1. Introduction

1.1 Immunosuppressant

Immunosuppressants are chemical or biological agents purposefully used to reduce or suppress the immune system [Fireman, et al., 2004]. These drugs have an array of uses from preventing the body from the rejection of organ transplant to treating various autoimmune disorders and various non-autoimmune inflammatory diseases. With dramatic increase and improvement in the efficacy of the outcome in both mortality and graft survival of solid organ transplantation, the rate of transplantation had increased worldwide because graft acceptance is the most crucial step for the restoration of the graft function in the long run. According to an International report on organ donation and transplantation activities, 153,863 solid organs were transplanted globally in 2019 http://www.transplant-observatory.org/wp-content/uploads/2021/04/glorep2019.pdf.

The significant increase in the number of transplantation has raised the demand of these immunosuppressants both in value and volume. On the basis of product, the Immunosuppressant Drugs market is primarily split into a. Calcineurin Inhibitors, b. Antiproliferative Agents, c. mTOR Inhibitors, d. Antibodies, e. Glucocorticoids.

Glucocorticoids are widely used to treat various auto-immune and inflammatory diseases as well as follow-up regimes during a solid organ transplant. Glucocorticoids like prednisone, methylprednisolone exhibit their anti-inflammatory and immunosuppressive potential both by genomic and nongenomic pathways [Xu-Amano, et al., 1993]. Cyclophosphamides are used in the treatment of autoimmune disorders like Systemic lupus

erythematosus. Cyclophosphamide suppresses the formation of antibodies by generating phosphoramide mustard, which can alkylate DNA and suppress cell division [Boldizsar, et al., 2010; Strauch, et al., 1959]. Cyclosporine and tacrolimus are enzyme inhibitors that suppress activated T-cells via inhibition of calcineurin. However, tacrolimus shows impressive potential over cyclosporine in its ability to inhibit IL-2 and T-cell activation [Kapturczak, et al., 2004; Treon and Chabner, 1996]. The Mycophenolic acids act as anti-proliferative agents by checking B-lymphocyte and T-lymphocyte proliferation by inhibiting inosine monophosphate dehydrogenase (IMPDH), a crucial key enzyme in de novo nucleotide synthesis [Kino, et al., 1987; Platz, et al., 1991]. Sirolimus and Everolimus which are inhibitors of mammalian target of rapamycin (mTOR), have an almost same mechanism. Once within the cell, they bind to FBK-12 forming a complex which binds mTOR and thereby inhibits the B-lymphocyte and T-lymphocyte to enter the cell division phase [Fine and Kushwaha, 2016; Gabardi and Baroletti, 2010; Ingle, et al., 2000; Sievers, et al., 1997].A wide range of polyclonal and monoclonal antibodies have been extensively used to prevent solid organ transplant rejection due to their ability to dramatically reduced the incidence of acute rejection and it also improve both short and long-term graft acceptance.

1.2 Market value of Immunosuppressant

Global immunosuppressant demand increases day by day. Immunosuppressants are the drugs that are administered to minimize the chances of organ rejection after transplantation. For almost every individual who receives an organ transplant, immunosuppressants are a must to be taken. This is because immune system reacts to transplanted organs as foreign objects and develops an immune response against it, thereby, destroying the organ and at the same time affect other body parts as well. These drugs

prevent hyper immune response of a patient's body. These drugs are also widely used by patients suffering from auto-immune disorders such as Crohn's disease, irritable bowel disease, and arthritis.

A new report published by Allied Market Research, entitled, "Immunosuppressant Market by Drug Class, Indication, And Distribution Channels: Global opportunity Analysis and Industry Forecast, 2019–2026," the global immunosuppressant market size was valued at 13,890 million dollar in 2018 and is expected to reach 42,511.37 million dollar by 2026, registering a compound annual growth rate (CAGR) of 14.7% from 2019 to 2026, in terms of value (https://www.alliedmarketresearch.com/press-release/immunosuppressants-market.html).

By drug class, the calcineurin inhibitors segment accounted for majority of the immunosuppressant's market share in 2018 and is expected to exhibit a prominent growth rate in the near future, owing to the fact that it is the most used immunosuppressant drug used after organ transplantation to suppress hyper immune response. The most widely prescribed calcineurin inhibitors drugs include cyclosporine and tacrolimus [Hatanaka, et al., 1988; Kolata, 1983].

