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Staphylococcus aureus Infection Associated with Necrotizing Fasciitis

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Chapter - 1

Staphylococcus aureus Infection Associated with Necrotizing Fasciitis

Ronald Bartzatt

Abstract

Necrotizing fasciitis is a bacterial infection that spreads rapidly and causes death of tissue. The perineum and limbs are the usual sites that are infected. A majority of cases will have involvement of more than a single bacterium. Notably, a methicillin-resistant Staphylococcus aureus (MRSA) will be involved in as many as one-third of cases. The type of bacteria involved defines which of the four levels of condition the necrotizing fasciitis; is identified. Type I includes the gram-positive Staphylococcus aureus, various anaerobes, and gram-negative Escherichia coli. Type II includes Streptococcus pyogenes, which could be in conjunction with staphylococcal co-infection. Type III involves *Vibrio vulnificus* and Type IV is a fungal infection. The appearance of methicillin-resistant Staphylococcus aureus (MRSA) is increasing in frequency by over 10% annually. Also of concern is the increasing appearance of community-acquired MRSA (CA-MRSA) in medical facilities, inclusive of hospitals. Greater vigilance for MRSA has been called for in various studies. In some studies, CA-MRSA is found to possess a high level of resistance to most antibiotics. Necrotizing fasciitis induced by CA-MRSA, has been identified internationally, inclusive of Taiwan, China, United States, Africa, and Asia. MRSA strains causing a necrotizing pneumonia was found to be susceptible to non-β-lactam antimicrobial agents. The use of broad-spectrum antimicrobials as well as rapid medical evaluation, with surgical debridement if necessary.

Keywords: Necrotizing fasciitis, staphylococcus aureus, MRSA

Introduction

The term necrotizing fasciitis (NF) describes a serious condition that can incorporate multiple bacterial involvement and always poses a serious threat to life and even loss of limb. Necrotizing fasciitis is a life-threatening and rapid progressing infection. A serious pathological effect of this condition leads to

the death of soft tissues of the affected limbs, and ultimately loss of affected limbs, arm, or hand[1]. It is the perineum and limbs that are commonly the origin site of infection[1]. Rapid diagnosis and clinical response is required for survival and reduction of tissue loss. The common treatment includes intravenous antibiotic usage applied with surgical removal of the infected tissue region [1]. Combinatorial usage of antibiotics can include vancomycin, gentamicin, penicillin G, and clindamycin [1].

Up to 80% of all cases involve more than a single type of bacteria presence [2]. Up to 33% of diagnosed cases involve methicillin-resistant *Staphylococcus aureus* (MRSA), which is *Staphylococcus aureus* that is resistant to the antibiotic methicillin [2]. The condition of NF occurs most often among older individuals, rarely among children, but found in equally among both genders [2].

Rapid identification of the symptoms is vital, and include swelling, fever, extraordinary pain, hardening of soft tissue and skin [1]. Diagnosis is enhanced by use of CAT scanning and magnetic resonance imaging. For clinical treatment of NF utilizing antibiotics, the drug panel should include broad-spectrum antibiotics, encompassing the spectrum of microbes including anaerobic bacteria, gram-positive (inclusive of MRSA), and gram-negative bacteria [3]. In some regions of the world the incidence of NF reaches to one in 100,000 [2].

There are four types of pathological conditions that are defined as necrotizing fasciitis [1, 4]. These four types are described in Table 1. Type I is the most commonly occurring condition of NF, and that occurs generally with older individuals. Interestingly, generally the cause of the Type I infection is not trauma [2]. The *Clostridium* species account or 10% of Type I classed infections and are the typical causation for the gas gangrene phenomena [2]. The Type II class of infection primarily involves the extremities [2]. The main microbe responsible *is Streptococcus pyogenes* with/without *Staphylococcus* participation [2]. The Type II condition is the infection that most likely is found in healthy and young individuals that have also suffered injury [1]. Type II infections spread rapidly and can result in toxic shock syndrome. Type III infections are caused by bacteria found in saltwater and are initiated by a break in the skin tissue. Type III infections can spread without recognizable skin pathology and spread as rapid as Type II infections. Investigators of Type IV infections have identified fungal involvement.

Table 1: Four Condition Classes of Necrotizing fasciitis

Type	Occurrence	Microbes Involved
I	70% to 80%	*Staphylococcus aureus*
		Streptococcus pyogenes
		Enterococci
		Escherichia coli
		Pseudomonas aeruginosa
		Anaerobes
		Bacteroides
		Clostridium perfringens
		Clostridium septicum
		Clostridium sordellii
II	20% to 30%	*Streptococcus pyogenes*
		Staphylococcus types
III	Less common	*Vibrio vulnificus*
IV	Described as fungal infection, that is less common	Fungal types reported

The incidence of necrotizing pneumonia (NP) in children has been occurring in increasing number of cases since the first report appearing in 1994 [5]. NP is an uncommon and quite severe complication of pneumonia [5]. *Staphylococcus aureus* and pneumococci are the most common pathogens associated with NP [5]. The condition of NP, is generally, characterized by destruction of the underlying lung parenchyma (alveoli, alveolar ducts and respiratory bronchioles) [5]. Many of children inflicted with this condition also have pleural effusion (unusual amount of fluid around the lung), empyema (pus in the pleural cavity) or pyopneumothorax (pleural collection of pus and air) [5]. The average age of patients identified having NF is from 38 to 44 years, with a male-to-female ratio of 2 to 3:1 [6]. The condition of NF is rarely spread from person to person, with skin breakage the common way of obtaining NF [6]. Rapid aggressive treatment of NF condition is vital for reduction of morbidity and mortality [6].

Staphylococcus Aureus

Staphylococcus aureus is a gram-positive bacterium that is round in shape and is frequently found in the upper respiratory tract, eye, nose, throat, hair, and the skin [2]. Staphylococcus aureus is known to be a common cause of dermal infections, as well as other types of infections that include abscesses, respiratory infections, and food poisoning [2]. Community associated MRSA, referred to as CA-MRSA (MRSA bacteria caused infection in people not having hospitalization or a medical procedure within the previous year), began

to be identified during the 1990's [7]. Since then, CA-MRSA has been recognized as distinct from hospital-associated MRSA [7]. Their increasing resistance to vancomycin is of considerable concern among clinicians [7]. However, strains of MRSA have been recognized as early as the 1960's [7]. Staphylococcus aureus is a major causation factor in both communities and hospitals [8]. Studies of these two associated infection areas are showing increased resistance to methicillin and various beta-lactams [8]. The MRSA types are associated with up to 75% of all childhood CA-MRSA that are identified in Taiwan [8].

MRSA is a significant cause of nosocomial infections across the globe [9], [10]. Outbreaks of CA-MRSA occur in diverse community settings and is worldwide [9]. The majority of CA-MRSA are skin and soft tissue infections that are graded as "mild", and are frequently susceptible to various non-beta-lactam antibiotics [9].

Studies of MRSA-positive individuals recognized in Finland from 1997 to 1999, have shown that 21% of MRSA patients presented CA-MRSA (out of a total of 526 MRSA-positive individuals) [10]. In northern Taiwan, between 1999 to 2001, as much as 33.7% of a total number of 104 cases of community acquired Staphylococcus aureus were found to be MRSA [11]. In northern Taiwan, most of the CA-MRSA showed high levels of resistance to most antibiotics, which include trimethoprim-sulfamethoxazole (65.7%), chloramphenicol (41.2%), and clindamycin (71.4%) [11].

International Incidence

Necrotizing fasciitis is an infection deeply located in the fascia (thin sheath of fibrous tissue enclosing a muscle or other organ), accompanied with secondary necrosis (death of most or all of the cells in an organ or tissue) within the subcutaneous tissues (lowermost layer of the integumentary system), and requires rapid drug and surgical intervention [12]. In a study in northern Taiwan, 53 patients diagnosed with NF and studied from 2001 to 2005, 20 showed strains of *Staphylococcus aureu*s, eight were methicillin sensitive *Staphylococcus aureus*, with 12 as MRSA [12]. The NF patients having been induced by CA-MRSA, the CA-MRSA isolates were susceptible to vancomycin, trimethoprim-sulfamethoxazole, and ciprofloxacin [12].

Within Los Angeles, California (USA), a dismaying number of NF conditions are caused by CA-MRSA [13]. Between 2003 to 2004, in Los Angeles, the median age is 46 years within a range of 28 to 67 years 13. Of some 843 patients, 71% were men and 86% were demonstrated to by mono-microbial for MRSA, with 40% of patients have blood cultures were positive

for MRSA [13]. In this study, all MRSA isolates were susceptible, at least *in vitro*, to trimethoprim-sulfamethoxazole, rifampin, and clindamycin [13]. Authors of this study pronounced that for suspected NF, and in areas where CA-MRSA is endemic, then treatment should include antibiotics active against CA-MRSA [13].

In another study, individuals having invasive infections of CA-MRSA, the clinicians determined that teicoplanin, linezolid, and vancomycin were effective therapeutic agents [14]. This study found that CA-MRSA infections in young individuals and having no healthcare associated risk factors are emerging worldwide [14]. Occasionally, life threatening cases of NF and NP, myonecrosis (necrotic damage specific to muscle tissue), and sepsis (life-threatening complication of an infection) are reported [14].

Other investigators have found that MRSA directed antibiotic therapy should be inclusive when providing clinical treatment for diagnosed and suspected NF [15]. This study showed that *Staphylococcus aureus* is a significant pathogen of mono-microbial incidences of necrotizing fasciitis [15]. Between 1998 to 2008, a total of 105 cases with NF were studied, 17% of the cases (18 total) were induced by mono-microbial *Staphylococcus aureus* [15]. MRSA has emerged as the actual predominant causative agent in contemporary time, with lower and upper limbs most commonly involved in the condition [15].

Other studies undertaken in Taiwan, have shown the MRSA is the predominant pathogen of identified NF [16]. A study completed from 2004 to 2008, with 247 cases of NF, *Staphylococcus aureus* accounted for 19.8% of the NF cases [16]. The patients included in the investigations that were presenting NF and infected with hospital associated MRSA required higher amputation rates, comorbidity (simultaneous presence of two chronic diseases), C-reactive protein levels, and lower extremity involvement, than those infected with CA-MRSA [16]. In this study, most patients did have coexisting medical conditions (diabetes mellitus, hypertension, chronic azotemia (abnormally high levels of nitrogen-containing compounds in the blood), or chronic hepatic disease.

A study conducted in northeastern China, between 2013 to 2016, some 39 patients treated for NF (7 female and 32 males) showed that diabetes mellitus and blunt trauma were the most common risk factors [17]. *Staphylococcus aureus* was the most common isolated bacteria (20 cases) [17]. The number of surgical treatments per patient ranged from 2 to 5, inclusive of debridement, were administered [17]. Again, early detection and aggressive debridement were essential for NF treatment [17].

Case Reports

A large urban hospital has determined that MRSA is a predominant issue for soft tissue infections [18]. Between 2001 to 2006, MRSA is an increasingly common pathogen in this hospital [18]. This study indicated that clinicians should maintain high suspicion, since MRSA is associated with presented necrotizing fasciitis. More so, rapid antibiotic intervention is required for favorable morbidity and mortality [18]. They found that among 74 cases of NF, as many of 39% of cases showed MRSA as the causative organism [18]. The average age of MRSA necrotizing fasciitis was 43+3 years, with no social variables to pre-dispose these patients to the MRSA [18]. Virtually all patients were subjected to surgical debridement and the overall mortality was 15%. Virtually 100% of the MRSA isolates were susceptible to vancomycin or rifampin, with 93% susceptible to sulfamethoxazole/trimethoprim, and 62% susceptible to clindamycin [18].

A case of periorbital (swelling in the tissues around the eyes) necrotizing fasciitis induced by CA-MRSA has been report [19]. Monomicrobial MRSA has to be considered in the event of periorbital necrotizing fasciitis with early diagnosis and prompt surgical/medicinal therapy [19]. This case required rapid necrotic tissue debridement and frontal sinus curettage [19]. CA-MRSA has been found to be a significant cause of NF in a Denver health center, with CA-MRSA accounting for 15% of NF cases [20]. In this study, five of 30 NF cases involving extremities were caused by MRSA, with none of the isolates inducible clindamycin resistance [20]. These patients required a median of six surgical procedures, within a range of 2 to 7 surgical operations [20].

In a Los Angeles area (California, USA), an alarming number of NF infections were caused by CA-MRSA [21]. Of a total of 843 patients showing MRSA in would cultures the median age was 46 years, with 71% being male [21]. Risk factors were noted in this study to be injection drug use (43%), previous MRSA infection (21%), diabetes (21%), chronic hepatitis (22%), cancer (7%), and acquired immunodeficiency syndrome (7%) [21]. The wound cultures were Monomicrobial for MRSA (86%), with 40% of patients that did have blood cultures done showing positive results for MRSA [21]. Virtually all MRSA isolates were susceptible, at least *in vitro*, to clindamycin, trimethoprim-sulfamethoxazole, and rifampin [21].

In China, a 15 year-old female teenager, was shown to have CA-MRSA induced necrotizing pneumonia [22]. This patient was successfully treated with linezolid, teicoplanin, and fosfomycin [22]. In America, a 11 year-old male presented a case of CA-MRSA induced necrotizing fasciitis, with the patient

showing no risk factors [23]. In another incidence, a 52 year old male arrived at an emergency room with 7-days of thigh pain and swelling [24]. This case was induced by CA-MRSA and despite aggressive support care, broad spectrum antibiotics, and surgical debridement, the patient died in less than 24 hours [24]. Another case involved severe MRSA necrotizing fasciitis in an AIDS patient [25]. A separate case reported CA-MRSA induced necrotizing fasciitis in a neonatal patient [26]. In yet another instance of neonatal necrotizing fasciitis, located on the back, was determined to be caused by CA-MRSA [27]. Appropriate antibiotic selection was considered to be vital by clinicians involved [27].

A case report from Wisconsin USA, described NF condition caused by methicillin sensitive Staphylococcus aureus in a 72-year-old diabetic male, that despite aggressive antibiotic intervention, surgical debridement, still required amputation [28]. In this case, the previously intravenous administered ciprofloxacin and clindamycin prior to presentation of NF, the patient still showed a deteriorating condition [28]. A methicillin-sensitive *Staphylococcus aureus* strain was eventually shown to be responsible [28].

Clinicians have reported that 4-year-old and 5-year-old children developed necrotizing fasciitis from minor lesions of the skin 29. A case in India, reported a 58 year-old male presenting NF in the left hand region had, caused by MRSA, and despite aggressive application of antibiotics (teicoplanin, clindamycin, vancomycin, linezolid, and daptomycin) and debridement, the limb required amputation for favorable outcome [30].

A report of Type II necrotizing fasciitis involving group A streptococcal bacteria have been noted, with the finding that treatment with strong broad-spectrum antibiotics administered intravenously is required [31]. For skin injuries, the antibiotics group bacitracin, which are polypeptides, have previously been shown to be effective to halt all strains of aerobic and anaerobic hemolytic *streptococci*, *D. pneumonia*, *Corynebacterium*, *Neisseria*, *Cl. perfringens*, *C. tetani*, and *Treponema pallidum* [32]. In addition, various strains of staphylococci, nonhemolytic streptococci, enterococci, and H. influenza are highly sensitive to bacitracin [32].

In another case, clinicians found that vancomycin was able to control and defeat MRSA infection [33]. In Peshawar Pakistan, post-surgical wounds may become infected within a period after 30 days of initial surgery [34]. An investigation, having the goal to defeat post-surgical wound infections in Pakistan, in a total of 210 incidences of infection were investigated, with the predominant microbes being *Escherichia coli* (26.2%), *Staphylococcus*

aureus (24.28%), *Pseudomonas spp*. (20.47%), MRSA (10%), *Proteus mirabilis* (7.14%), *Escherichia coli* ESBL producer (3.81%), *Acinetobacter* (3.33%), *Proteus vulgaris* (2.38%), *b-Streptococci* (1.43%), and *Klebsiella pneumoniae* (0.95%) [34]. In that study, the antibiotics linezolid, vancomycin, amoxicillin, cefoperazone, and meropenem were the most effective agents for treating post-surgical wounds [34].

The appearance of MRSA can appear from unexpected sources, and thereby, be a danger in environments where skin injuries can occur. In one report coming from Punjab, India, The prevalence of MRSA bacteria found in a retail meat operation posed an appreciable threat to individuals exposed to the infected meat in the normal business operation of the facility [35]. This exposed the possibility of serious dermal infection where not expected. There are global concerns of the spread and infection by MRSA strains [36]. Within a hospital in Nowy Targ Poland, methicillin sensitive Staphylococcus aureus was found in all of the hospital's wards [36].

Implementation of improved standards were required to control that situation. Investigators of another study indicated the abundant infections in communities, livestock, and hospitals by MRSA [37]. In addition, MRSA is a major cause of central venous catheters infections and such strains have been resistant to doxycycline, clindamycin, azithromycin, amikacin, trimethoprim-sulfamethoxazole, gentamycin, tobramycin, and ofloxacin [37]. A notable study indicated that derivatives of ciprofloxacin inhibited the growth of necrotizing fasciitis associated penicillin-resistant Escherichia coli [38]. That study showed that derivatives of ciprofloxacin induced more than 60% bacterial cell death at concentrations of less than 1.0 micrograms/milliliter [38]. These investigators demonstrated that these derivatives of ciprofloxacin showed much improved tissue penetration compared to the parent compound ciprofloxacin. The study indicated that the ciprofloxacin derivative showed the potential of penetrating tissue fast and possibly in a manner aiding the effective treatment of active necrotizing fasciitis infection [38]. The burden of *Staphylococcus aureus* is known to be high in Africa [39]. Another investigation has shown that bacteriostatic and bactericidal effects obtained from ethyl acetate root bark extracts of *Terminalia avicennioides*, were effective therapeutic agents in MRSA treatment [40]. Further studies concerning effective therapeutics and administration methods will be beneficial in the clinical treatment of NF.

Conclusion

Necrotizing fasciitis poses a highly dangerous pathological condition that is difficult to identify and often fatal if not met with rapid and aggressive antibiotic treatment and debridement where necessary. Because of the new

findings, identifying origins of *Staphylococcus aureus* infections that culminates into NF condition, further observations and studies are needed to maintain a clinical useful state for treatment of patients. Further elucidation of successful treatments should be presented and examined in detail to identify successful clinical responses. Case reports are very valuable for comparison of treatment regimens. Case reports are needed to not only enhance the final outcome result for the patient, but to up-date approaches in treatment. Care providing facilities should be constantly examining current case reports to up-date/improve clinical treatment protocols. Recognition of the condition is vital for favorable outcome. Case reports will enhance the means and protocols for identifying the very dangerous condition of necrotizing fasciitis. Generally, further studies are required to ascertain the most effective approach of clinical treatment of necrotizing fasciitis infection.

