration range of 2-16µg/ml for both the methods A and B respectively. The proposed methods were statistically validated and found to be useful for the routine determination of ETX in tablets.

Keywords : Etoricoxib, Spectrophotometry, Tablets, Validation.

INTRODUCTION

Etoricoxib is a specific type of an anti-inflammatory drug most commonly used for the relief of the pain and swelling suffered by individuals^{1,2}. Chemically it is 5-chloro-6'-methyl-3-[4-(methylsulfonyl)phenyl] -2,3'-bipyridine³. Literature review revealed very few analytical methods including HPLC⁴, HPTLC⁵, LC-MS⁶, Capillary Zone Electrophoresis⁷ and Ultra Performance Liquid Chromatography⁸ for quantification of ETX in pharmaceutical dosage forms. In the present work, two simple and sensitive spectrophotometric methods (A and B) have been developed for the estimation of ETX in bulk drug and pharmaceutical dosage form. Method A is Area Under Curve method. Method B is First derivative spectroscopic method⁹. Spectrophotometric parameteres are established for standardization of the methods including statistical analysis of data.

MATERIALS AND METHODS:

EXPERIMENTAL :

Instrument: All spectral and absorbance measurements were made on Shimadzu UV-Vis spectrophotometer - 1650.

Standard solution of ETX: A 1mg/ml stock solution of ETX was prepared by dissolving 100 mg of drug in 100ml of ethanol.

Sample preparation :

Twenty tablets were weighed. A quantity equivalent to 100mg of ETX was weighed accurately, transferred to a beaker, dissolved in ethanol, filtered through whatmann filter paper No.1 into a 100ml volumetric flask and made up to volume with ethanol to get a concentration of 1mg/ml.

Assay :

Method A :

Area Under Curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 215 nm and 244nm. The area under curve between 215nm and 244nm were calculated by inbuilt software. Aliquots of stock solution of ETX were suitably diluted with ethanol to give varying concentrations ranging from 2-16µg/ml. The solutions were scanned in the spectrum mode in the wavelength range of 200-400nm, as shown in fig-1. The calibration curve was obtained by plotting concentration versus AUC as shown in fig-2. The amount of ETX was computed from the calibration curve.



Fig. 2 : Calibration curve of ETX by method A

Concentration (ug/ml)

Method B :

The stock solution was diluted suitably with ethanol to give a series of concentration ranging from $2-16\mu g/ml$ of ETX. The above solutions were scanned in the range of 200-400nm and the resultant spectra were derivatised to get the first order spectra as shown in fig-3. The amplitude of the corresponding concentrations were measured in mm. The calibration curve was constructed by plotting amplitude versus concentration as shown in fig-4. The amount of ETX was computed from calibration curve.

Sample Analysis :

Pharmaceutical formulation of ETX was successfully analysed by the proposed methods. Appropriate aliquots were subjected to the above methods and the amount of ETX was determined from the calibration curve.



Fig. 3 : First derivative overlay spectrum of ETX by method B



Fig. 4 : Calibration curve of ETX by method B

RESULTS AND DISCUSSION :

The optical characteristics like absorption maxima and the regression characteristics like slope (b), intercept (c), correlation co-efficient (r), percent relative standard deviation (%RSD) and standard error (SE) were calculated and the results are summarized in Table - 1. The results of sample analysis are furnished in Table - 2. The results of sample analysis showed that the drug determined by the proposed methods was in good agreement with the label claim proving the accuracy of the proposed methods.

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery calculated. The results are furnished in Table - 2. The results indicate that there is no interference of other ingredients present in the formulation. Thus the proposed methods are simple, sensitive, economical, accurate and reproducible and useful for the routine determination of ETX in bulk drug and its pharmaceutical dosage forms.

PARAMETERS	METHOD A	METHOD B
Absorption maximum/wavelength range (nm)	215-244	236
Linearity range (µg/ml)	2-16	2-16
Limit of detection[LOD](µg/ml)	1	1
Limit of quantification[LOQ](µg/ml)	2	2
Correlation coefficient(r)	0.9995	0.9998
Standard deviation	0.1217	0.4237
Standard error	0.0430	0.1498
% Relative standard deviation	0.05	0.133
Ruggedness	0.002029	0.007071
Regression equation y=mx+c	0.2568x+0.7142	4.29x+1.25
Intercept (c)	0.7142	1.25
Slope(m)	0.2568	4.29

Table 1 : Optical and statistical parameters by methods A and B.

Table 2 : Assay and recovery of ETX in dosage form.

Method	Labelled amount (mg)	Amount obtained (mg)*	Percentage recovery**
А	60	59.97	100.01
В	60	59.92	100.03

*Average of six determinations **Average of three determinations

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