1.3 Approval of Mycophenolic Acid as Immunosuppressant

Mycophenolic Acid and its derivatives such as mycophenolate mofetil (MMF) and mycophenolate sodium used as immunosuppressive drugs for preventing the rejection of organ transplantation [Ardestani, et al., 2010; Sievers, Rossi, 1997]. At the early 1990s, several needs of immunosuppressant remained undone [Mele and Halloran, 2000]. In the starting of 1980s cyclosporine and anti-CD3 antibodies resulted in improved one year graft

survival rates. However, the rejection of acute allograft remained frequent and it was a leading cause of graft loss during the first year and a risk factor for the development of chronic rejection and long term graft loss [Foegh, 1990; Gulanikar, et al., 1992; Lindholm, et al., 1993; Naimark and Cole, 1994; Palmer, et al., 1985; Tesi, et al., 1993; Vanrenterghem, 1995]. The U.S. Food and Drug Administration approved mycophenolate mofetil (MMF) for use as immunosuppressive drug for organ transplants patient in 1995 [Alani, et al., 2009; Bentley, 2000].

1.4 Mycophenolic Acid as Compared with others Immunosuppressants

In clinical practice, the most effective use of immunosuppressive drugs in transplant patients to be a major challenge. Mycophenolic acid is the most commonly used immunosuppressive agent [Eugui, et al., 1991; Gong, et al., 1999; Lamba, et al., 2014; Pou, et al., 2001; Senda, et al., 1995], in various combinations with other immunosuppressants [Shaw, et al., 2001; Shaw, et al., 2004]. A combination of two or three immunosuppressive drugs of different classes used to treat various immunosuppressive diseases. A combination of calcineurin inhibitors and corticosteroids or anti proliferative agents either azathioprine or mycophenolic acid are most frequently used for treatment. In primary immunosuppression, MPA replaced azathioprine, because MPA has stronger immunosuppressive potency than azathioprine [Srinivas, et al., 2007; Wagner, et al., 2015].

1.5 Other Roles of Mycophenolic Acid

Clinical trials using mycophenolic acid as an anticancer medication yielded relatively minor results, as previously stated. Mycophenolic acid used as immunosuppressive drugs, prescribed to prevent acute and chronic rejection of organ transplantation [Benjanuwattra, et

al., 2020]. Mycophenolic acid is used as anti psoriasis agent, anti tumour agent and antiproliferative agent [Végső, et al., 2007]. Anti ageing property is also found in mycophenolic acid.

1.5.1 Treatment of Psoriasis Using Mycophenolic Acid

Mycophenolic acid is also used for the treatment of psoriasis [Epinette, et al., 1987; Geilen and Mrowietz, 2000; Gomez, et al., 1979; Lebwohl and Ali, 2001; Lynch and Roenigk, 1977; Marinari, et al., 1977; SPATZ, et al., 1978; Van Scott, 1976]. Oral mycophenolic acid was first used in the treatment of psoriasis in 1973 [Kitchin, et al., 1997]. The reasoning was that previous medications that slowed cellular replication had a positive effect on psoriasis when given systemically. Jones *et.al*, investigated that most of the patients showed improvement in psoriasis after third week of treatment. They found that after mycophenolic acid therapy, patients are gradually relapsed to their former state. Most of the patients encountered adverse reaction when the dose of mycophenolic acid increased [Jones, et al., 1975]. The mycophenolic acid and curcumin conjugate as a promising antiproliferative and anti-inflammatory therapeutic agent for the treatment of psoriasis [Yuyun, et al., 2021].

Mycophenolic acid, reformulated as mycophenolate mofetil (MMF), was first used to treat psoriasis nearly three decades ago and has recently been rediscovered by dermatologists [Liu and Mackool, 2003; Shoji, et al., 1994; Strathie Page and Tait, 2015]. MMF has recently been reported to show promise for several dermatologic conditions, including psoriasis [Dauden, et al., 2004; Sweeney, 1977], pemphigus vulgaris [Baskan, et al., 2009; Bystryn, 1999; Stanley, 1999], pyoderma gangrenosum [Baliu-Piqué and Mascaró Jr, 2017; Eaton and Callen, 2009; Husein-ElAhmed, et al., 2010; Lee and Cooper, 2004; Nousari, et al., 1998],

bullous lichen planus [Nousari, et al., 1999; Tursen, et al., 2004], and even connective tissue diseases like lupus erythematosus [Eickenberg, et al., 2012; Look, et al., 2013; Sagcal-Gironella, et al., 2011; Sherwin, et al., 2012; Ye, et al., 2021] and dermatomyositis [Edge, et al., 2006; Hornung, et al., 2012; Wang, et al., 2021], as a relatively well-tolerated immunosuppressive used in organ transplant recipients.