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Chapter - 2
Recent Trends in Cancer Biology

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Chapter - 2

Recent Trends in Cancer Biology

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Abstract

Disparities in cancer treatment and outcomes mirror socio-economical, racial, ethnic, and academic backgrounds. Recent years have seen an explosion of new discoveries of the diverse molecular and biological changes underlying cancer development and progression. These insights are changing our understanding of the complex pathways that regulate cancer cell biology, the interactions of tumors with their microenvironment, and the mechanisms that normally restrain tumourigenesis. Importantly, researchers are translating these findings into novel approaches towards cancer diagnosis, prognosis, and therapies.

Keywords: Cancer biology, innovative treatment, gene therapy

Introduction

Cancer has probably been around for as long as people do. But over the years, we have greatly improved our ability to test for the disease and treat it. More people living longer who get cancer. Some are healed. Exciting advances pave the way for better treatments and perhaps more cures.

It is currently clear that bound tumors are sustained by a rare population of cancer stem cells that share one amongst the process properties of traditional stem cells the flexibility to renew themselves. Self-renewal is what permits stem cells to persist throughout the lifespan of the organism and to produce new cells for tissue genesis, maintenance, and regeneration following stress or injury. These properties are precise what cancer stem cells exhibit in initiating and maintaining malignant growth and, sadly, to make a tumor once the cancer stem cells escape treatment.

A key question so is whether or not cancer stem cells are invariably derived from traditional stem cells that run amok, manufacturing cancer cells rather than traditional cells. This idea is actually plausible as a result of several cancer-stem-cell populations' specific cell-surface proteins that are also found on traditional stem cells. However, stem cells won't be the sole

supply of cancer stem cells. Krivtsov and colleagues [1] report the generation of cancer stem cells by a leukemia-associated macromolecule that has found how to trick non-stem cells into deed stem-cell-like behavior and supporting tumor formation.

Most of the cancer researchers today are finding ways for new approaches in treatment. The approach for treatment has been improved from the utilization of nanoparticles to gene-targeted therapy.

Immunotherapy: Your immune system is hunting down and killing bacteria and viruses like invaders. It also looks for cancers as they form and destroys them. But for your immune system, cancer has developed ways to hide. It may grow despite the efforts of your body to stop it. Immunotherapy helps attack cancer in your immune system. This treatment works in various ways, including cancer prevention from hiding Boosting immune system.

Checkpoint Inhibitors: Your system has fighters known as T cells. They obtain and destroy foreign cells, together with cancer. Healthy cells have proteins known as checkpoints on their surface that allow the fighters to grasp they are friendly. Cancer cells generally use checkpoints to cover from your system.

Immunotherapy medicine known as stop inhibitors block checkpoints on cancer cells to assist T cells realizes cancer. Stop inhibitors treat many styles of cancer, together with bladder, colorectal, head and neck, kidney, liver, lung, lymphoma, melanoma, and abdomen.

Monoclonal Antibodies: Weiner said that Monoclonal antibody-based therapies are starting to fulfill the promise predicted more than 20 years ago with the advent of core technology. Tumor cell surface antigens such as B-cell idiotypes, CD20 for malignant B cells, CD33 for leukemic blasts, and HER2/neutrophils for breast cancer cells have been shown efficacy in clinical trials. Multiple antibody-based strategies have shown promising efficacy in recent clinical trials. Unconjugated immunoglobulins directed against CD20 induce partial and complete responses in up to 50% of patients with advanced, indolent non-Hodgkin's lymphoma When such antibodies are conjugated to appropriate radionuclide' are administered in therapeutic doses, the proportions of complete and overall responses increase considerably. In patients with relapsed or refractory acute myelogenous leukemia, conjugates composed of anti-CD33 antibodies and the chemotherapy agent, calicheamicin, show promising activity.

The RNA interference (RNAi) is an evolutionarily preserved cellular defense in most eukaryotes, including humans, to control the expression of

foreign genes. Monoclonal antibodies used in cancer treatment are designed in a laboratory to target certain antigens that live on the surface of cancer cells-foreign substances in the body. These antigens can target the antibodies to latch on the cancer cells and act as a "call to arms" in the immune system for other disease-fighting warriors. The Food and Drug Administration (FDA) has approved more than a dozen monoclonal antibodies to fight various types of cancer, including breast, head and neck, lung, liver, bladder and melanoma, and Hodgkin's cancers. More research is going on in this area to neutralize the side effects and provide an effective environment in the treatment of breast cancer.

Adoptive Cell Transfer: This technique uses your own immune cells to treat your cancer. Doctors take the cells from your blood or neoplasm. They modify them during work so that they are higher ready to bind to and attack cancer cells. Automobile T-cell medical aid is one kind. It's approved to treat bound varieties of leukemia and malignant neoplastic disease.

Immune System Modulators: They boost your body's response against cancer. Associate degree example is cytokines-substances that management immune cells' growth and activity. Examples include:

- **Interleukins:** Which facilitate immune cells communicate with one another
- **Interferons:** Which activate cancer-fighting immune cells

Cancer Vaccines

Rather like vaccines facilitate your body notices contagious disease or acute anterior poliomyelitis viruses, cancer vaccines facilitate your system to notice cancer cells.

Vaccines are made up of your cancer cells. Doctors take them out of your body and use them to create the vacuum. Once it's able to facilitate your cells notice and attack cancer, it goes back to your body.

One cancer vacuum, sipuleucel-T (Provenge), treats prostatic adenocarcinoma that has to unfold. Different vaccines against breast, lung, and brain cancers area unit being studied in clinical trials.

Preventive vaccines are available to protect against human papillomavirus (HPV) and serum hepatitis, which might cause liver disease.

Finding an efficient cancer vaccine has tried tougher than researchers had thought. As a result of cancer cells have many ways to cover from your system, they'll be arduous to trace down.

Researchers are currently watching simpler ways in which to relinquish cancer vaccines. One methodology is to mix them with substances known as an adjuvant to assist those works higher.

Cancer vaccines similar to vaccines facilitate your body to realize contagious disease or infectious disease viruses, cancer vaccines facilitate your system to realize cancer cells.

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Gene-Based Treatments

Cancer analysis now not follows a one-size-fits-all approach. It's become far more customized, with analysis on genes driving the trend. Doctors currently understand that one willcer-like breast or carcinoma-can are available in many alternative genetic varieties. Genomics, the study of changes to genes in your DNA, is giving doctors necessary clues regarding however your cancer can act and the way to best treat it. Doctors explore for changes known as mutations inbound cancer genes. Gene-based treatments. It works for individuals with specific sequence changes within cancer. For instance, the medicine vemurafenib (Zelboraf), dabrafenib (Tafinlar), and encorafenib (Braftovi) treat individuals with skin cancer WHO have a chromosomal mutation referred to as BRAF. The medicine stops the production of BRAF supermolecule that the mutation causes. Trastuzumab (Herceptin) works identical method against breast cancers that overproduce the HER2 sequence. Scientists area unit learning new treatments supported sequence changes in cancer cells. These studies may lead to even a lot of targeted sequence therapies within the future.

Some of the Recent Works Carried out by Various Researchers on Cancer are

Gomez L [3] worked on mouse models for breast cancer metastasis. The reason for carrying his work on breast cancer was more patients are suffering from breast cancer. Though the early diagnosis can prevent the spread of cancer from one organ to other it is difficult to prevent the relapse of breast cancer. It is in the metastasis phase specific molecular changes occurring in the cancer cells and the surrounding cells that play an important role in

detachment from tumor cells and special invasion to tumor stroma, other blood vessels, extravasation and colonization of target organ and finally metastatic outgrowth. The treatment approaches have shifted from genetical level to transplantation surgery. She employed mouse models for the state of metastasis. The changes that are observed in the mammary gland during pregnancy and lactation was easily known where the cells of mammary gland cells proliferate and extend into fat pads where the branches develop into alveoli forming milk. After that phase the mammary alveoli involute. The changes observed facilitate the incidence of cancer. Alteration of the expression or function of adhesion molecules responsible for the adhesion of breast cancer cells to themselves, to stromal cells, or to tumor matrix, including integrin family members, immunoglobulin-domain cell adhesion molecules (such as L1 and NCAM), cadherin family members, or other cell surface receptors (such as CD44), contributes predominantly to late-stage tumor progression and metastatic dissemination of cancer cells.

Experiments on xenograft transplantation are conducted in immunocompromised mice to investigate the growth and metastasis of human breast cancer cell lines *in vivo*. After the completion of study she identified that changes in the cell adhesion played an important role in the incidence of cancer. Forced expression of E-cadherin prevents tumor cell migration and invasion, whereas inhibition of E-cadherin function enhances tumor cell invasion and metastatic dissemination. E-cadherin is irreversibly lost in more than 85% of invasive lobular breast cancer associated with an invasive phenotype, and in the remaining 15%, the retention of E-cadherin is associated with dysfunctional adhesion. Chemotherapy was only used to inhibit the growth of the cells. New aims in treating cancer were finding a new method for safer treatment by using smaller molecules, antigenomic, antibodies and viral therapy. The treatment approaches changes based on a number of prognostic factors, including endocrine tumor status, other possible actionable targets (androgen receptor, BRCA mutation), hormonal status, prior therapy (adjuvant and metastatic), disease-free interval, comorbidity, performance status, metastatic disease burden, patient preferences, pre-toxicity, geographical accessibility. Immunotherapy has been shown to induce remarkable responses in the treatment of advanced malignant melanoma.

Imran ali [4] worked on imidazole rings as antitumor agents. His aim was to identify the new usage of the drug as the anti-cancer agent which has fewer side effects and is more beneficial to patients with high safety and efficacy.the uses of imidazole as anticancer drugs have been drastically

increased because of their relative potency in treating cancer. Among the number of targets for cancer treatment DNA being the most efficient target for beneficiary therapy. In this approach, imidazole is effective in binding with the DNA protein easily and are most effective in halting cell division. At higher concentrations, they inhibit the synthesis of membrane proteins. A number of researches proved imidazole anti-cancer drugs.

Jamalian and his colleagues worked on the combination of imidazoles with other drugs like 1, 8-acridine which are effective anti-cancer drugs by incorporating electron withdrawing groups to imidazoles. Sarkarzadeh worked on different combination molecules for anti-cancer effect by MTT assay for breast, cervical and colon cancer and proved that imidazolyl-2-yl moiety on 11^{th} carbon position of a dihydropyridine ring showed superior excellent antiproliferative activity.

Piao Zhu [5] worked on the usage of sonodynamic therapy in experimental cancer. In his research, he used ultrasound radiations for the cytotoxic effects of agents. Ultrasound radiations fall on the cells and altering the permeability of the cells where the proliferation of cancer cells is inhibited by apoptosis. The radiation can even be strengthed by using microbubbles and chemotherapy also.

Liu [6] worked on the effect of cyclin-dependent kinase 4 inhibitors for anti-cancer treatment. The cyclins and cyclin-dependent kinases play an important role in the regulation of the cell cycle. The cyclin complex is an important regulator for the G1 phase of cell division. It was observed that in cancer cells the complex protein has been deregulated. He also proposed that work in the field of cyclin protein may improve the cancer treatment regimen and can also be an effective approach.

QingliBie [7] worked on gastric cancer. He found out that there is a specific type of mucosa cells or group 2 innate lymphoid cells in cancer patients. The changes in the cells may lead to the cause of cancer. In gastric cancer patients, TH_2 phenotype was also found. He worked on the genetic phenotype of gastric cancer patients. He also made a potential analysis of samples and comparison from different gastric cancer patients. The expression levels of RORα, GATA3, T1/ST2, IL-17RB, CRTH2, IL-33, IL-5, and IL-4 mRNA were significantly increased in patients, but no significant changes were found in the expression of ICOS, CD45, and IL-13, and the correlation between RORα or IL-13 and other related factors such as ICOS and CD45 was positive. The increased frequency of ILC2s was also found in the PBMC of patients by flow cytometry. In addition, the mRNA of

Arg1 and iNOS were also significantly increased in patients. After thorough research and comparative analysis revealed that targeting ILC2s in gastric patients may be efficient treatment.

Colorectal cancer cases are also found to be increased day by day. Zhexuan [7] worked on the efficacious treatment of colorectal cancer. After a thorough investigation, he found that angiogenesis was found to be an effective treatment in colorectal cancer. It was becoming a major approach for treatment in clinical trials alone and in combination with other therapies. Prof Sir John burn worked on hereditary colorectal cases. He collected a group of patients who are hereditary colorectal and treated them with aspirin 600mg daily for up to 25 months reduces the risk of colorectal cancer. The study was conducted in a randomized study taking into consideration all the pros and cons of the patients. The comparison was done with aspirin placebo studies. After the completion of the study, he revealed that the patients who took aspirin were found less incidence with colorectal cancer. The delayed effect was observed.

Diane Feskanich [8] worked on the efficacy of vitamin D in colorectal cancer. He found out that the precursors of vitamin D 1,25dihydroxyvitamin D and 25 hydroxyvitamin D plays an important role in the treatment of colorectal cancer. For his study, he selected women volunteers who are incident with colorectal cancer and grouped based on their age, incidence of cancer, the position of cancer at colon and genetic hinderance conditions. He grouped the study into two cases and carried out the study where each group was given with individual metabolite and blood samples were collected frequently from the study. He identified the cases who are taking 25 hydroxyvitamin D has a low risk of cancer.

Han [9] worked on "Requirement for ERK Activity in Sodium Selenite-induced Apoptosis of Acute Promyelocytic Leukemia-derived NB4 Cells". He carried his work on promyelocytic leukemia cells. He identified the requirement of an extracellular signal-regulated protein kinase in causing sodium selenite-induced apoptosis. Patrick *et al*. worked on the importance of selenium as an anti-cancer drug which kills the cells by apoptosis. Recent researches also showed the importance of sodium selenite as potent anti-cancer drugs. The effect was calculated by the amount of phosphorylation of protein molecules which indicate a sign of apoptosis. After his thorough research, he proved that the concentration of selenium is responsible for causing apoptosis. At low concentration, it facilitates the growth of cells and at high concentration, it kills cancer cells.

Xi ang [10] worked on the effect of imitab on acute lymphoblastic leukemia. He worked on the benefit of a combination of drugs for anti-cancer work. For his

work, he selected rifampicin and determined the effect in combination with imitab for anti-cancer effect. The combination of drugs was success full because of synergism. The effect kills the cancer cells with fewer side effects and also overcome the resistance.

Kazufumi Suzuki [11] worked on the function of the p53 gene and their role in cancer treatment. P53 is one of the most mutated genes in cancer research. It is also found that this gene is inactivated in almost all cancer patients. He worked on applications of p53 gene. P53 plays an important role in cancer. It identifies the damaged DNA and induces apoptosis in cells. CD95 a death receptor plays a role in the apoptosis. The apoptosis of cancer cells by chemotherapy is due to activation of CD95 by p53 gene activity. The p53 gene also causes cell arrest at G1 and G2 phases. p53 promotes the self-renewal process of neural stem cells and hemopoietic stem cells. Loss of p53 promotes leukemia by aberrant self-renewal. Mammary stem cells with a targeted mutation in the p53 gene reported in reactivation of gene which acts as a tumor growth inhibitor. Still more researches in gene modulation may lead to drastic development in cancer treatment [12]. p53 is a nuclear transcription factor with a pro-apoptotic function and is considered as a classical type tumor suppressor. But the mutant p53 gene has oncogenic potential. In general, p53 inhibits cell division by apoptosis which prevents the propagation of cells by DNA damage. The binding capacity of p53 to DNA is responsible for its tumor suppressive function. When damage is observed in cell DNA then the p53 gene accumulates in the cell by phosphorylation and acetylation and gets activated within the cell. The functionally active p53 gene again activates its target sites and induce cell cycle arrest. In the meantime, DNA repair is observed. If the damage is beyond repair then the DNA transmission to daughter cells is inhibited. If the repair of DNA is done then the cell becomes to its normal pathway. In the majority of cases the genetic alterations are observed where the binding capacity of p53 to DNA binding protein is altered then the gene loses its ability and the tumor cells grow where the cell division is not under control.

Nikolaev [13] *et al.* discovered Parc (P53-associated, Parkin-like cytoplasmic protein) as a large cytoplasmic protein. Based on their findings, Park's NH2-terminal region binds to p53's COOH-terminal region. Park had an intrinsic activity of the ligase of the E3 ubiquitin protein. It is associated with most cytoplasmic p53 and acts as a p53 cytoplasmic anchor protein. Knock mediated by siRNA.

Mihara [14] *et al.* found that, in response to DNA damage, a fraction of p53 is translocated from the cell nucleus into mitochondria in cancer cells

undergoing apoptosis. Within mitochondria, p53 directly promotes the permeabilization of the outer mitochondrial membrane by forming complexes with BclXL and Bcl2 and thereby releasing cytochrome c into the cytoplasm. Additional studies have shown that MDM2-mediated monoubiquitination of p53 promotes its mitochondrial translocation where p53 undergoes rapid deubiquitination by mitochondrial HAUSP, generating non-ubiquitinatedp53 apoptotically active. Down-regulation of HAUSP leads to increased resistance to DNA-induced apoptosis in conjunction with mitochondrial suppression of p53.Thus, targeting p53 to mitochondria, which causes the dysfunction of mitochondria, might be one of the transcription-independent pro-apoptotic pathways mediated by p53.

Iwabuchi [15] *et al.* identified 53BP1 as a novel p53-binding partner. 53BP1 binds to the central region of p53 (amino acid residues 80-320). Unlike wild type p53, 53BP1 does not interact with p53 mutants (R175H and R273H) derived from cancer. Subsequent studies revealed that 53BP1 improves transcriptional activation with p53 mediation.

Rappoid [16] *et al.* found that 53BP1 is rapidly phosphorylated by ATM in response to DNA damage, and forms discrete nuclear foci containing phosphorylated histone variant H2AX (γH2AX). γH2AX localizes at the sites of DNA strand breaks. Therefore, 53BP1 is closely involved in the early DNA damage-signaling pathway. Reactivation of mutant p53 may have provoked tumor suppressing property and an area of research in this field may be very fast in treatment.

Immunotherapy [17] in cancer started a century ago but the beneficial results are observed nowadays in pre-clinical trials and the trails in human volunteers are in progress which may eradicate cancer completely. The data from preclinical trails and early human trails proved the ability of cancer vaccines to induce a human response that is tumor specific. The human immune system has the potential to protect the body against uncontrolled cell growth that was observed in tumors. Tumor-associated antigens (TAA) displayed on human malignancieshelp the immune system to identify the tumor cells. T-cell-mediated immune responses seem to have a greater potential for eradicating tumor cells.