1.5.2 Treatment in Tuberous Sclerosis

Inosine monophosphate dehydrogenase inhibitor such as mycophenolic acid is also used for treatment of tuberous sclerosis. Recent studies investigated the genetic tumour syndrome tuberous sclerosis complex in mouse tumour model and multiple cells [Valvezan, et al., 2020].

1.5.3 Reduction in Occurrence of Skin Cancer

Recent studies investigated that mycophenolic acid inhibiting the proliferation of non immune cells. Mycophenolic acid reduced the proliferation in various call lines such as smooth muscle cells, renal tubular cells, mesangial cells and fibroblasts [Morath, et al., 2006]. Kim *et.al,* investigated the effect of rapamycin and mycophenolic acid on expression of inflammation related factors. The combination of mycophenolic acid and rapamycin is used in inflammatory skin diseases [Kim, et al., 2015]. Several studies demonstrate that the single drug introduction of tacrolimus, mycophenolic acid, and interleukin 2 (IL2) receptor antibodies more than a decade ago did not raise the risk of cancer following renal transplantation. However, there is limited information on their carcinogenic implications when used as part of a powerful immunosuppressive regimen [Braconnier, et al., 2012]. Although research to support the role of newer pharmaceuticals in squamous cell skin cancer

(SCSC) risk is scant, organ transplant patients have a significantly increased risk of squamous cell skin cancer, which is mostly due to immunosuppressive medications used to avoid graft rejection. They found in this study that organ transplant patients on newer regimens, such as mycophenolic acid and tacrolimus, do not have the elevated risk of SCSC formerly related with azathioprine [Coghill, et al., 2016].

1.5.4 Anti Tumour Effect of Mycophenolic Acid

Mycophenolic acid, an ancient antibiotic that has recently been discovered to have significant efficacy against a variety of tumours in mice and rats, substantially inhibits DNA synthesis in the L strain of fibroblasts [Ando, et al., 1968; Domhan, et al., 2008; Franklin and Cook, 1969; Sweeney, et al., 1972]. During regular screening experiments using mould filtrates for action against mice fibroblasts, mycophenolic acid emerged as a promising anti-cancer chemical [Carter, et al., 1969; Jones and Mills, 1971; Planterose, 1969; Suzuki, et al., 1976].

1.5.5 Role of Mycophenolic Acid against Human Immunodeficiency Virus (HIV)

Mycophenolic acid, a specific inhibitor of de novo guanosine nucleotide synthesis in T and B lymphocytes, has been hypothesised to suppress HIV replication in vitro by depleting the substrate for reverse transcriptase (guanosine nucleotides) [Schläpfer, et al., 2003]. Mycophenolic acid caused apoptosis and cell death in a substantial proportion of activated CD4+ T cells, demonstrating that it can reduce HIV infection in vitro through both virological and immunological processes [Chapuis, et al., 2000]. Mycophenolic acid inhibits viral replication and may reduce the immunopathology caused by viruses [Kaur, et al., 2005].

Abacavir [Heredia, et al., 1999; Margolis, et al., 1999; Margolis, et al., 2002] and amdoxovir [Borroto-Esoda, et al., 2004] with inosine monophosphate dehydrogenase inhibitor mycophenolic acid have potent and synergistic anti-HIV efficacy [Coull, et al., 2001; Hawley, et al., 2013; Hossain, et al., 2002]. In patients with active glomerulonephritis despite infection management, mycophenolic acid may show to be a novel nonsteroid treatment [Tiong, et al., 2020; Ui, et al., 2005].