Tumor cells are unstable genetically and have efficient protective mechanisms in cells. A small number of tumor cells undergo apoptosis and release apoptotic bodies containing TAA that are taken up by immature antigen presenting cells. These antigen-presenting cells slowly get matured and result in the activation of T-cells. Activated T cells produce specifically

targeted proteins that act against the proteins that also recognize the tumor cells and kill them. But cancer cells target the immune system and affect the immune mechanism. So a mechanism is to be developed to reactivate the immune system and T cells in acting against the tumor cells.

Initially, by peptide vaccination, cytotoxic T lymphocytes get activated and increase the production of TAA-specific T cells and increase the affinity to bind with the tumor cells. Thus the binding of antibodies to tumor cells is increased by converting a subdominant epitope into a dominant one.

Immunization with non-peptide antigens have also been observed in case of melanoma. In this case, Gangliosides play an important role. They are related glycolipid antigens expressed on normal cells and highly specific to tumor cells. Among them, GM2 has been found to be most immunogenic and is the target of several vaccination clinical trails. The antibody production was associated with disease-free interval and survival.

In the case of patients with multiple interactions, the immune system cannot recognize and eliminate tumor cells. Tumor cells can themselves induce tolerance also. This facilitates tumor growth. In these cases, cellular vaccines have an approach. Cellular vaccines are in the stage of ex vivo and are in the early stages of development. They create proper microenvironment to overcome tumor-induced tolerance.

Tumor vaccines were first found for autologous and allogeneic tumor cells. The main reason is that they have all the relevant tumor antigens needed by the immune system for an effective anti-tumor response. A specific cell-based immunization allows the development of a vaccine.

In vitro and preclinical trials proved that genetically modified tumor cells showed increased immunogenicity. The trails on human volunteers are going on. The goals of the treatment are to evaluate the safety and decline of the toxicity of tumor cells. This approach is new and is safer with less toxicity.

The most active [18] and safer vaccine compounds are synthetic long peptides that correspond to tumor viral antigen or tumor-associated viral antigen. These peptides induce T cells to stimulate the production of specific antigen presenting cells. Thus formation CD4 and CD8 T cells act against the tumor cells and inhibit their growth.

Conclusion

Cancer genes play a subtle game of subversion that we could ultimately use against them. By appropriating mechanisms we can modify the action of

the genes and cancer growth can be prevented Many of the scientists working on the various preventive mechanisms of cancer that are essential to the functions they want to gain (here self-renewal), cancer genes teach us how these processes work and give us the opportunity to identify molecular targets that could be used for cancer treatment.

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Chapter - 3
Packaging Material Science

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Chapter - 3

Packaging Material Science

Preeti Singh, Praveen Kumar and Dr. Dharmendra Ahuja

Abstract

Pharmaceutical packaging material Science plays a vital and very important role in the stability of the products and also help in transportation, shipping. Packaging should be such that it should maintain the outer appearance of the goods & products, should be inert in nature, should not be fragile, and should have good mechanical strength and stability of shelf life. Glass is the most commonly use packaging material for the packing of any type of product. The selection of packaging material is very important parameter during the study of stability testing of the dosage form. Packaging may also be designed as the collection of different components (E.g.: bottle, vial, etc.) surrounding the pharmaceutical product till its use. Now recent time biodegradable polymers are also used in the packaging material to minimize the pollution instability. Function of packaging is also helpful for the product identification. Quality test for packaging materials required depends on the type of component used in the packaging manufacturing. Components are in direct contact with the dosage form for use Primary type packaging. Packaging system classification based on dosage capacity of the container depends on the single and multiple dosing. Desirable attributes of packaging material for the protection of product from environmental conditions e.g.: light, gases, moisture. The choice of primary and/or secondary packaging materials will depend on the degree of protection required, compatibility with the contents, the filling method and cost, but also the presentation for over-the-counter (OTC) drugs.

Keywords: Packaging material, pharmaceutical dosage form, biodegradable polymers, packaging, and product. Desirable attributes unit-dose packaging, quality tests for packaging materials

Introduction

The process by which the pharmaceuticals are suitably placed so that they should retain their therapeutic effectiveness from the time of their

packaging till they are consumed is termed as packaging. Packaging means a collection of different packaging materials which encase the pharmaceutical product from the time of manufacturing to the end of the user. Packaging is a multiple user means provide presentation, protection, identification information, about a product during storage, carriage, display and until the product is consumed.

Packaging: Refers to economic ways of protecting, presenting, identifying, providing information, containment, convenience and stability of a product until it is consumed or throughout its life.

Pharmaceutical Packaging: Science, art and technology of enclosing or protecting products for distribution, storage, sale and use.

Packaging may also be designed as the collection of different components (Eg: bottle, vial, etc.) surrounding the pharmaceutical product till its use.

Functions of Packaging

- **Product Identification:** Packaging greatly helps in identification of products
- **Product Protection:** Packaging protects the contents of a product from spoilage, breakage, leakage, etc.
- **Facilitating the use of Product:** Packaging should be convenience to open, handle and use for the consumers
- **Product Promotion:** Packaging is also used for promotional and attracting the attention of the people while purchasing
- **Marketing:** The packaging and labels can be used by marketers to encourage potential buyers to purchase the product
- **Convenience:** Packages can have features that add convenience in distribution, handling, stacking, display, sale, opening, re-closing, use, dispensing, reuse, recycling, and ease of disposal
- **Barrier protection:** A barrier from oxygen, water vapour, dust, etc., is often required. Permeation is a critical factor in design. Some packages contain desiccants or oxygen absorbency to help extend shelf life. Keeping the contents clean, fresh, sterile and safe for the intended shelf life is a primary function
- **Security:** Packaging can play an important role in reducing the security risks of shipment. Packages can be made with improved tamper resistance to deter tampering and also can have tamper-

evident features to help indicate tampering. Packages can be engineered to help reduce the risks of package pilferage

Desirable Attributes of Packaging Material

Protection of product from environmental conditions:

- Light
- Gases
- Moisture
- Solvent loss
- Sterility

Safety:

- Non toxic
- Must not impart taste and odour
- FDA approved

Compatibility:

- Must not be reactive with product

Performance:

- Adaptable with common high speed packaging machines
- Must meet tamper-resistance and tamper- evident requirements
- Transit worthy
- Economical
- Eco-friendly

FDA: The drug packages must "maintain the standards of identity, strength, quality and purity of a drug for its intended shelf life."

Basis for Selection of Packaging Material

1. Facilities available
2. Route of administration
3. Ultimate use of the product
4. Physical form of the product
5. Stability of the material
6. Contents
7. Cost of the product

Hazards Due to Packaging

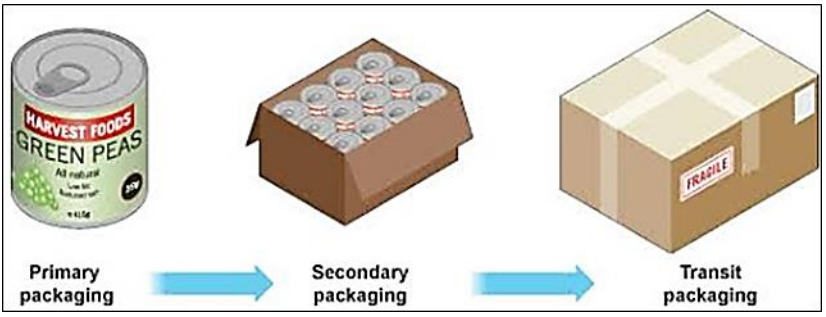
1. **Biological**
    - Microbiological hazards
    - Chemical hazards
2. **Mechanical**
    - Shocking or impact damage
    - Compression
    - Vibration
    - Electrical conductance
    - Abrasion
3. **Environmental**
    - Moisture
    - Temperature
    - Pressure
    - Atmospheric gases
    - Light
    - Solid airborne contaminants

Types of Packaging

1. **Primary Packaging:** Components are in direct contact with the dosage form. Examples include liners, bottles, desiccant in bottles and blister films. These components protect the drug from the environment.

2. **Secondary Packaging:** Packaging outside the primary packaging used to group primary packages together. Examples include cartons, overwraps for blisters etc.

3. **Tertiary Packaging:** Used for bulk handling, warehouse storage and transport shipping. Most common form is palletized unit load that packs tight containers together.



Packaging System Classification Based on Dosage Capacity of the Container

1. **Single-Unit Container:** Contains only a unit quantity of medication used at one time
2. **Multiple-Unit Container:** Encloses multiple doses and permits multiple withdrawals of oral dosage forms

Special Packaging Currently in Use Includes

1. **Unit-Dose Packaging:** This packaging guarantees safer medication by reducing medication errors; it is also more practical for the

patient. It may be very useful in improving compliance with treatment and may also be useful for less stable products.

2. **Device Packaging:** Packaging with the aid of an administration device is user-friendly and also improves compliance. This type of packaging permits easier administration by means of devices such as pre-filled syringes, droppers, transdermal delivery systems, pumps and aerosol sprays. Such devices ensure that the medicinal product is administered correctly and in the right amount.

Types of Packaging Materials

1. Glass

- Widely used for drug packing
- Composed of soda ash, sand, limestone and cullet
- Preparation of glass includes: Si, Al, Na, K, Ca, Mg, Zn & Ba

Advantages

- Hygienic
- Suitable for sterilisation
- Relatively non-reactive
- Transparent
- Accept variety of closures
- Good protection power
- Easily labelled
- Can be used on high speed packaging lines
- Do not deteriorate with age
- Impermeable to atmospheric gases and moisture
- Withstand various temperature and pressure in sterilisation
- Economical
- Easily available
- Can be sealed hermetically or by removing closures
- Neutral after proper treatment

Disadvantages

- Relatively heavy
- Fragile so easily can be broken
- Release alkali to aqueous preparations

- Photosensitive drug cannot be protected in the transparent glass container. Amber colour glass container is required in this case
- As glass is a chemical substance, sometime it reacts with the product contained inside it

Types of Glass

1. **Type I (Neutral or Borosilicate Glass)**
    - Borosilicate Glass is produced by replacing the sodium oxide by boric oxide (B2O2) and some lime by alumina (Al2O3) in the basic components of glass
    - Least reactive
    - High melting point and can withstand high temperature
    - Resistant to chemical substance
    - Higher ingredients and processing cost therefore used for more sensitive pharmaceutical products such as parenteral or blood products
    - Mostly ampoules and vials are made up of Type I glass
2. **Type II (Treated Soda Lime Glass)**
    - Type II glass is made from commercial soda-lime glass that has been de-alkalized or treated to remove surface alkali
    - The de-alkalizing process is known as "Sulfur treatment"
    - Sulfur treatment neutralizes the alkaline oxides on the surface, rendering the glass more chemically resistant
    - Higher chemical resistance but not as much as type I
    - Cheaper than Type I
    - Acceptable for most products accept blood products and aqueous pharmaceutical with a pH less than 7
3. **Type III (Soda Lime Glass)**
    - It is ordinarily glass prepared from silicon dioxide, soda ash and lime stone and is generally referred to as soda- lime glass
    - Glass containers are untreated and made of commercial soda-lime glass of average or better than average chemical resistance
    - It is cheapest in quality
    - This type of glass is not suitable for alkali sensitive products

- Has average or slight better than average resistance and is suitable for non- aqueous parenteral and non-parenteral products
- Type III glass containers are normally dry sterilized before being filled

4. Type IV (General Purpose Soda Lime Glass)

- It is general purpose soda-lime glass used for oral and topical preparation
- It has lowest hydraulic resistance and is suitable for solid products, some liquids and semi solids and not for parenteral

5. Coloured Glass

- Coloured glass is obtained by adding small amounts of metals during fusion of glass
- Coloured glass is used for light sensitive products which do not allow the UV rays to pass through it
- Coloured glass should not be used for parenteral preparation because it becomes difficult to check clarity in such preparations

6. Neutral Glass

- It is another commercial variety of glass available in between soda-lime glass and borosilicate glass
- It is resistant to alkalis
- Resistant to weathering
- Withstand to autoclaving
- It is used for the manufacture of multiple dose vials and transfusion bottles etc.

2. Plastics

- They are used as primary and secondary packaging materials. Plastics are made of polymers which are

1. Durable
2. Easily moulded into varieties of shapes and sizes
3. Flexible Unbreakable and Biocompatible in many applications
4. These materials can be transformed into finished products, such as bottles, containers, films, hoses, coatings, lacquers, etc.
5. Mostly all types of dosage forms solid, liquid, and semi-solids dosage forms

Advantages

- Low in cost
- Light in weight
- Durable
- Pleasant to touch
- Flexible facilitating product dispensing
- Odourless and inert to most chemicals
- Unbreakable
- Leak proof
- Able to retain their shape throughout their use
- They have a unique suck-back feature, which prevents product doze
- Ease of transportation
- They are poor conductor of heat
- They are resistant to inorganic chemicals
- They have good protection power

Disadvantages

- Plastics appear to have certain disadvantages like interaction, adsorption, absorption lightness and hence poor physical stability
- All are permeable to some degree to moisture, oxygen, carbon dioxide etc. and most exhibit electrostatic attraction, allow penetration of light rays unless pigmented, black etc.
- **Other Negative Features Include: Stress Cracking:** A phenomenon related to low density polythene and certain stress cracking agents such as wetting agents, detergents and some volatile oils
- **Panelling or Cavitation:** Where by a container inward distortion or partial collapse owing to absorption causing swelling of the plastic dimpling following a steam autoclaving operation
- **Crazing:** A surface reticulation which can occur particularly with polystyrene and chemical substances (e.g. isopropyl myristate which first causes crazing and ultimately reaches of total embitterment and disintegration)
- **Poor Key of Print:** Certain plastics such as the poly olefins need pre-treating before ink will key. Additives that migrate to the surface of the plastic may also cause printing problem

- **Poor Impact Resistance:** Both polystyrene and PVC have poor resistance. This can be improved by the inclusion of impact modifiers such as rubber in case of polystyrene and methyl methacrylate butadiene styrene for PVC

Types of Plastic Materials

1. **Thermoplastic Type:** On heating, they are softening to viscous fluid which hardens again on cooling. Resistant to breakage and cheap to produce and providing the right plastics are chosen will provide the necessary protection of the product in an attractive containers. E.g. polyethylene, PVC, polystyrene, polypropylene, polyamide, polycarbonate
2. **Thermosetting Type:** When heated, they may become flexible but they do not become liquid. During heating such materials form permanent crosslinks between the linear chains, resulting in solidification and loss of plastic flow. E.g. Phenol formaldehyde, urea formaldehyde, melamine formaldehyde

Polyethylene

- This is used as high and low density polyethylene
- Low density polyethylene (LDPE) is preferred plastic for squeeze bottles. Properties: Ease of processing, barrier to moisture, strength/toughness, flexibility, ease of sealing
- High density poly ethylene (HDPE) is less permeable to gases and more resistant to oils, chemicals and solvents. Properties: Stiffness, strength/toughness, resistance to chemicals. It is widely used in bottles for solid dosage forms. Drawback: prone to stress cracking in the presence of surfactants or vegetable or mineral oils

Polypropylene

- It has good resistance to cracking when flexed
- Good resistance to heat sterilization
- It is colourless, odourless thermoplastic material with excellent tensile properties even at high temperature
- Excellent resistance to strong acids and alkalis
- Low permeability to water vapour
- Permeability to gases is intermediate between polyethylene HD and un-plasticized PVC

- Suitable for use in closures, tablet containers and intravenous bottles

Polyvinyl Chloride

- Versatility, ease of blending, strength/toughness, resistance to grease/oil, resistance to chemicals, clarity
- Used as rigid packaging material and main component of intravenous bags
- **Drawback:** Poor impact resistance which can be improved by adding elastomers to the plastics but it will increase its permeability

Poly Vinylidene Chloride (PVDC)

- Excellent barrier properties against: moisture, water vapour, UV light, aroma, inorganic acids, alkalis, aqueous salt solutions, organic water soluble acids, aliphatic hydrocarbons, esters of long chain fatty acids, detergent base materials, emulsifying agents and wetting agents
- Good thermoform ability
- PVDC is very cost-effective, as coating weight can be customized depending on the requirements of the barrier properties
- Medical grade and non-toxic
- High levels of transparency which improves the aesthetics of the product

Polystyrene

- Versatility, insulation, clarity, easily foamed ("Styrofoam")
- It is also used for jars for ointments and creams with low water content
- Drawback: Chemicals like isopropyl myristate produce crazing (a fine network of surface cracks) followed by weakening and eventually collapsible of the container

Product-Plastic Interactions

1. Permeation
2. Leaching
3. Sorption
4. Chemical reaction
5. Alteration in the physical properties of plastics or products

Constituents of Plastic Containers

1. Monomer residues
2. Catalysts
3. Accelerators
4. Solvents
5. Extenders
6. Fillers
7. Slip additives
8. Anti-slip additives
9. Antistatic agents
10. Anti-blocking agents
11. Release agents

1. Metals

- Metal containers are used solely for medicinal products for non-parenteral administration
- Metal is strong, opaque, impermeable to moisture, gases, odors, light, bacteria, and shatterproof, it is the ideal packaging material for pressurized containers
- It is resistant to high and low temperatures
- They include tubes, packs made from foil or blisters, cans, and aerosol and gas cylinders
- Aluminium and stainless steel are the metals of choice for both primary and secondary packaging for medicinal products
- Form an excellent tamper evident containers

A) Aluminium

- It is relatively light yet strong
- Barrier to light and chemicals
- Impermeable and easy to work into a variety of formats, depending on its thickness
- Thickest aluminium is used for rigid containers such as aerosol cans and tubes for effervescent tablets
- Intermediate thickness is when mechanical integrity is still important but the pack should be capable of being reformed under a

reasonable force. E.g. Collapsible tubes for semi solid preparations or roll on screw caps.

- Thinnest aluminium is used in flexible foils that are usually a component of laminated packaging material
- **Disadvantages and Their Overcome Solution:** Major disadvantage is its reactivity in raw state, although it rapidly forms a protective film of aluminium oxide it is still liable to corrosion (when exposed to some liquids and semi solid formulations, particularly at extreme pH or if the product contains electrolytes.
- **Overcome:** To overcome this problem, Aluminium is lined with epoxide, vinyl or phenolic resins. They are work hardening like collapsible tubes are made by impact extrusion which tends to make aluminium less flexible. Overcome: To overcome, flexibility has to restore by an annealing stage.

B) **Tin:** Tin containers are preferred for food, pharmaceuticals and any product for which purity is considered. It is the most chemically Inert of all collapsible metal tubes.

C) **Lead:** Lead has the lowest cost of all tube metals and is widely used for non-food products such as adhesives, inks. Paints and lubricants. It should never be used alone for anything taken internally because of the risk lead poison. With internal linings, lead tubes are used for products such as chloride tooth paste.

D) **Linings:** If the product is not compatible with bare metal, the interior can be flushed with wax-type formulation or with resin solutions, although the resins or lacquers are usually sprayed on. A tube with an epoxy lining costs about 25% more than the same tube uncoated. Wax linings are most often used with water-based products in tin tubes, and phenolic, epoxides, and vinyl are used with aluminium tubes, giving better protection than wax, but at a higher cost.

2. Rubber

- Excellent material for forming seals, used to form closures such as bungs for vials or in similar applications such as gaskets in aerosol cans.

Categories of Rubbers:

1) **Natural Rubbers:** Suitable for multiple use closures for injectable products as rubber reseals after multiple insertion of needle.

Disadvantages are:

- It doesn't well tolerate multiple autoclaving becoming brittle and leads to relative degree of extractable material in presence of additives
- Risk of product absorbing on or in to a rubber
- It has certain degree of moisture & gas permeation

2) Synthetic Rubber: Have fewer additives and thus fewer extractable and tends to experience less sorption of product ingredients.

- Are less suitable for repeated insertions of needle because they tend to fragment or core pushing small particles of the rubber in to the product. E.g. Silicone, butyl, bromobutyl, chlorobutyl etc.
- Silicone is least reactive but it does experience permeability to moisture and gas. Softer rubbers experience less coring and reseal better, harder rubbers are easier to process on high speed packaging lines.

3. Fibrous Materials

- The fibrous materials are the important part of pharmaceutical packaging.
- Fibrous materials include: Papers, Labels, Cartons, Bags, and Outers etc.

The Applications as well as Advantages of Cartons include:

- Increases display area
- Provides better stacking for display of stock items
- Assembles leaflets
- Provides physical protection especially to items like metal collapsible tubes
- Fibberboard outers either as solid or corrugated board also find substantial application for bulk shipments
- Regenerated cellulose film, trade names Cellophane & Rayophane, is used for either individual cartons or to assemble a no. of cartons

4. Films, Foils and Laminates

- Applicable to tablets, capsules, pills, etc.
- It's a good substitute for PVC sheet

- No cracking, delamination or pinholes
- It has the quite good blocking properties effectively protecting drugs from water vapour, oxygen and ultraviolet
- It can extend the storage period of drugs
- It is particularly suitable for packing moisture-sensitive drugs or those sold in the hot and humid areas
- Taking out a part of the drugs from the drug boards without any impact on other well-packaged drugs
- It is used by cold-moulding packaging machines
- It is shaped easily by changing the mould
- Nice appearance can upgrade drug's image

5. Blister Pack

- Blister packaging is a type of pre-formed plastic packaging commonly used as unit dose packaging for pharmaceuticals such as tablets, capsules or lozenges

Blister packs consist of two principal components:

1) The cavity made from either plastic or aluminium
2) The lidding, made from paper board, paper, plastic or aluminium. The cavity contains the product and the lidding seals the product in the package.

There are two types of forming the cavity into a base web sheet: thermoforming and cold forming:

a) Thermoforming: In the case of thermoforming, a plastic film or sheet is unwound from the reel and guided though pre-heating station on the blister line. The temperature of the pre-heating plates (upper and lower plates) is such that the plastic will soften and become mouldable

b) Cold Forming: In the case of cold forming, an aluminium-based laminate film is simply pressed into a mould by means of a stamp. The aluminium will be elongated and maintain the formed shape. Advantage of cold form foil blisters is that the use of aluminium is offering a near complete barrier for water and oxygen, allowing an extended product expiry date.

- The disadvantages of cold form foil blisters are the slower speed of production compared to thermoforming and the lack of transparency of the package and the larger size of the blister card.

Materials used in blister packaging:

1. PVC (Polyvinyl Chloride)
2. PCTFE (Polychlorotrifluroethylene)
3. COC (Cyclic olefin copolymers)
4. PVDC (Polyvinylidene chloride)
5. PP (polypropylene)
6. PE (polyethylene)
7. PETg (glycol-modified polyethylene terephthalate)

Advantages

1. Product integrity
2. Product protection
3. Tamper evidence
4. Reduce possibility of accidental misuse
5. Patient compliance

Strip Packaging

- Strip packaging is an alternative form of pack for a unit dosage.
- It is a method of enclosing the product concerned between the two web of material so that each is contained between separate compartment
- Two web of material may not be necessary to be identical
- It is commonly used for the packaging of tablets and capsules
- A strip package is formed by feeding two webs of a heat sealable flexible film through a heated crimping roller
- The product is dropped into the pocket formed before forming the final set of seals
- A continuous strip of packets is formed which is cut to the desired number of packets in length
- The materials used for strip package are cellophane, polyester, polyethylene, polypropylene, polyvinylchloride

Closures

- Closures are the devices by means of which containers can be opened and closed

- Proper closing of the container is necessary because
- It prevents loss of material by spilling or volatilization
- It avoids contamination of the product from dirt, microorganisms or insects
- It prevents deterioration of the product from the effect of the environment such as moisture, oxygen or carbon dioxide
- **Material used for Closures are:** The closures for containers meant for storage of pharmaceutical products are generally made from the following basic materials
- *Cork *Glass *Plastic *Metal

Quality Tests for Packaging Materials

- The majority of chemical testing is required on primary component
- The type of testing required depends on the type of component used

1) **Glass, Vials and Ampules:** The USPXXII requirements for glass containers are chemical resistance and light transmission. The requirements vary from country to country.
2) **Plastic Primary Components:** The testing is more extensive with plastic components, requiring both biological and physicochemical test. This is because the plastic components contain other substance such as plasticizers, stabilizers, antioxidants, pigment, lubricants, etc.
3) **Water Attack Test:** This test is used only with containers that have been exposed to sulphur dioxide fumes under controlled humidity conditions. Such a treatment neutralizes the surface alkali. Now the glass becomes chemically more resistant. The principle involved in the water attack test is to determine whether the alkali leached form the surface of a container is within the specified limits or not. Since the inner surface is under test entire container (ampoule) has to be used. The amount of acid that is necessary to neutralize the released alkali from the surface is estimated, the leaching of alkali is accelerated using elevated temperature for a specified time. Methyl red indicator is used to determine the end point. The basic is acid-base titration.

Tests for Glass Containers

1. **Powdered Glass Test:** It is done to estimate the amount of alkali leached from the powdered glass which usually happens at the

elevated temperatures. When the glass is powdered, leaching of alkali is enhanced, which can be titrated with 0.02N sulphuric acid using methyl red as an indicator.

- **Step 1: Preparation of Glass Specimen:** Few containers are rinsed thoroughly with purified water and dried with stream of clean air. Grind the containers in a mortar to a fine powder and pass through sieve no. 20 and 50.
- **Step 2: Washing the Specimen:** 10gm of the above specimen is taken into 250 ml conical flask and wash it with 30 ml acetone. Repeat the washing, decant the acetone and dried after which it is used within 48hr.
- **Procedure:** 10gm sample is added with 50ml of high purity water in a 250ml flask. Place it in an autoclave at 121 ^{o}C ± 2 ^{o}C for 30min.Cool it under running water. Decant the solution into another flask, wash again with 15ml high purity water and again decant. Titrate immediately with 0.02N sulphuric acid using methyl red as an indicator and record the volume.

2. Hydrolytic Resistance of Glass Containers

- Rinse each container at least 3 times with CO2 free water and fill with the same to their filling volume
- Also fill & Cover the vials and bottles and keep in autoclave
- Heat to 100 ^{o}C for 10min and allow the steam to issue from the vent cork. Rise the temp from 100 ^{o}C to 121 ^{o}C over 20min
- Maintain the temp at 121 C to 122 ^{o}C for 60min
- Lower the temp from 121 ^{o}C to 100 Cover 40 min venting to prevent vacuum
- Remove the container from autoclave, cool and combine the liquids being examined
- Measure the volume of test solution into a conical flask and titrate with 0.01M HCl using methyl red as an indicator
- Perform blank with water and the difference between the titration represents the volume of HCl consumed by the test solution.

3. Arsenic Test

- This test is for glass containers intended for aqueous parenterals
- Wash the inner and outer surface of container with fresh distilled

water for 5 min. Prep test as described in the test for hydrolytic resistance for an adequate no. of samples to produce 50ml

- Pipette out 10ml solution from combined contents of all ampoules to the flask
- Add 10ml of HNO3 to dryness on the water bath dry the residue in an oven at 130 ^{o}C for 30min cool and add 10ml hydrogen molybdate reagent
- Swirl to dissolve and heat under water bath and reflux for 25min
- Cool to room temp and determine the absorbance at 840nm
- Do the blank with 10ml hydrogen molybdate
- The absorbance of the test solution should not exceed the absorbance obtained by repeating the determination using 0.1ml of arsenic standard solution (10ppm) in place of test soln

4. Thermal Shock Test

- Place the samples in upright position in a tray
- Immerse the tray into a hot water for a given time and transfers to cold water bath, temp of both are closely controlled
- Examine cracks or breaks before and after the test
- The amount of thermal shock a bottle can withstand depends on its size, design and glass distribution
- Small bottles withstand a temp differential of 60 to 80 ^{o}C and 1 pint bottle 30 to 40 ^{o}C.
- A typical test uses 45 C temp difference between hot and cold water.

5. Internal Bursting Pressure Test

- The most common instrument used is American glass research increment pressure tester
- The test bottle is filled with water and placed inside the test chamber
- A scaling head is applied and the internal pressure automatically raised by a series of increments each of which is held for a set of time
- The bottle can be checked for predetermined pressure level and the test continues until the container finally bursts

6. Leakage Test

- Drug filled container is placed in a container filled with coloured solution (due to addition of dye)which is at high pressure compared to the pressure inside the glass container so that the coloured solution enters the container if any cracks or any breakage is present.

Test for Rubbers

1. Fragmentation Test

- Place a 4ml of water in each of 12 clean vials
- Close a vial with closure and secure caps for 16 hrs
- Pierce the closure with 21 SWG hypodermic needle. Repeat the operation 4 times for each closure
- Count the number of fragment visible on the rubber
- Total number of fragment should not be more than 10 except butyl rubber

Tests For Closures: Preparation of Sample (SOL-A): Wash closures in 0.2% w/v of anionic surface active agents for 5min. Rinse 5 times with distilled water and add 200ml water and is subjected to autoclave at 119 to 123^{o}C for 20 to 30min covering with aluminium foil. Cool and separate solution from closure (solution-A)

1. **Sterility Test:** When treated closures are subjected to sterilization test at 64-66 ^{o}C and a pressure of about 0.7 KPa for 24hr
2. **Fragmentation Test:** For closures for aqueous place a vol of water corresponding to the nominal vol minus 4 ml in each of 12 clean vials. Close the vials with the 'prepared' closures & allow to stand for 16 hours. For closures for dry preparations close 12 clean vials with the 'prepared' closures. Using a hypodermic needle with an external diameter of 0.8 mm inject 1 ml of water into the vial and remove 1 ml of air. Carry out this operation 4 times with new needle each time Pass the liquid in the vials through a filter with a pores size of 0.5 µm. No. of fragments is NMT 10 except in the case of butyl rubber closures where the total no. of fragments is NMT 15
3. **Self-Sealability Test:** This test is applicable to closures intended to be used with water close the vials with the 'Prepared' closures. For each closure, use a new hypodermic needle with an external

diameter of 0.8 mm & pierce the closure 10 times, each time at a different site. Immerse the vials upright in a 0.1% w/v solution of methylene blue & reduce the external pressure by 27KPa for 10 min. Restore the atmospheric pressure and leave the vials immersed for 30 minutes. Rinse the outside of the vials. None of the vials contains any trace of coloured solution

4. **PH of Aqueous Extract:** 20ml of solution A is added with 0.1ml bromothymol blue when it is added with a small amount of 0.01M NaOH which changes the colour from blue to yellow. The volume of NaOH required is NMT 0.3ml and if it is done with HCl, the volume of HCl needed should NMT 0.8ml
5. **Light Absorption Test:** It must be done within 4hrs of preparing solution A. It is filtered through 0.5µ filter and its absorbance is measured at 220 to 360nm.Blank is done without closures and absorbance is NMT 2.0
6. **Reducing Substances:** 20ml of solution A is added with 1ml of 1M H2SO4 and 20ml of 0.002M KMnO4 and boil for 3min then cool and add 1gm of potassium iodide which is titrated with sodium thio-sulphate using starch as an indicator. Blank is done and the difference between titration volumes is NMT 0.7ml.
7. **Residue on Evaporation:** 50ml of solution A is evaporated to dryness at 105 ºC. Then weigh the residue NMT 4mg

Test for Plastics

1. **Leakage Test/Collapsibility Test:** Applicable to containers which are to be squeezed in order to remove contents. Yield 90% of its contents at required rate of flow at ambient temp. Fill 10 containers with water Fit with closures keep them inverted at room temp. 24hrs No signs of leakage
2. **Clarity of Aqueous Extract:** Clarity of aqueous extract Select unlabelled portion from a suitable containers Cut these portions into strips Wash it with extraneous matter by shaking with two separate portions of distilled water Transfer to flask-previously washed with chromic acid Rinse with distilled water add 250ml d.w. Cover the flask autoclave at 121Ċ, 30min Colourless, free from turbidity.
3. **Transparency:** Fill 5 containers with dil. Suspension. The cloudiness of the diluted suspension in each container is detectable when viewed through the containers as compared with a container of the same type filled with water Evaporate 100ml extract Allow it to dry at 105Ċ Residue weighs not more than 12.5mg

4. **Water Vapour Permeability:** Fill 5 containers with nominal volume of water and heat seal the bottles with aluminium foil. Weigh accurately each container and allowed to stand for 14 days humidity- 60±5% temp. 20Ċ and 25Ċ. Reweigh the containers. Loss in weight in each container is NMT 0.2% Specifications and tests of plastic container materials: Barium Heavy metals Tin zinc.

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Chapter - 4
Formulation and Comparative Evaluation of Sublingual Tablets of Ondansetron Hydrochloride

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Chapter - 4

Formulation and Comparative Evaluation of Sublingual Tablets of Ondansetron Hydrochloride

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Abstract

Sublingual tablets were prepared as novel formula using two different methods called direct compression and sublimation method. The sublingual route is capable of producing a rapid onset of action. Ondansetron hydrochloride is a selective inhibitor of type 3 serotonin (5-hydroxytryptamine) receptors (5-HT_3) that exhibits antiemetic activity. Formulations are evaluated for physicochemical parameters, disintegration time (DT), dissolution, wetting time. Accelerated Stability studies were carried out at 40±20 °C/75%±5% RH for 6months. Compatibility studies of drug and polymer were performed by DSC and FTIR which shows that there is no possible interaction between them. Preformulation study of API was satisfactory. Compressibility and flow properties were in acceptable range. Post compressional parameters were found to be satisfactory. In case of direct compression and sublimation method DT was found to be within 30-60sec and 22-55sec while wetting time was found to be 14sec and 10sec respectively. Tablets prepared by sublimation method were vacuum dried at 60 °C for 12hrs, generating pores. Direct compression containing crospovidone (6%w/w), sublimation containing 6% w/w camphor and marketed product showed the maximum release of 92%, 100% and 95% in 20 min respectively. Dissolution profile of sublimation method is better than direct compression and marketed product which follows zero order release kinetics with Fickian diffusion. The optimized formulation was found to be stable when exposed to stability studies. Sublimation method showed better results because of increased porosity of the tablets due to which the absorption of water took place resulting in rapid disintegration forming solution when in contact with saliva leading to quick absorption.

Keywords: Sublimation, direct compression, sublingual, stability, Ondansetron

1. Introduction

Treatment of many acute diseases or a chronic illness has been mostly achieved by delivering drugs using various pharmaceutical dosage forms along with many formulations, including tablets, capsules, pills, suppositories. Oral route is perhaps the most preferred to the paediatric and geriatric patients. However this route provides problems for a few drugs which include GIT-pH conditions and the enzymes bound to GIT membranes are a few factors responsible for the bioavailability problems. Also the problem of first pass metabolism is one of the disadvantages leading to poor bioavailability. Certain problems can be solved by modifying the formulation or by changing the routes of administration. Parenteral, mucosal and transdermal routes circumvent hepatic first-pass metabolism which also provide alternative routes for the systemic delivery of drugs [1]. One of the formulations are tablets which are intended for oral administration. Some are swallowed, chewed, dissolved or dispersed in water before being administered and some are retained in the mouth where the active substance is liberated.

Advantages of Tablet as a Dosage form

1) It is more convenient to the patient because of easy administration
2) Tablets are essentially dry dosage forms having longer shelf life than other formulations
3) It provides different drug release pattern
4) Manufacturing of tablets is a cost-effective process
5) The tablet form is easy to handle and transport

Looking at the anatomy of oral cavity it is the first part of digestive system of human body and also has good accessibility and reasonable patient compliance.

The fluids secreted in the oral cavity are-

1) **Saliva:** The total average volume of saliva produced daily in an adult is around 750 ml. The flow rate of saliva differs and varies with different factors. The average resting flow rate for whole saliva is 0.3 ml/min (range 0.1-0.5 ml/min). Chemically, saliva is 99.5% water and 0.5% solutes.
2) **Crevicular Fluid:** The crevicular fluid it is a fluid secreted from the gingival glands of oral cavity.
3) **Mucus:** It is a thick secretion composed mainly of water, electrolytes and a mixture of several glycoprotein, which themselves are

composed of large polysaccharides bound with smaller quantities of protein. It is secreted over many biological membranes of body for example, throughout the gastrointestinal tract walls. Mucus is secreted by special type of epithelia called mucosa.

All the layers of the oral mucosal membranes contain a large amount of protein in the form of keratin. Keratinized and non-keratinized tissues (table 1) occupy about 50% and 30% respectively of the total surface area of the mouth [1, 2].

Table 1: Composition and state of keratinization of oral mucosa

Tissue	State of Keratinization	Composition
Buccal mucosa	Non-keratinized	Few neutral, but mainly polar lipids, particularly cholesterol sulphate and glucosylceramides
Sublingual mucosa	Non-keratinized	
Gingiva mucosa	Keratinized	Neutral lipids i.e., ceramides
Palatal mucosa	Keratinized	

Sublingual glands also known as the salivary glands are present in the floor of mouth below the tongue producing mucin which helps to promote the production of saliva. Because of the secretion of the glands, the interior area of the mouth is kept lubricated, which is necessary for chewing and swallowing food. The lubrication and binding functions of the sublingual glands are important. A secretion from the glands mix with food as it is chewed, making the material slippery and easily swallowed. Because of the saliva content of the masticated food, it can move without difficulty into the throat and on to the digestive tract. Low levels of saliva production decrease swallowing and will increase the potential for food to lodge in the throat [2].