1.6 Side Effects of Mycophenolic Acid

When the dose of mycophenolic acid increased, most of the patients have adverse effect [Mourad, et al., 2001; van Agteren, et al., 2008; Wieland, et al., 2000]. Many side effects associated with the mycophenolic acid such as gastrointestinal side effects in renal transplant patients. Following renal transplantation, gastrointestinal (GI) adverse events are common, and all immunosuppressive regimens have been linked to them [Hao, et al., 2008]. Mycophenolate mofetil or enteric-coated mycophenolate sodium are immunosuppressive drugs that have been linked to the best kidney transplant results. Independent of the formulation or method of administration, the gastrointestinal events produced by both mycophenolate mofetil and enteric-coated mycophenolate sodium could be linked to MPA [Davies, et al., 2007].

1.7 Objectives of the Study

Mycophenolic acid is an important immunosuppressive drug that prevents the rejection of organ transplantation. It is a secondary metabolite, obtained by fermentation of *Penicillium brevicompactum*. The study was performed to give a detailed insight into the production process of mycophenolic acid using different fermentation modes and after that purification of mycophenolic acid using column chromatography.

The study has been conducted with following objectives:

- **To investigate optimized media for mycophenolic acid production using different carbon and nitrogen sources.**

  Secondary metabolite biosynthesis has been reliant on carbon and nitrogen sources; however the outcomes are uneven and vary depending on the strain and growth conditions. Fermentation medium was optimized for better production of mycophenolic acid using one variable at time (OVAT) method. Different carbon sources, nitrogen sources and precursors were used for mycophenolic acid production studies. Because these nutrients are closely related with the formation of biomass and metabolites, it is assumed that the culture nutrients carbon and nitrogen sources have a major influence in fermentation productivity.

- **To evaluate batch fermentation kinetics using optimized media in a stirred tank bioreactor and to study the growth, production and substrate consumption profile.**

  Kinetics studies of mycophenolic acid production using batch fermentation mode has been performed to evaluate the specific growth rate, the specific substrate

uptake rate, and specific product formation rate. The yield of MPA on substrate and biomass were also evaluated for batch fermentation process.

- **To study morphological changes during the growth and production of mycophenolic acid production process in fermentation broth.**

  The morphological changes in fungal growth during mycophenolic acid have been performed. Batch fermentation was conducted for mycophenolic acid production in 3.7 L stirred tank bioreactor. Fermentation broth was sampled and morphological variations were measured during 10 days of fermentation period for mycophenolic acid production. At high cell mass concentration, microorganisms grow as long, thin, branched threads of mycelium. The mycelial suspensions constitute viscous non-Newtonian fluid. A cluster of entangled hyphae constitute mycelia that are dispersed discretely in the broth.

- **To evaluate broth hydrodynamics during mycophenolic acid fermentation.**

  The two most essential factors for all rheological changes in the broth during the production process are morphological variations and biomass content. The clumped growth of *Penicillium brevicompactum* causes a substantial rise in broth viscosity, which limits free cell cultivation investigations for mycophenolic acid synthesis. The culture obtained via 3.7 L STR was then subjected to rheological investigation, which revealed that the relationship between shear stress and shear rate is best characterized by the Power Law model.

- **To evaluate different strategies for mycophenolic acid production process by different fermentation modes i.e., Batch, Fed Batch and Continuous modes in stirred tank bioreactor.**

  Mycophenolic acid is due to its immunosuppressive and biological activities, a potential compound. It is the secondary metabolite produced in submerged cultivation by the microfungus *Penicillium brevicompactum*. Batch, fed-batch, and continuous mode of cultivation for mycophenolic acid production were performed and compared in the current work. To increase productivity, mycophenolic acid production was studied in batch, fed-batch, and continuous bioreactors.

- **To study the purification process for mycophenolic acid from the fermentation broth using column chromatography.**

  A metabolite's downstream process costs roughly 70% of the total. In this instance, this study intended to reduce the cost of the method while boosting the quality and yield of the final product. The crude fermentation broth has been taken for solvent extraction processes using different solvents. MPA is observed to be insoluble in water. Mycophenolic acid purification from fermented broth has been done by using column chromatography technique. Different columns such as Alumina, Silica gel, and Kieselguhr (Diatomaceous Earth) columns have been used for purification of mycophenolic acid. Glass columns of length 50 cm having an internal diameter of 3 cm have been used, with a length to diameter ratio of 16.67. The length, diameter, and the flow rate have been kept similar in all cases. Column dimension is an important factor for separation.