Absorption means transfer of drug from its site of administration to the systemic circulation, so it is obvious that absorption is directly proportional to the membrane layer thickness. The mucosal layer thickness of Sublingual > Buccal > Gingival > Palatal are 100-200, 200, 250, 500-600 micrometer respectively. Because of the high permeability and the rich blood supply, the sublingual route is capable of producing a rapid onset of action which makes it an appropriate route for drugs with short delivery period and in frequent dosing regimen. The drug is released in to saliva and its subsequent spreading may cause the drug to be absorbed across the oral cavity.

Drug absorption through sublingual route involves placing the drug under the tongue which is rapidly absorbed via the blood vessels. Majority of sublingual formulations are absorbed by simple diffusion; where the

sublingual area acting like tissue paper, rapidly soak the substance. Mechanism of drug absorption occurs via oral mucosa, and transported through the facial veins, internal jugular vein, and brachiocephalic vein and then drained in to systemic circulation. This involves passive diffusion into the lipoidal membrane. The absorption of the drug through the sublingual route is 3 to 10 times more than oral route and is only surpassed by hypodermic injection. For such formulations, the small volume of saliva is sufficient to result in tablet disintegration in the oral cavity. Sublingual absorption is mostly rapid in action, but also short acting in duration.

Process is required for drug absorption through sublingual route. Small particles which dissolve in water, does not process problem in permeation and diffusion, and so move freely between the tissues of the body. Active transportation leads to rapid metabolism.

Fast-Disintegrating Sublingual Tablets as Drug Delivery System

Paediatric and geriatric patients find it easy to have tablets that disintegrate or dissolve rapidly in the patient's mouth which skips difficulty in swallowing and in situations where liquids are not available. Only small volume of saliva is sufficient for tablet disintegration in the oral cavity. The medication can then be absorbed partially or entirely into the systemic circulation from blood vessels in the sublingual mucosa (fig. 1) [3].

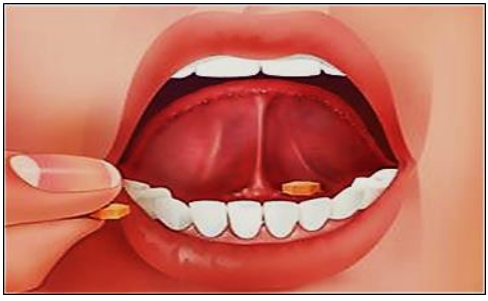


Fig 1: Sublingual route of administration

Advantages

- A relatively rapid onset of action possible as compared to the oral route
- Liver is bypassed and also drug is protected from degradation
- Improved patient compliance
- Low dosage gives high efficacy as hepatic first pass metabolism is avoided and also reduces the risk of side effects

- The large contact surface of the oral cavity contributes to rapid and extensive drug absorption
- Due to rapidity in action these sublingual dosage forms are widely used in emergency conditions
- Rapid absorption and higher blood levels due to high vascularization
- They also present the advantage of providing fast dissolution or disintegration in the oral cavity, without the need for water or chewing

Disadvantages

- Sublingual administration of drugs interferes with eating, drinking and talking; this route is generally considered unsuitable for prolonged administration
- Only small doses can be accommodated easily
- Not suitable for sustain release formulations
- Patient is uncooperative or unconscious
- The patient should not smoke while taking Sublingual medication, because smoking causes vasoconstriction of the blood vessels. This will decrease the absorption of the medication

Factors Affecting the Sublingual Absorption

- **Lipophilicity of Drug:** For a drug to be absorbed completely through sublingual route, the drug must have slightly higher lipid solubility than that required for GI absorption is necessary for passive permeation
- **Solubility in Salivary Secretion:** In addition to high lipid solubility, the drug should be soluble in aqueous buccal fluids i.e. biphasic solubility of drug is necessary for absorption
- **pH and pKa of the Saliva:** As the mean pH of the saliva is 6.0, this pH favors the absorption of drugs which remain unionized. Also, the absorption of the drugs through the oral mucosa occurs if the pKa is greater than 2 for an acid and less than 10 for a base
- **Binding to Oral Mucosa:** Systemic availability of drugs that bind to oral mucosa is poor
- **Thickness of Oral Epithelium:** As the thickness of sublingual epithelium is 100-200μm which is less as compared to buccal thickness. So the absorption of drugs is faster due to thinner

epithelium and also the immersion of drug in smaller volume of saliva

- **Oil to Water Partition Coefficient:** Compounds with favorable oil to water partition coefficients are readily absorbed through the oral mucosa. An oil to water partition coefficient range of 40 to 2000 is considered optimal for the drugs to be absorbed sublingually [2]

Method of Preparation of Sublingual Tablet Formulations

Various techniques can be used to formulate sublingual tablets. Direct compression is one of the techniques which require incorporation of a superdisintegrants into the formulation, or the use of highly water soluble excipients to achieve fast tablet disintegration. Direct compression does not require the use of water or heat during the formulation procedure and is the ideal method for moisture and heat labile medications. Conventional equipment, commonly available excipients and a limited number of processing steps are involved in direct compression. Also high doses can be accommodated and final weight of tablet can easily exceed that of other production methods.

The disintegration and solubilisation of directly compressible tablets depends on single or combined action of disintegrants, water soluble excipients and effervescent agent. Disintegration efficacy is strongly affected by tablet size and hardness. Large and hard tablets have disintegration time more than that usually required. As a consequence, products with optimal disintegration properties often have medium to small size and /or high friability and low hardness [3]. The major drugs which are currently marketed as sublingual tablets are in table 2.

Present research work deals with developing sublingual tablet formulation of ondansetron hydrochloride (OND HCl) which has antiemetic action, enhancing the bioavailability and bypassing the first pass effect.

Table 2: Composition of tablet with dosage limit

Sublingual		
Brand Names	**Tablet**	**Dose**
Tenormin	Isoproterenol HCl	10-15 mg
Isordil	Isosorbide di nitrate	25-5 mg
Nitroquick	Nitroglycerin	0.15-0.6 mg
Microtab	Nicotine	2mg
Subuter	Buprenorphine	2-8mg

2. Material Methods

Pure API is obtained from Gen Pharma Ltd; rest all the raw materials are obtained from college source.

Methods

1. Preformulation Study of the Drug

Organoleptic Properties: The Drug was studied for organoleptic properties such as color, odour and appearance.

Melting Point: The melting point of drug was determined by melting point apparatus using capillary method.

UV-Spectroscopy: Stock solution (100 μg/ml) of OND HCl was prepared in 6.8 pH phosphate buffer. This solution was further diluted, UV spectrum was recorded in the range 200-400nm by using UV double beam spectrophotometer. The wavelength of maximum absorption (λ max) was determined. From the stock solution (100 μg/ml) of drug standard solutions in the range 4-24μg/ml were prepared by appropriate dilution with 6.8 pH phosphate buffer. The absorbance of each standard solution was determined spectrophotometrically. Beer-Lambert's plot was constructed.

2. Compatibility Studies [3, 4]

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a useful analytical technique utilized to check the chemical interaction between the drug and other excipients used in the formulation. The sample (1mg) was powdered and mixed with 10mg of dry powder of potassium bromide this mixture was taken in a sampler and the spectrum was recorded by scanning in the wavelength region of 4000-400cm^{-1}.

Powder X-Ray Diffraction (PXRD)

PXRD patterns for drug, physical mixture, solid dispersion and formulation were recorded using D_8 advanced model of Bruker Axs Company fitted with a copper target, a voltage of 40 kV, and a current of 30 mA. The scanning rate was 1°/min over a 2 θ range of 1-50°.

Differential Scanning Calorimetry (DSC)

DSC analysis of the samples was carried out using JAPEDSC, Perkin, Elmer (USA). Samples (6.5-10 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10 °C/min over the temperature range of 5 and 300 °C. DSC analysis was carried out under nitrogen gas flow of 20 lb/in.

Scanning Electron Microscopy (SEM)

Surface morphology of drug was determined by SEM (Juol JSM 6360A apparatus). The photo was obtained at different magnification levels to study the surface morphology.

3. Characterization of Durg Polymer [5-8]

Identification

Organoleptic properties, melting point and FTIR spectroscopy were used as identification tests as mentioned in preformulation study of the drug.

Determination of Powder Characteristics

Samples were subjected for studies of powder characteristics like bulk density, tapped density, compressibility index and angle of repose.

4. Development of Fast Disintegrating Sublingual Tablet [5-9]

Preparation of Tablet by Direct Compression

Sublingual tablets were prepared by direct compression (table 3) method using different excipients like lactose (binder), aspartame (sweetening agent), Cross carmellose sodium, sodium starch glycolate, crospovidone (super disintegrant), magnesium stearate (Lubricant), micro crystalline cellulose (diluent). Compositions of various formulations are shown in table. All the ingredients were weighed and mixed in mortar with the help of pestle. Then the blended material was slightly compressed on the 7mm flat biconvex punch using a tablet machine. The total weight of the formulation was maintained 150 mg per tablet.

Table 3: Formula for Direct compression

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
OND HCL	4	4	4	4	4	4	4	4	4
CCS	3	6	9	-	-	-	-	-	-
SSG	-	-	-	3	6	9	-	-	-
CP	-	-	-	-	-	-	3	6	9
MCC (102)	101	96	91	101	96	91	101	96	91
Mannitol	38	40	42	38	40	42	38	40	42
Aspartame	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Flavour	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mg stearate	1	1	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1	1	1
Total	150	150	150	150	150	150	150	150	150

Preparation of Tablet by Sublimation

Fast dissolving sublingual tablet of OND HCl were prepared by sublimation method using camphor as sublimating agent (table 4). Three formulations of ondansetron containing camphor [6, 8] in different proportions were prepared. All the ingredients were passed through # 60 mesh separately. The drug and the diluents was mixed in small portion of both each time and blending it to get uniform mixture and set aside. The other ingredients were weighed and mixed in geometrical order, mixed thoroughly with lubricant the tablets of 150mg prepared by direct compression using 7mm single punch machine. Then its initial and final weight was taken into consideration and was dried for 5hrs for sublimation of camphor.

Table 4: Formula for Sublimation

Ingredients	F1	F2	F3
OND HCL	4	4	4
Camphor	3	6	9
MCC	89	86	83
Mannitol	50	50	50
Aspartame	1.5	1.5	1.5
Flavour	0.5	0.5	0.5
Mg stearate	1	1	1
Talc	1	1	1
Total	150	150	150

Table 5: Ranges of angle of repose

Flow Property	Angle of Repose
Excellent	25-30
Good	31-35
Fair	36-40
Passable	41-45
Poor	46-55
Very poor	56-65

5. Evaluation of OND HCl Fast Disintegrating Sublingual Tablets [7, 8]

Pre Compression Parameters

Bulk density, tapped density, carr's or compressibility index and angle of repose.

Post Compression Parameters

Hardness

The crushing strength or hardness of the tablets was measured with help of a Monsanto hardness tester and expressed in kg/cm^2.

Thickness

Three tablets were taken from each formulation and their thickness was determined by using screw gauge.

Uniformity of Weight

Weight variation test was done with 20 tablets. It is the individual variation of tablet weight from the average weight of 20 tablets.

Friability

The friability of tablets using 10 tablets as a sample was measured using a Roche Friabilator. Tablets were rotated at 25 rpm for 4 minutes or up to 100 revolutions. The tablets were then reweighed after removal of fines and the percentage of weight loss was calculated.

Drug Content

Two tablets were weighed and finely powdered. The quantity equivalent to 10 mg of drug was weighed accurately and taken in 100 ml volumetric flask. 50ml of 6.8 phosphate buffer was added, shaken for 5 min, made up to 100 ml with 6.8 phosphate buffer, and filtered. 1 ml of above solution was diluted to 10ml in a volumetric flask and the drug concentration was determined at 248 nm by using UV spectrophotometer.

Wetting Time

The wetting time of the tablets was measured using a very simple process. Five circular tissue papers of 10cm diameter were placed in a Petri dish with a 10cm diameter. Ten milliliters of water containing a water-soluble dye (eosin) was added to the Petri dish. A tablet was carefully placed on the surface of tissue paper. The time required for water to reach the upper surface of the tablet was noted as the wetting time.

Water Absorption Ratio

A piece of tissue paper folded twice was kept in a Petri dish (internal diameter 6.5cm) containing 6 mL of purified water. The tablet was placed on the tissue paper and allowed to wet completely. The wetted tablet was removed and reweighted.

In vitro Disintegration Time [9]

Disintegration time for sublingual tablet was determined using USP disintegration apparatus with phosphate buffer of pH 6.8. The volume of medium was 900ml and temp was 37± 0.2 °C. The time in seconds taken for complete disintegration of the tablet with no palatable mass remaining in the apparatus was measured. To comply the test all tablets should disintegrate within 3 minutes.

In vitro Drug Release Study

In vitro drug release study of sublingual fast disintegrating tablets was carried out using the Paddle apparatus method. The dissolution test was carried out using 900 ml of 6.8 pH phosphate buffer, at 37 ± 0.5 ºC and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at 2, 4, 6 up to the 30 min and withdrawn volume was replaced with fresh dissolution media. The % release of drug was calculated.

Drug Release Kinetics [10]

The data of the *in vitro* release was fit into different equations and kinetic models to explain the release kinetics from sublingual tablets.

Stability Study [12-18]

The fast disintegrating sublingual tablets were subjected to stability as per ICH guidelines at 40 ± 1 ºC, 50 ± 1 ºC, 37± 1 ºC and RH 75% ± 5%. The tablets were withdrawn after a period of 15 days and analysed for physical characterization (visual defects, Hardness, friability, disintegrations dissolution etc) and drug content.

3. Result and Discussion

Preformulation Study of Drug

Characterization of Drug

The sample of OND HCl received was studied for its organoleptic characters such as colour, odour, appearance, its melting point. The results are presented in Table 6. Results were found to be similar to that mentioned in literature (table 6).

Table 6: Comparison of results of identification tests

Identification Test	Observed Result	Reported Standard
Appearance	Crystalline powder	Crystalline powder
Colour	White almost white powder	White almost white powder
Odour	Odourless	Odourless
Melting point	231-235°C	232 °C

Standard Calibration Curve in Distilled Water

Standard calibration curve (table 7) of OND HCl in water was obtained by constructing graph between absorbance and concentration (µg/ml). It obeys Beer Lambert's Law within the concentration range of 2-12µg/ml. The linear co-relationship between absorbance and concentration was found in the range of 2-12µg/ml (r^2 =0.9983, y=0.0425) for Distilled water at 248 nm.

Table 7: Calibration curve in Distilled water (n=3)

Conc (µg/ml)	Absorbance
0	0.00
2	0.096±0.02
4	0.1691±0.71
6	0.2645±0.04
8	0.3584±0.01
10	0.4265±0.07
12	0.5075±0.23

Calibration Curve in Phosphate Buffer pH 6.8

Standard calibration curve (table 8) of OND in phosphate buffer pH 6.8 was obtained by constructing graph between absorbance and concentration (µg/ml). It obeys Beer Lambert's Law within the concentration range of 2-12µg/ml. The linear co-relationship between absorbance and concentration was found in the range of 2-12µg/ml (r^2 =0.9983, y=0.0425) for phosphate buffer pH 6.8 at 248 nm.

Table 8: Observation of calibration curve in phosphate buffer pH 6.8 (n=3)

Conc (µg/ml)	Absorbance
0	0.00
2	0.108±0.09
4	0.1763±0.01
6	0.275±0.23
8	0.3368±0.33
10	0.4201±0.01
12	0.5057±0.02

Table 9: Characteristics IR peaks of OND HCl

Functional Group	Frequency (cm^{-1})
C=O	1740
NH	3334 (bending)
C=C	1434 (stretch)

C-H	2900 (bending)
C-N	1227 (stretch)

FTIR Spectroscopy

The spectrum was recorded in the wavelength region of 4000-400 cm^{-1} using FTIR spectrophotometer. The FTIR spectrum of OND HCl is shown in Fig. 2 and interpretation of FT-IR spectra. Characteristic peaks of OND HCl were observed it can be concluded that the IR spectrum of drug complies with its chemical structure given below.

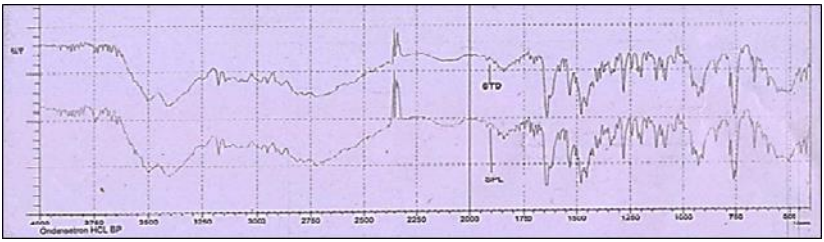
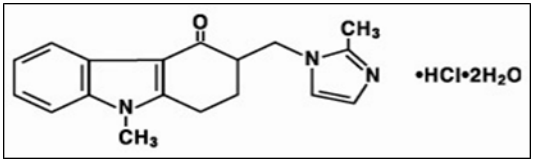


Fig 2: FTIR spectra of OND HCL



OND HCL

XRD

Peaks reveal characteristic crystalline peaks of pure drug as in fig 3.

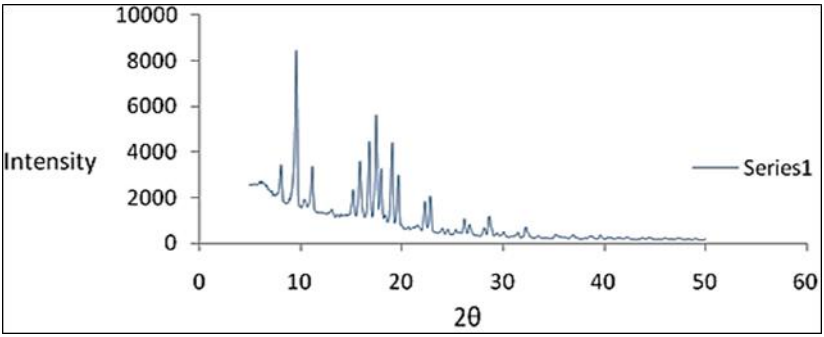


Fig 3: XRD spectra of OND HCL

DSC Analysis

DSC thermogram of OND HCL was shown in fig.4, 5. The DSC

thermogram of OND showed an endothermic peak at 124 ºC corresponding to its melting point.

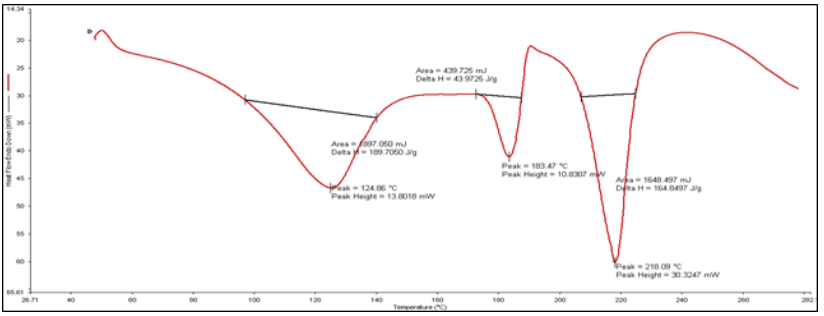


Fig 4: DSC thermogram of OND HCl

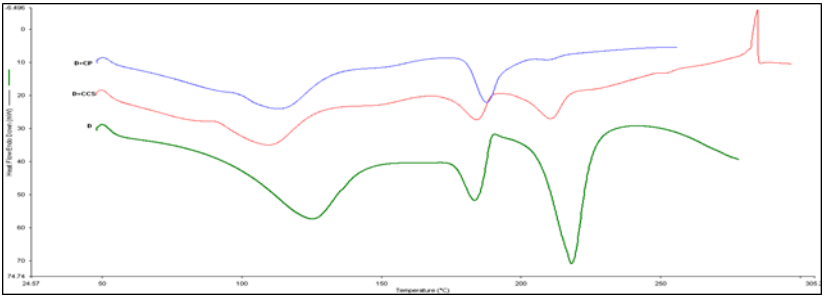


Fig 5: DSC thermogram of placebo, drug, formulation

Evaluation of Ondansetron Sublingual Tablet Formulation [19, 20]

Precompression Parameters

Characterization of Prepared Fast Disintegrating Sublingual Tablets by Direct Compression Method

The results of (table 10) angle of repose and compressibility index (%) ranged from (29.22 to 32.32) and (10.19 to 14.02) respectively. The results of bulk density and tapped density ranged from (0.22 to 0.28) and (0.24 to 0.29) respectively.

Table 10: Evaluation of Precompression parameters by direct compression (n=3)

Formulations	Angle of Repose (θ)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Compressibility (%)	Hausner's Ratio
F1	32.5±0.55	0.22 ±0.16	0.26±0.42	14.87±0.36	1.14±0.16
F2	32.1±0.54	0.28 ±0.12	0.32 ±0.39	11.62±0.34	1.13±0.23
F3	31.4±0.56	0.24 ±0.16	0.24 ±0.14	12.02±0.23	1.12±0.13
F4	32.6±0.82	0.25 ±0.14	0.29 ±0.27	13.62±0.36	1.16±0.14

F5	30.9±0.56	0.25 ±0.14	0.28 ±0.27	10.58±0.30	1.14 ±0.14
F6	30.2±0.56	0.26 ±0.27	0.26 ±0.24	10.59±0.28	1.13 ±0.21
F7	28.8±0.32	0.46 ±0.16	0.30±0.18	13.44±0.36	1.15±0.21
F8	30.4±0.32	0.28 ±0.27	0.28 ±0.16	10.84±0.32	1.12 ±0.21
F9	29.7±0.76	0.26 ±0.15	0.29±0.15	10.46±0.32	1.12 ±0.14

Characterization of Prepared Fast Disintegrating Sublingual Tablets by Sublimation Method

The results (table 11) of angle of repose and compressibility index (%) ranged from (24.22 to 28.32) and (4.9 ± 0.18 to 5.02± 0.16), respectively. The results of bulk density and tapped density ranged from (0.41to 0.44) and (0.42 to 0.46) respectively.

Table 11: Evaluation of Precompression parameters by sublimation (n=3)

Formulations	Angle of Repose (θ)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Compressibility (%)	Hausner's Ratio
F1	24.54±0.55	0.436±0.16	0.459±0.42	5.011±0.19	0.949±0.15
F2	25.56±0.54	0.411±0.12	0.432±0.35	4.861±0.13	0.951±0.21
F3	23.17±0.50	0.428±0.14	0.450±0.27	4.889±0.28	0.951±0.25
F4	26.56±0.56	0.423±0.16	0.446±0.29	5.157±0.15	0.948±0.24
F5	25.47±0.32	0.429±0.16	0.456±0.29	5.921±0.14	0.940±0.14
F6	28.81±0.76	0.418±0.18	0.442±0.34	5.43±0.13	0.945±0.13
F7	24.42±0.50	0.415±0.11	0.437±0.35	5.034±0.36	0.949±0.24
F8	26.64±0.56	0.421±0.12	0.443±0.35	4.966±0.12	0.956±0.13
F9	28.52±0.76	0.430±0.16	0.458±0.34	6.114±0.13	0.938±0.24

Evaluation of Post Compression Parameters

Direct Compression Method

General Appearance

All the tablets showed good appearance. Surface was elegant, smooth and uniform. All the tablets were odorless and had bland taste.

Size and Shape

All the tablets showed circular shape having size 7±0.10mm.

Tablet Thickness

Thickness of all batches (F1 to F9) varied from 3.18±0.15mm.

Tablet Hardness

Hardness of all batches was kept constant from 4.0 to 4.18 (kg/cm^2).

Friability

Friability of the developed tablets increased with increased in super disintegrating agent and diluent from 0.20±0.73 to 0.24±0.54.

Uniformity of Weight

Tablets from each batch showed uniformity of weight as per IP limits. Each tablet sample was analyzed in triplicate (n=3).

Disintegration Test

Tablet of each batch showed that immediate decreased in disintegration time with increase concentration of superdisintegrant and diluents was observed. Disintegration time of the formulations F1 to F9 was varied form 11±0.087 to 13±0.58 sec.

Wetting Time

Wetting time of optimized batch was found to be 12-15 sec (table12 and fig 6).

Table 12: Evaluation of Post compression parameters by direct compression (n=3)

Formulations	Thickness (mm) (n=3)	Hardness (kg/cm^2)	Friability (%)	Weight Variation (mg)	Drug Content (%)	Disintegration Time (min)	Wetting Time (sec.)	Water Absorption Ratio
F1	3.18 ±0.02	4.0 ±0.23	0.266 ±0.24	153.27 ±0.94	98.44 ±0.15	4.20±1.28	13±0.9	130.07 ±2.1
F2	3.10 ±0.09	4.17 ±0.16	0.213 ±0.32	147.96 ± 0.86	99.9 ±0.15	4.5±1.25	12±1.12	127.36 ±1.9
F3	3.09 ±0.04	4.0 ±0.43	0.203 ±0.26	150.60 ±1.14	96.25 ±0.26	3.5±1.30	15±1.72	125.20±1.4
F4	3.18 ±0.03	4.0 ±0.58	0.266 ±0.34	153.27 ±0.87	98.44 ±0.56	4.4±1.28	14±1.43	130.07 ±1.2
F5	3.10 ±0.03	4.17 ±0.59	0.213 ±0.27	147.96 ±0.86	99.9 ±0.36	4.5±1.27	12±1.22	127.36 ±1.9
F6	3.09 ±0.02	4.0 ±0.58	0.203 ±0.23	151.60 ±0.83	96.25 ±0.89	3.0±1.36	12±1.72	125.20±2.2
F7	3.18 ±0.04	4.0 ±0.59	0.246 ±0.35	151.46 ±0.73	99.65 ±0.56	5.5±1.37	15±0.98	111.58 ±2.4
F8	3.20 ±0.09	4.17 ±052	0.257 ±0.37	151.72 ±0.86	96.84 ±0.59	4.0±1.25	17±0.94	107.73 ±2.4
F9	3.10 ±0.09	4.0 ±0.35	0.223 ±0.24	150.3 ±0.86	99.29 ±0.36	3.5±1.30	12±0.94	97.19 ±1.9

Sublimation Method

General Appearance

All the tablets showed good appearance. Surface was elegant, smooth and uniform. All the tablets were odorless and had bland taste.

Size and Shape

All the tablets showed circular shape having size 7±0.10mm**.**

Tablet Thickness

Thickness of all batches (F1 to F9) varied from 3.18±0.15mm.

Tablet Hardness

Hardness of all batches was kept constant from 2.4 to 3.40 (kg/cm^2).

Friability

Friability of the developed tablets increased with increased in super disintegrating agent and diluent from 0.16±0.73 to 0.26±0.54.

Uniformity of Weight

Tablets from each batch showed uniformity of weight as per IP limits. Each tablet sample was analyzed in triplicate (n=3).

Disintegration Test

Tablet of each batch showed that immediate decreased Disintegration time with increase concentration of superdisintegrant and diluent. Disintegration time of the formulations F1 to F3 was varied form 11±0.087 to 13±0.58 sec.

Wetting Time

Wetting time of optimized batch was found to be 11 to 13 (table13 and fig. 6, 7).

Table 13: Evaluation of Post compression parameters by sublimation

Formula Tions	Thickness (mm) (n=3)	Hardness (kg/cm^2)	Friability (%)	Weight Variation (mg)	Drug Content (%)	Disintegration Time (Sec)	Wetting Time (Sec.)	Water Absorption Ratio
F1	3.1 ±0.02	2.3 ±0.14	0.235 ±0.29	153.5 ±0.51	98.41 ±0.23	24.0±2.8	13±1.7	153.73
F2	3.2 ±0.08	2.6 ±0.11	0.228 ±0.30	151.33 ±0.29	98.65 ±0.36	28.0±2.1	12±1.9	140.38
F3	3.0 ±0.09	3.2 ±0.15	0.137 ±0.35	153.07 ±0.35	97.31 ±0.56	48.0±2.9	11±1.2	142.55

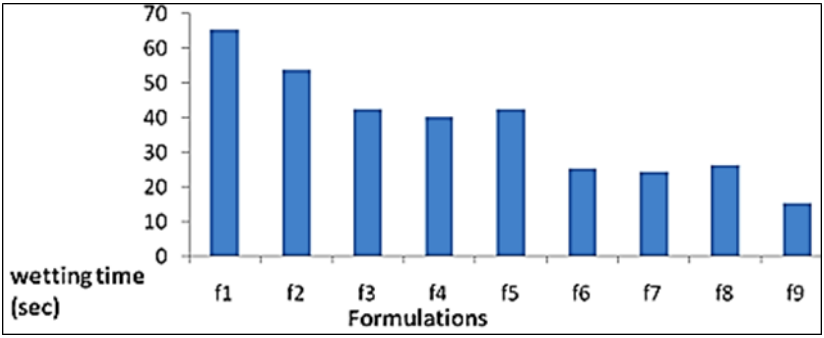


Fig 6: Wetting time profile of OND formulations of F1-F9 by Direct Compression

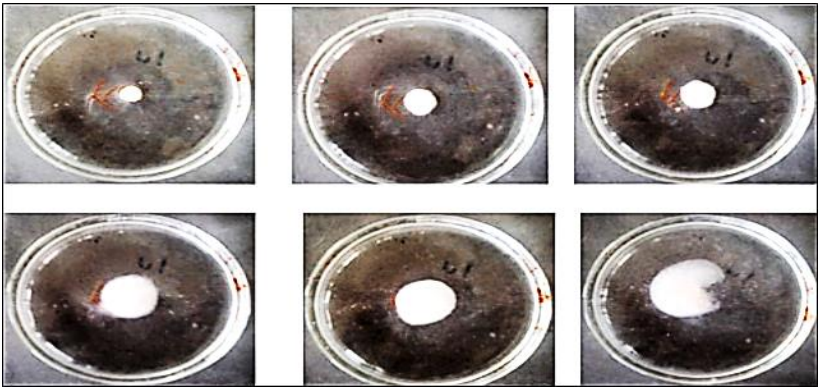


Fig 7: State of Tablet while measuring Wetting Time

In vitro Drug Release Study

In vitro dissolution of all the prepared FDT formulations was conducted and the dissolution data obtained reported that F9 formulation showed the maximum release in 20 min of about 96.98% and hence F9 formulation came out to be the best formulation among the others (Table 14). So, the F9 formulation was further compared to the market formulation for its *in vitro* dissolution release.

Table 14: Drug Release Kinetic Studies by sublimation method

Batch Code	Zero Order	First Order	Higuchi	Hixson Crowell	Korsmeyer Peppas	
	R^2	R^2	R^2	R^2	R^2	n
F1	0.9973	0.9868	0.976	0.7494	0.9997	0.5578
F2	0.990	0.9757	0.9974	0.9801	0.9963	0.4368
F3	0.9151	0.9030	0.9404	0.9858	0.9528	0.3431
F4	0.9251	0.9063	0.9456	0.9130	0.9512	0.2490
F5	0.9722	0.9625	0.9894	0.9660	0.9954	0.2405

F6	0.9897	0.9796	0.9983	0.9835	0.9993	0.3353
F7	0.9953	0.9850	0.9969	0.9895	0.9973	0.4545
F8	0.9566	0.9555	0.9575	0.9560	0.9545	0.3228
F9	0.9970	0.9837	0.9953	0.9952	0.9837	0.2927

Sublimation [20]

In vitro dissolution of all the prepared FDT formulations was conducted and the dissolution data obtained reported that F9 formulation showed the maximum release in 20 min of about 99.98% and hence F9 formulation came out to be the best formulation among the others (Table 13). So, the F9 formulation was further compared to the market formulation for its *in vitro* dissolution release.

Comparison of Optimized Batch with Marketed Tablets [21-24]

The optimized tablet formulation (F9) was compared with marketed conventional tablet of OND HCl *in vitro* drug release profile. Percentage of drug dissolved in 20 min for F9 was about 99.98% and for marketed product it showed 97.14% Mean. Results of comparative release profile is given in Fig. 8.

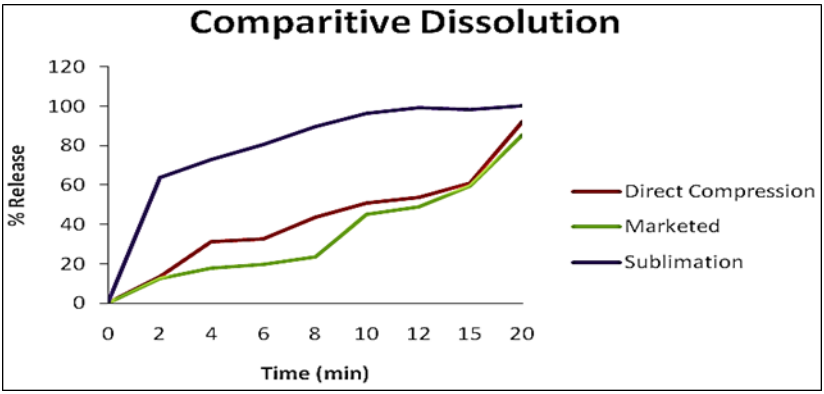


Fig 8: Comparative Dissolution Profile of Formulation (F9) and Market (MP)

Drug Release Kinetics

In vitro drug release data for all the formulations, F1 to F9 were subjected to release kinetic study according to zero order, first order equation and Korsmeyer -Peppas models. The R^2 and n values were given in Table 14.

Among the zero order and first order equations the value of regression correlation coefficient (R^2) were found to be higher in zero order equation. Hence the drug release from all the formulations followed zero order release

kinetics. In case of Korsmeyer-Peppas model, the results indicated that release exponent 'n' values are less than 0.5 (n<0.5). This indicates that Fickian type diffusion is the predetermining mechanism of drug release. So, overall data showed that all the formulations followed zero order release kinetics with Fickian diffusion mechanism.

P-X-Ray Diffractometry

Powder X-ray diffraction studies were done to reveal the crystalline modification of the drug during formulation of tablets. The more intensive peak was obtained at 18.84(2θ) for Ondansetron standard. Similarly in sublingual tablets and in physical mixture a peak at about 18.74(2θ) appears it showed the crystalline nature of the drug in formulations which was shown in Fig 9.

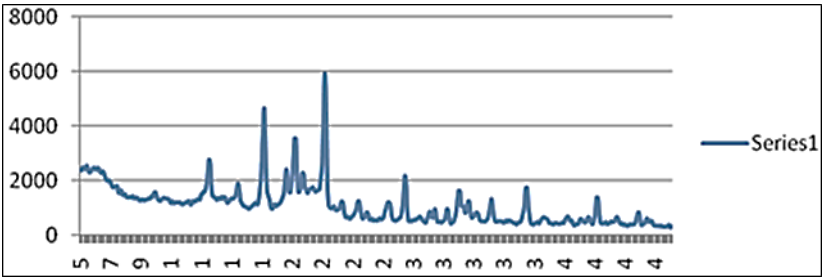


Fig 9: X-Ray diffractometry of F9 formulation

Scanning Electron Microscopy (SEM)

Surface morphology of OND HCl formulation was determined by SEM (Juol-JSM 6360A apparatus) the photo was obtained at different magnification levels to study the surface morphology (fig. 10, 11).

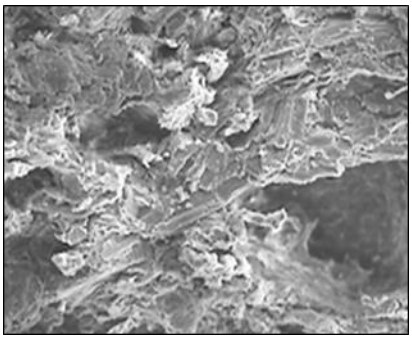


Fig 10: SEM Before sublimation

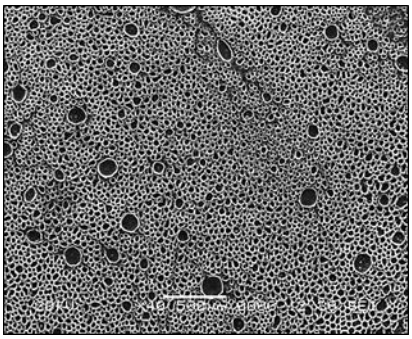


Fig 11: SEM After sublimation

Stability Studies

The stability studies of optimized formula were carried out at 40±2 ºC and 75±5% RH [18] using stability chamber for six months. The different parameters that were studied are disintegration time, hardness, friability, drug content and dissolution rate. The optimized formulation was found to be stable in terms of physical appearance, drug content, disintegration time and *in vitro* drug release in Table 15.

Table 15: Stability Studies for optimized formulation

Parameter	Initial	At 40 ºC and 75% RH
Weight variation	150.59	150.34
Thickness (mm)	3.15	3.14
Hardness (kg/cm^2)	4.08	4.06
Friability (%)	0.223	0.201
Disintegration time (sec)	22	20
Wetting time (sec)	12	11
% drug content	99.29	99.21
% CDR	99.98	98.92

4. Conclusion

From the above studies, it was concluded that the Fast disintegrating sublingual tablet of the drug formulated with the use of different superdisintegrants (Cross carmellose sodium, Sodium starch glycolate, Crospovidone) in their different concentrations prepared by direct compression and camphor as sublimating agent in sublimation method. These superdisintegrants and sublimating agents accelerate disintegration/ dissolution of tablets by virtue of their ability to absorb a large amount of water when exposed to an aqueous environment. But amongst both methods sublimation method showed good and efficient results because in sublimation, Camphor was used as a sublimating agent which increases the porosity of the tablets due to which the absorption of water takes place at high rate that results in breaking of tablets and therefore faster disintegration/dissolution.

However various compatibility tests like XRD showed crystalline pattern of the Ondansetron, FTIR studies showed no evidence of any chemical interactions between drug and the excipients and DSC studies further provided useful information about the drug and excipients compatibility studies.

Fast disintegrating sublingual tablets of OND HCl was successfully prepared by using sublimation method. The optimal batch F9 exhibited the disintegration time of 12 sec and friability of 0.16%. It is concluded that by

adopting a systematic formulation approach, an optimum can be reached in the shortest time with minimum efforts. Drug release mechanism data showed that all the formulations followed zero order release kinetics with Fickian diffusion mechanism. Sublingual formulation gives faster onset of action as compared to other formulation.

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Chapter - 5
Current Biomarker of Cardiovascular System

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Chapter - 5

Current Biomarker of Cardiovascular System

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Abstract

Cardiovascular disease (CVD) is a leading cause of death worldwide and continues to increase in prevalence compared to previous decades, in part because of the aging of the world population. Advances in biomarker research and developments related to CVD over the past 30 years have led to more sensitive screening methods, a greater emphasis on its early detection and diagnosis, and improved treatments resulting in more favorable preclinical *In vivo*. However, the use of biomarkers for different purposes in CVD remains an important area of research that has been explored by scientists over the years and many new developments are still underway. Therefore, a detailed description of all CVD biomarkers that are currently being used or investigated for future use in the field of cardiovascular medicine is out of scope for any review article. In the present review, we do not intend to replicate the information from previous exhaustive reviews on biomarkers including their preclinical utility; any putative novel biomarker is utilized in clinical practice. In addition, we will summarize information regarding recent novel heart failure biomarkers in current practice, which are undergoing scrutiny before they can be available for preclinical use, and their impact on clinical outcomes.

Keyword: Cardiovascular, prognosticate, atherosclerosis, morbidity

Introduction

CVD are the leading cause of morbidity and mortality in the United States. Primary prevention and secondary prevention of CVD are public health priorities. Substantial data indicate that CVD is a life course disease that begins with the evolution of risk factors that in turn contribute to the development of subclinical atherosclerosis. Subclinical disease culminates in overt CVD. The onset of CVD itself portends an adverse prognosis with greater risk of recurrent events, morbidity, and mortality [1]. It is also

increasingly clear that although clinical assessment is the keystone of patient management such evaluation has its limitations. Biomarkers are one such tool to better identify high-risk individuals, to diagnose disease conditions promptly and accurately, and to effectively prognosticate and treat patients with disease [2]. This review provides an overview of the molecular basis of biomarker discovery and selection and the practical considerations that are a prerequisite to their clinical use.

Definition of Biomarker

The National Institute of Health Consortium in 2001 defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [3]. Subsequently, in 2009 the American Heart Association outlined the extensive criteria for how newer biomarkers should be evaluated in a standardized fashion before their clinical use can be recommended [4]. The characteristics of an ideal biomarker to be used for a given purpose in any disease condition with a special emphasis on CVD are detailed in previous reviews [5].

Biological Marker (Biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

Type 0 Biomarker: A marker of the natural history of a disease and correlates longitudinally with known clinical indices.

Type I Biomarker: A marker that captures the effects of a therapeutic intervention in accordance with its mechanism of action.

Surrogate end Point (Type 2 Biomarker): A marker that is intended to substitute for a clinical end point; a surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) on the basis of epidemiological, therapeutic, pathophysiological, or other scientific evidence [6].

Biomarkers play an important role in the evaluation of disease as well as in the development of drug treatments for disease conditions. In the late phases of drug development, biomarkers can even be helpful in determining the accurate doses for any given drug. In more recent times, biomarkers are being considered as surrogate end points for preclinical trials as well. Biomarkers are traditionally classified on the basis of their intended use as screening, diagnostic or prognostic [7]. Desired characteristics of a novel biomarker according to their intended use are also displayed in Figure 1.

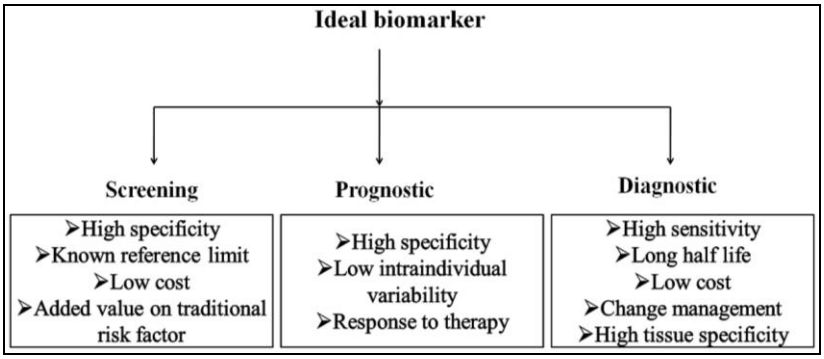

Fig 1: Ideal characteristics of a biomarker according to their intended use

As many factors play a role in the system's functionality, it is imperative for researchers to consider multiple cardiovascular biomarkers when trying to understand underlying molecular mechanisms and complete cardiovascular functionality. Despite advances in various *in vitro* and in silico experimental methods, in fields such as pharmacology, toxicology, and physiology it is acknowledged *in vivo* experimental models are required to understand the complete picture on how a new drug, medical device, or alternate therapy affects the body. This chapter provides an overview of common cardiovascular biomarkers using preclinical *in vivo* models including:

- Cardiac Contractility
- Heart Rate Variability
- Arrhythmia
- Systemic Blood Pressure
- Pulse Wave Velocity
- Baroreflex Sensitivity

Cardiac Contractility

Cardiac contractility is the force of cardiomyocyte contraction resulting from the interaction between actin and myosin at the cellular level. Contractility is a significant area of research due to its direct relationship to heart stroke volume (the amount of blood pushed out of the left ventricle during each heartbeat). Contractility is influenced by many factors, including the autonomic nervous system, afterload, preload, heart rate, or pharmacological drugs. As cardiac contractility plays a role in the body's cellular oxygenation, researchers continue to study how intrinsic and extrinsic factors affect contractility, particularly when it comes to drug safety assessment [8]. Current applications exploring cardiac contractility include:

- Heart Failure
- Cardiac Hypertrophy
- Cardiomyopathy
- Myocardial Infarction (MI)
- Hypertension
- Myocardial Ischemia
- Arrhythmias
- Drug Safety

Rodents, canines, and nonhuman primates remain the most common models used to evaluate cardiac contractility in whole animal studies. Left ventricular dP/dtmax is a common, robust, translatable, and sensitive indicator of changes in cardiac contractility. dP/dtmax measures the maximal change of left ventricular pressure (LVP) over time and presents as a wave form (Figure 2). LVP can be collected by implanting a pressure catheter into the left ventricle in both anesthetized and conscious animal models. Echocardiograms can also be used in conjunction with anesthetized models, but due to cost and expertise required, they remain a less accessible collection tool [9]. Additional physiologic endpoints researchers typically collect with LVP include systemic blood pressure, electrocardiogram (ECG), temperature, and activity.

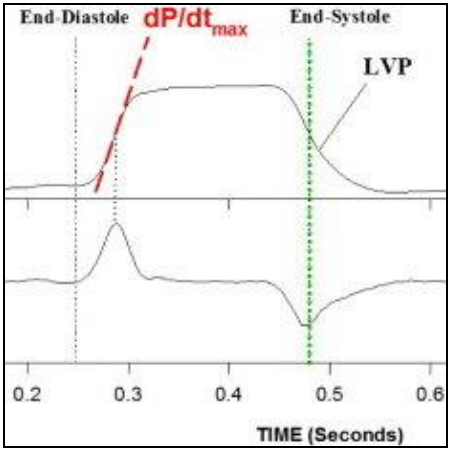

Fig 2: dP/dt indicates the change in pressure over time [17]

Heart Rate Variability

Heart rate variability (HRV) is the physiologic phenomenon of variation in the time interval between heartbeats and is measured by looking at

variation in the beat-to-beat interval. Heart rate and blood pressure spontaneously fluctuate even while resting or during steady-state conditions. HRV allows observation of specific frequencies resulting from fluctuations and provides insight to autonomic function. HRV is one method used to help diagnose cardiovascular (myocardial infarction, congestive heart failure, coronary artery disease, hypertension) and non-cardiovascular diseases (stroke, diabetes, alcoholism, cancer, glaucoma, etc.). High HRV is an indication of healthy autonomic and cardiovascular response. Low HRV may indicate the sympathetic and parasympathetic nervous systems are not properly coordinating to provide an appropriate heart rate response [10].

HRV can be affected by the following factors:

- Reflexes (baroreceptors, chemoreceptors, cardiopulmonary receptors)
- Respiration
- Renin-angiotensin System
- Physical or Mental Stress
- Exercise
- Cardiovascular and Non-Cardiovascular Disease States
- Age
- Drugs (beta-blockers, atropine, glycosides, anesthetics, etc.)

HRV analysis requires a series of successive heart beat intervals. HRV is typically derived from the R-R intervals of ECG signals (Figure 3) or inter-beat-intervals from systolic to systolic peaks of blood pressure signals. Analysis methods for HRV data exist in the time domain and frequency-domain. Each method of analysis is very different but contains a wealth of information. Note, the quality of analysis results is highly dependent on the quality of the original data and performance in detecting cardiac cycles. False detections or missed detections can have a profound effect on the results.

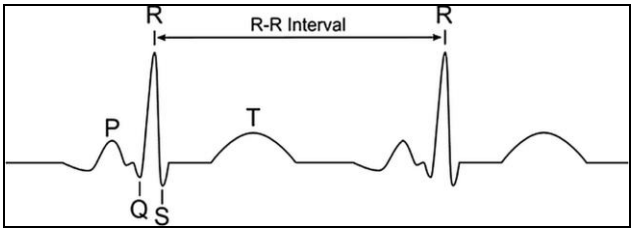

Fig 3: HRV may be calculated from the ECG's R-R Interval [18]

Arrhythmia

Arrhythmia is an irregular rate or rhythm disturbance in the heart's conduction system. Examples of acute causes of arrhythmia include stress, anxiety, or ingestion of stimulants. Examples of chronic comorbidities of arrhythmia may include disease, shock, infection, or structural changes of the heart. Many arrhythmias are benign and have no clinical significance. However, some have serious implications and may lead to cardiac arrest or sudden death. Researchers are particularly interested in monitoring arrhythmias, due to changes in the incident rate or arrhythmia type which may indicate disease progression or treatment effect. Arrhythmias are identified by recording and reviewing an ECG signal and assessing the rate, regularity, and morphology of the heartbeat. As the heart beats, cellular membrane polarity changes in the electrical conduction system throughout the heart, leading to depolarization and repolarization of atrial and ventricular cardiac cells by causing them to contract and relax. Figure 4 shows the conduction system of the heart. The cardiomyocyte chain reaction of depolarization ultimately facilitates the heart's ability to oxygenate blood and pump it throughout the arterial tree. This contraction (depolarization) and relaxation (repolarization) can be measured using electrodes placed in different combinations and configurations on the chest and limbs to produce a series of ECG complexes. An ECG complex is comprised of different waves which represent the electrical activity in specific regions of the heart [11].

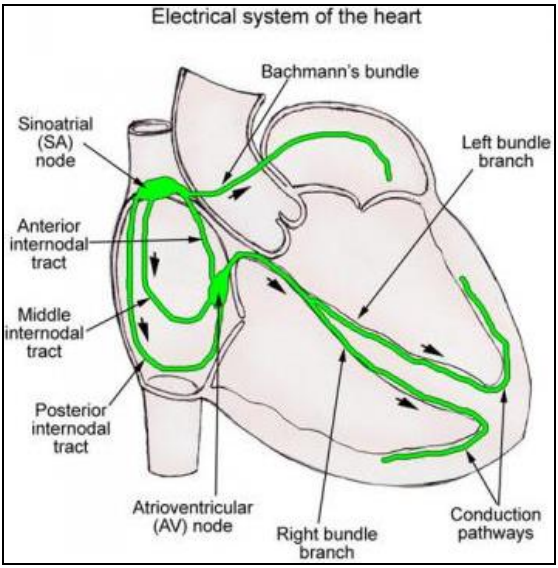

Fig 4: Conduction network of the human heart [19]

Systemic Blood Pressure

Blood pressure is a surrogate endpoint in the clinic, meaning it is "a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy" [12]. As blood pressure is a strong factor in many cardiovascular diseases, regulating it can improve the patient's overall well-being. Figure 5 shows a diagram of an aortic pressure waveform. Blood pressure is also a highly translatable endpoint from animal models to humans and is used in a wide variety of applications including the desire to understand mechanisms behind various cardiovascular diseases, stress responses, metabolic disorders, sleep apnea, drug safety evaluation, and more. In 2005, the American Heart Association published a paper on recommendations for measuring blood pressure in both humans and animal models. These recommendations recognize telemetry as the most reliable method for measurement in animal models over indirect methods of measurement, such as a tail cuff solution [13]. In addition, the ICH S7a guidelines for safety pharmacology studies stress a preference for unrestrained, conscious methods, such as telemetry, for *in vivo* studies [14].

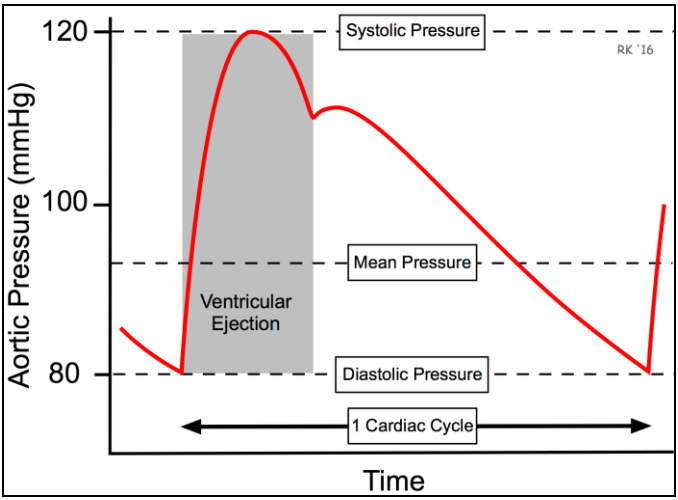

Fig 5: Diagram of aortic pressure waveform [20]

Pulse Wave Velocity

Pulse Wave Velocity (PWV) is a measure of the rate at which pressure waves move down a blood vessel, and increased speed correlates with arterial stiffness. It has been established as a highly reliable indicator for

cardiovascular morbidity and mortality in a variety of adult populations including older adults, patients with end-stage renal disease, diabetes, and hypertension. During systole of the heart, contraction of the left ventricle and ejection of blood into the ascending aorta acutely dilates the aortic wall and generates a pressure wave which moves along the arterial tree. The velocity of this movement gives a measurement of arterial compliance. With age, or changes in the arterial wall, these vessels become stiffer and the speed at which pressure waves move through the system increases. In addition, there are reflected pressure waves which move back towards the heart at the end of the systolic period. When pressure waves move faster through the arteries, the reflected waves will also move quicker. As higher systolic pressure is needed to overcome the afterload, the cardiovascular system must work harder. PWV can be collected using two pressure catheters placed a known distance from one another, referred to as the Pulse Wave Distance. The time it takes the pressure wave to go from the upstream catheter to the downstream catheter provides the Pulse Transit Time (PTT). PWV can then be calculated by dividing distance by transit time, providing a measure of cardiovascular health. Figure 6 shows a diagram of pulse wave velocity measurement [15].

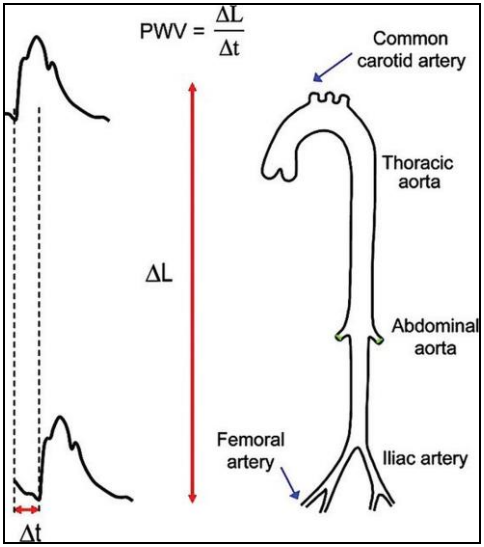

Fig 6: Diagram of pulse wave velocity measurement between carotid and femoral [21]

Baroreflex Sensitivity

Baroreflex is the fastest mechanism to regulate acute blood pressure changes by controlling heart rate, contractility, and peripheral resistance.

Baroreceptors are mechanoreceptors located in the carotid sinus and aortic arch (Figure 7). Their function is to sense pressure changes by responding to variations in tension of the arterial wall. The baroreflex mechanism is a fast response to changes in blood pressure. The baroreflex or baroreceptor sensitivity (BRS) index quantifies how much control the baroreflex has on the heart rate. BRS can be valuable in assessing the development and progression of cardiovascular diseases [16]. Reduced BRS can indicate:

- Neurological Disorders
- Hypertension
- Coronary Artery Disease
- Myocardial Infarction (MI)
- Heart Failure
- End-organ Damage
- Progression of Underlying Disease

BRS requires beat-to-beat information from both blood pressure and RR interval. Systolic blood pressure is typically derived from systemic arterial pressure, where as the RR interval is derived from ECG. The spectral analysis method to assess baroreceptor sensitivity outputs the gain and phase of the transfer function. Gain corresponds to the effectiveness with which the baroreflex is able to maintain constant conditions. Phase is the time lag between systolic blood pressure and RR.

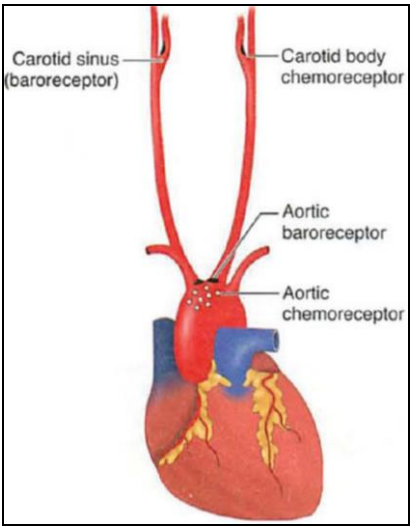

Fig 7: Diagram showing location of the carotid baroreceptors [22]

Conclusion

In summary, there are numerous CVD biomarkers that are currently available and that have clinical use as diagnostic, prognostic or predictive biomarkers. Several of these biomarkers have to be vigorously tested to assess their preclinical utility across a varying spectrum of patients with atherosclerotic CVD and who have with different comorbidities. Biomarker may be able to reflect pathophysiologic process of heart disease, and also may be able to provide meaningful information about prognosis and assist guide preclinical decision making without duplicating any information that is already available clinically.

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Chapter - 6
Uraria picta: A Comprehensive Review and Its Pharmacological Action

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Chapter - 6

Uraria Picta: A Comprehensive Review and Its Pharmacological Action

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Abstract

In present times, focus on herbal research has increased worldwide. *Uraria picta* is an important medicinal herb that is extensively used in dasamula and is becoming popular. Flavonoids, alkaloids and pterocarpans are the key constituents of *Uraria picta* and mainly alleged for its broad beneficial actions. Other than for given treatments, the herb is suggested as remedy for a variety of other ailments. The present review is an effort to provide complete information on Phytochemicals screening, traditional uses, pharmacology relating to preclinical studies and other informations.

Keywords: *Uraria picta*, Description, Phytochemicals, Pharmacological actions, Formulations & Dosage

Introduction

Uraria picta of family Leguminosae sub-family Fabaceae is a well-liked ayurvedic healthful plant that additionally glorious by name Prishniparni in Sanskrit. It's one amongst a part of dasamula that precisely means that ten roots and is a well-recognized ayurvedic drug of Indian system of medicines used for treating general fatigue, antioxidant, analgesic and anti-inflammatory like medical conditions [1]. *Uraria picta* also used in other Ayurvedic formulations like Abana, Amritarishta, Angamardana prashamana kashaya churna, Dashamoola taila, Vyaghri taila, Madhyama Narayana taila, Dasamularishta and Shira Shuladi vajra Ras [2, 3]. Other vernaculars were given to *Uraria picta* such as Citraparni, Prishniparni, and Simhapuri etc. Ayurvedic texts clearing up its morphological characteristics, attributes and benefits [4].

Uraria picta is a local of tropical zone including Nepal, Srilanka, Northern Australia, China, and Burma. This suffruticose herb which grows up to 1.5 meters tall is originate in dry grassland, west places, and open

deciduous forests and in all plains of India extending from Himalayas to Ceylon, Malayasia and Phillipines [5]. *Uraria picta* consists of different phytoconstituents present in different extracts exhibit a number of biological profiles and guard from most of the chronic diseases [6, 7].

Botanical Description

It is an vertical, small branched, perpetual herb, 90-180cm tall, stems with short, rough hairs, leaves imparipinnate with 5-9 leaflets (lowermost leaves often 1-3-foliolate); leaflets narrowly lanceolate, 7-25cm long (lowermost smaller), often variegated, shiny and hairless above, rough hairy. below; margins entire, inflorescence a long terminal densely many-flowered spike-like raceme, up to 55 cm long, covered in long whitish hairs, flowers pink, bluish or reddish, fruit 5-9 mm long, folded into 3-6 segments, brown to black, turning greyish-white when old [8].

Table 1: Scientific Classification [9]

Kingdom	**Plantae**
Subkingdom	Viridiplantae
Super order	Rosanae
Order	Fabales
Class	Magnoliopsida
Subclass	Rosidae
Family	Leguminosae
Sub-family	Fabaceae
Genus	*Uraria*
Species	*Picta* - (Jacq.) DC

Table 2: Classical Categorization [10]

Charak Samhita	**Sushrut Samhita**	**Vagbhata**
Angamardana Prashamana-group of herbs that help to relieve body aches.	Vidarigandhadi gana,	Haridradi gana
Shothahara-group of herbs having ant-inflammatory properties.	Haridradi group of herbs	-
Sandhaneeya-group of herbs that are used in fractures and dislocations	-	-

Table 3: Vernacular Names [11]

Regions/Language	**Names**
Sanskrit	Citraparni, Kalasi, Dhavani, Prishniparni, Galvanina
Bengali	Salpani, Chhalani, Chakule
Gujarat	Pithava

Hindi	Pithava, Dabra
Telugu	Murele Honne, Andale home, Prushniparni
Malayalam	Oril
Marathi	Pithava, Prishniparni
Oriya	Prushnipamee, Shankar Jata
Punjabi	Detedarnee
Tamil	Oppai
Kannada	Kolakuponna

Table 4: Ayurvedic Properties [10]

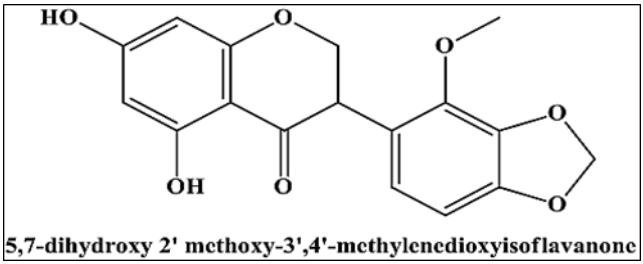
Hindi/Sanskrit		**English**	
Rasa	**Madhura/Tikta**	**Flavor**	**Bitter, Sweet**
Guna	Laghu	Physical Property	Light
Virya	Ushna	Effectiveness	Hot
Vipaka	Madhura	Metabolic Property (After Digestion)	Sweet

Fig 1: *Uraria picta* Plant

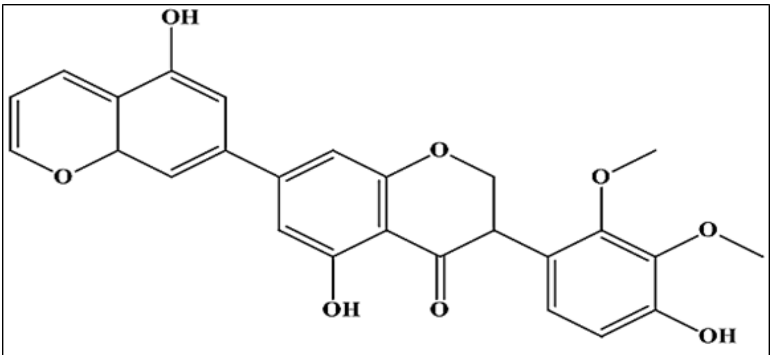
Biochemical Investigation

Rahman *et al*. (2007) isolated and elucidated the structures of 2 new isoflavones (5,7-dihydroxy 2' methoxy-3',4'-methelenedioxyisoflavanone; 4',5-dihydroxy-2',3'-dimethoxy-7-(5-hydroxychromen-7yl)-isoflavanone) fig. 1, following UV, IR, MS and 1D and 2D NMR scrutiny from roots of *U. picta*. Those compounds were found effective (MIC starting from twelve.5-200.0_g/ml) against gram-positive (*Staphylococcus aureus* CTC10788 and *Bacillus subtilis* NCTC8236), gram-negative (*E. coli* NCTC90001 and *Proteus vulgaris* NCTC4175) and fungi (*Aspergillus niger* NCPF3149 and *Candida albicans* IMII49007). Except the two new compounds the authors additionally found six antecedently illustrious compounds [12]. Yadav *et al*. (2009) quantified rhoifolin (7-[4, 5-dihydroxy-6-(hydroxymethyl)-3-(3,4,5-trihydroxy-6-methyloxan-2-yl) oxyoxan-2-yl] oxy-5-hydroxy-2-(4-hydroxyphenyl) chromen-4-one) fig. 2, quantity by RP-LC technique from

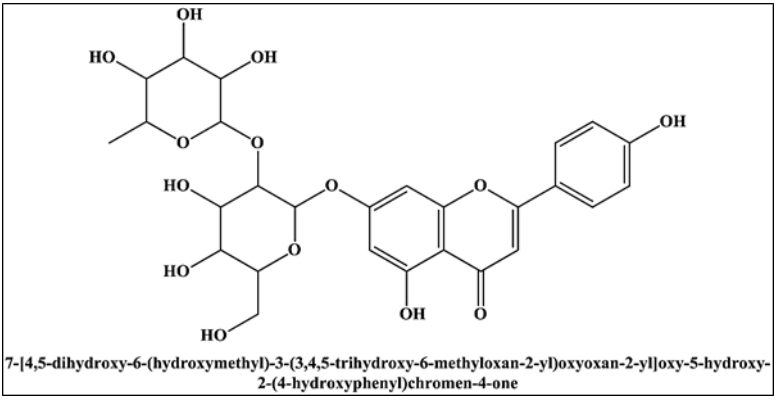
arid, thinly powdered aerial part of the plant variety and reported the best quantity (0.571% w/w) from methanolic extract with mega sonication [13].



5,7-dihydroxy 2' methoxy-3',4'-methylenedioxyisoflavanone



4',5-dihydroxy-2',3'-dimethoxy-7-(5-hydroxychromen-7yl)-isoflavanone



7-[4,5-dihydroxy-6-(hydroxymethyl)-3-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)chromen-4-one

Fig 2: Biochemical Investigation

Phytoconstituents

Preliminary phytochemical screening truly helps in analytic and characterizing the chemical constituents gift within the plant extracts and also the information of the chemical constituents of plants is enviable to know herbal medicine and their preparations and at last in discovering the

particular worth of folkloric remedies. The plant is reported to possess the existence of alkaloids, flavonoids, steroids, terpenoids, phenols and saponins all told plant components. Tannins were absent in stem and roots whereas internal organ glycosides were absent in roots [9].

Traditional Uses

Uraria picta simmering is prescribed for cough, chills and fevers. The leaves are well thought-out antiseptic and utilized in gonorrhea. The roots and pods are utilized to treat prolapse of anus in infants; the pods are employed for the treatment of sore-mouth in kids. It's used for the treatment of urinary diseases, tumors, edema, burning sensation and issue in respiration. Its paste, mixed with water, is employed as a remedy for snake bite [14]. It's a well-recognized ayurvedic drug of Indian system of medicines used for treating general fatigue, antioxidant, analgesic and anti-inflammatory like medical conditions [1].

Ailment & Pests

Water stagnation causes underdeveloped growth, curling and browning of leaves; but, the plants recover simply once the stress amount is over. No illness or insect pests are according within the crop inflicting serious injury. However, 0.1% $HgCl_2$ untreated seeds were according to be infected by *Fusarium* spp. and *Alternaria* spp [15].

Pharmacological Actions

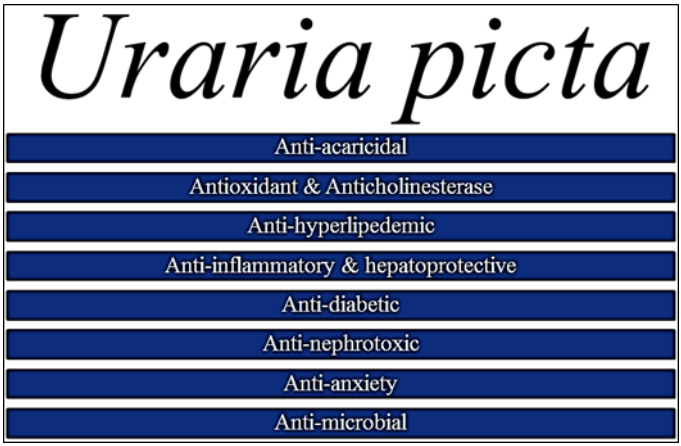


Fig 3: Pharmacological Actions of *Uraria picta*

1. Anti-Inflammatory & Hepatoprotective Activities

Methanolic roots extract showed important activity against each models of inflammation. *Uraria picta* methanolic extract (400, 200, and 100 mg/kg,

p.o.) reduced inflammation in egg albumin and formalin treated in rats paw edema in dose reliant method. Administration of paracetamol 2000 mg/kg induced liver injury in rats, and so, amplified the amount of enzymes alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) within the blood. Administration of methanolic extract of *Uraria picta* 400, 200 and 100 decline the amount of enzymes ALT, ALP and AST noticeably that were found comparable to standard drug silymarin 100 mg/kg [16].

2. Antioxidant and Anticholinesterase Activities

The aqueous extract of *Uraria picta* with key enzymes (acetylcholinesterase and butyrylcholinesterase) linked with Alzheimer's disease *in vitro*. Inhibition of cholinesterase enzymes is another method employed in treating/managing Alzheimer's illness. The study thus done to analyze the interaction of binary compound extract of *Uraria picta* with key enzymes (acetylcholinesterase and butyrylcholinesterase) connected with Alzheimer's illness *in vitro*. Inhibition of acetylcholinesterase and butyrylcholinesterase, the overall phenolic content and radical scavenging skills were assessed *in vitro*. The extract suppressed acetylcholinesterase and butyrylcholinesterase in a very dose dependent manner. Present within the extract are phenol and flavonoids. The extract conjointly scavenged 2, 2 - azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical (ABTS*) and hydroxyl radical (OH*) in a very dose-dependent manner. Inhibition of acetylcholinesterase, butyrylcholinesterase and also the exhibited inhibitor properties may build *U. picta* extract an honest suggests that in treating/managing Alzheimer's illness [17].

3. Anti-Acaricidal Activity

Ethomedical uses of the plant, *Uraria picta* (Leguminosae), in African nation embrace the management of ectoparasites in man and domestic animals. Total and fractionated extracts of this plant are assessed for acaricidal activity on *Ixodes ricinus*. All the extracts were acaricidal to the take a look at organisms. Methanolic extract of this plant could be a less assailable pesticide compared to the liquid extract. Similarly, the base-forming-soluble non-polar fraction of the methanolic extract exhibited bigger acaricidal activity than the alkaline insoluble non-polar fraction. However, the polar fraction of the methanolic extract exerted no detectable acaricidal activity. The acaricidal property of this plant is owing to quite one category of chemical compounds found to incorporate phenolic resin, flavonoid, alcohol and hydrocarbon derivatives [18].

4. Antihyperlipidemic Activity

Chronic administration of Abana, an Indian herbomineral preparation containing *U. picta* as ingredient, showed a major hypolipidemic activity in rats. Serum β lipoprotein fat elements and apoprotein levels were considerably lowered, LDL being a lot of affected than terribly low-density lipoprotein. However, serum HDL lipids and apoproteins were to some extent amplified. The drop within the lipoprotein elements of serum and liver were attended with belittled level of serum free fatty acids and liver lipolytic enzyme activities. Abana caused marked inhibition in hepatic biogenesis of cholesterol and increased the excretion of soiled bile acids. The modes of action of Abana as a cardioprotective and hypolipidemic agent were explained earlier [19].

5. Anti-Diabetic Activity

A study was done to research the Oral Glucose Tolerance Test (OGTT) action on aqueous extract of *U. picta* leaves on a high calorie diet fed 120 Sprague-Dawley rats animal model to induce Type-II diabetes. The administered quantity of *U. picta* within the regular diet fed animals considerably enhanced the glucose clearance rates. Elevated quantity ought to be checked in future studies still because the insulin sensitivity of the plant extracts [20].

6. Anti-Nephrotoxic Activity

An investigational study was done with aqueous extract of *Uraria picta* (250 and 500 mg/kg/body wt.) to assess the anti-nephrotoxic action with the assistance of 24 Healthy wistar rats animal model. Treatment with the aqueous extract of *Uraria picta* (250 and 500 mg/kg/body wt.) containing polyphenolic compound and carbohydrates can be considerably reduces the elevated levels of urine urea, BUN levels and conjointly elevated levels of serum creatinine and urine creatinine compared to acetaminophen group. The activity evoked by the extract can be because of its ability to activate antioxidant enzymes. The findings counsel the potential use of the aqueous extract of *Uraria picta* as a unique therapeutically helpful nephroprotective agent [21].

7. Anti-Anxiety Activity

Administration of various forms of extracts of *Uraria picta* leaves in doses 200 mg/kg, 400 mg/kg and 600 mg/kg to rats, of that 400 mg/kg and 600 mg/kg showed fine CNS connected pharmacologic activity like anti-anxiety this can be because of plant has free radical scavenging and

antioxidant potential. The study was compared with standard anti-anxiety drug diazepam [22].

8. Anti-Microbial Activity

Two isoflavanones 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone, and 4',5'-dihydroxy-2',3'-dimethoxy-7-(5-hydroxychromen-7yl)-isoflavanone along with 6 compounds including isoflavanones, triterpenes and steroids were isolated from roots of *Uraria picta*. The structures of these compounds where established unambiguously by UV, IR, MS and a series of 1D and 2D NMR analysis. The minimum inhibitory concentrations (MIC) for these compounds were found to be in the range of 12.5-200 lg /ml against bacteria (both Gram positive and Gram negative) and fungi [12].

Nutritive Importance

The nutritionary capabilities of 4 wild legumes in North-Eastern India as well as *U. picta* utilization seed protein concentrate (SPC) to judge contents of amino acids, ash, starch, sugar, fibre, phosphorus, ether extractive and calories additionally as for *in vitro* enzymatic digestibility by pepsin-trypsin enzyme system. Results indicated promising nutritionary potential of those SPCs [23].

The quantity of essential amino acids (nutritional worth obtained from chemical score that was concerning 87.0%) from seeds of *U. picta* and located it on the point of cultivated legumes (garden pea, horse bean, kidney bean, amongst others) and cereals (bread-wheat, rice and barley). The authors additionally reported that even if the lipid content of seed was low (1.6%), however the seed oil contains massive proportions of essential and long chain fatty acids. Therefore from the potential nutrient worth of the seeds of *U. picta* the species could also be enclosed within the diet of rural/tribal populations [24].

Important Formulations

- Amritarishta Ayurvedic medicine is valuable in all type of fever, chronic fever, malaria; frequent fevers and additional complaints of inflamed the liver of the spleen or digestive disorder, night sweating, and weakness
- Angamardana prashamana Kashaya Churna
- Dashamula Taila is used superficially for massaging body. It is fine for the skin and can be used by each one. It helps to treat skin troubles, ache caused due to vata and kapha disproportion

- Vyaghri Taila
- Dashamularishta nourishes body and gives potency. It is superior for reproductive system of both males and females and it improves fertility

Dosage OF *U. picta* According to Condition

Powder of whole plant is taken in amount of 20-50 grams for preparing decoction.

- **Cold:** For cold, simmer 10-20 grams of powder of total plant in 400 ml of water. Bubble it till it becomes 1/4 of original measure. Strain this decoction. Put in some sugar and sip lukewarm
- **Bony Fracture:** Obtain Prishniparni root powder 5 grams + turmeric 2 grams. Act recurrently for one month
- **Poison:** Clean liquid of total plant taken in a quantity of 10-30 ml gives relief
- **Spleen swelling, Liver and abdominal diseases**: Simmer 10-20 grams of powder of total plant in 400 ml of water. Bubble it till it becomes 1/4 of original amount. Strain this decoction, put in some sugar and drink [25]

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