Chapter 1 INTRODUCTION

Gout is one of the leading diseases worldwide. This disease is also known as the “king of diseases and disease of Kings” because; most of the kings were having this disease due to their high consumption of purine rich diet. Recently it has been estimated that 0.3% population of India and above 8.3 million people in the United States at around 3% of the populations are suffering from an inflammatory disease called as ‘Gout’ (Punzi, Scanu et al. 2012; Edwards and So 2014). The global epidemiology data is represented in Fig. 1.1 indicating the prevalence of gout. Gout is one of the most common auto-inflammatory arthritis disorder caused due to the high consumption of protein rich diets such as red meat, seafoods, fish, and beans etc particularly in the improper functioning of kidney patients. Along with these, alcohol consumption, obesity, hypertension, use of diuretic drugs and a low dose of aspirin also contribute to gout.

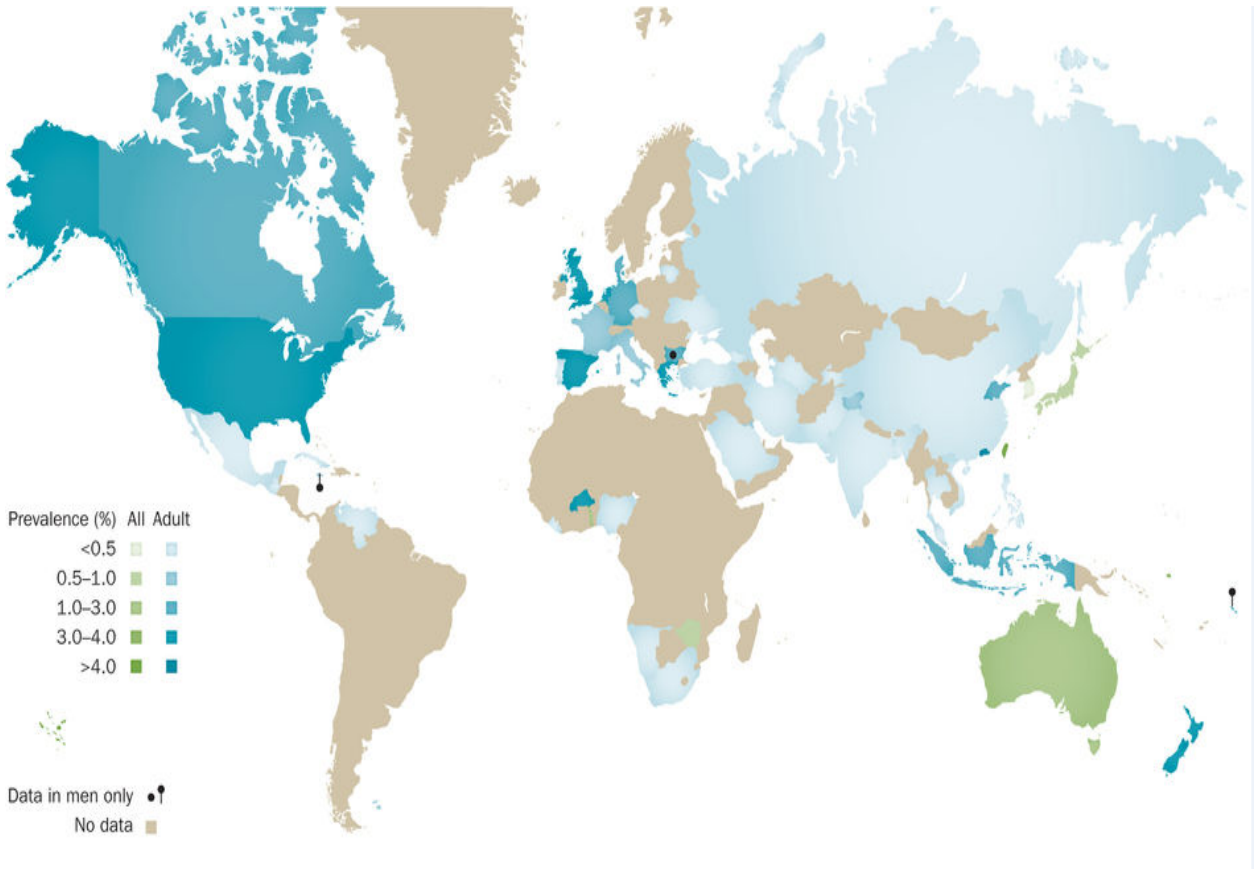

Fig. 1.1 Global epidemiology data of Gout

The development of gout caused due to hyperaccumulation of uric acid crystals (especially monosodium urate crystals (MSU)) in the joints and serum (Nuki 2002; Garay, El-Gewely et al. 2012; Taylor 2013). The gout also results in the development of Lesch-Nyan syndrome (Luo, Do et al. 2006; Zhang and Yin 2014). Along with MSU crystals, two other types of crystals also contribute to gout as calcium pyrophosphate dehydrate leads to pseudogout and basic calcium phosphate (BCP/hydroxyapatite) (Marianayagam, Koduri et al. 2014). Hypertriglyceridemia is being observed in 80% gout populations (Falasca 2006). The disease is most probably common in the middle-aged men and is increasing in the elder populations along with postmenopausal women (VanItallie 2010; Smith, Bracken et al. 2011). Bone erosion

is commonly observed in the chronic gout disorder due to the accumulation of MSU which results in the joint damage and deformity (Chhana, Callon et al. 2010) shown in Figure 1.2.

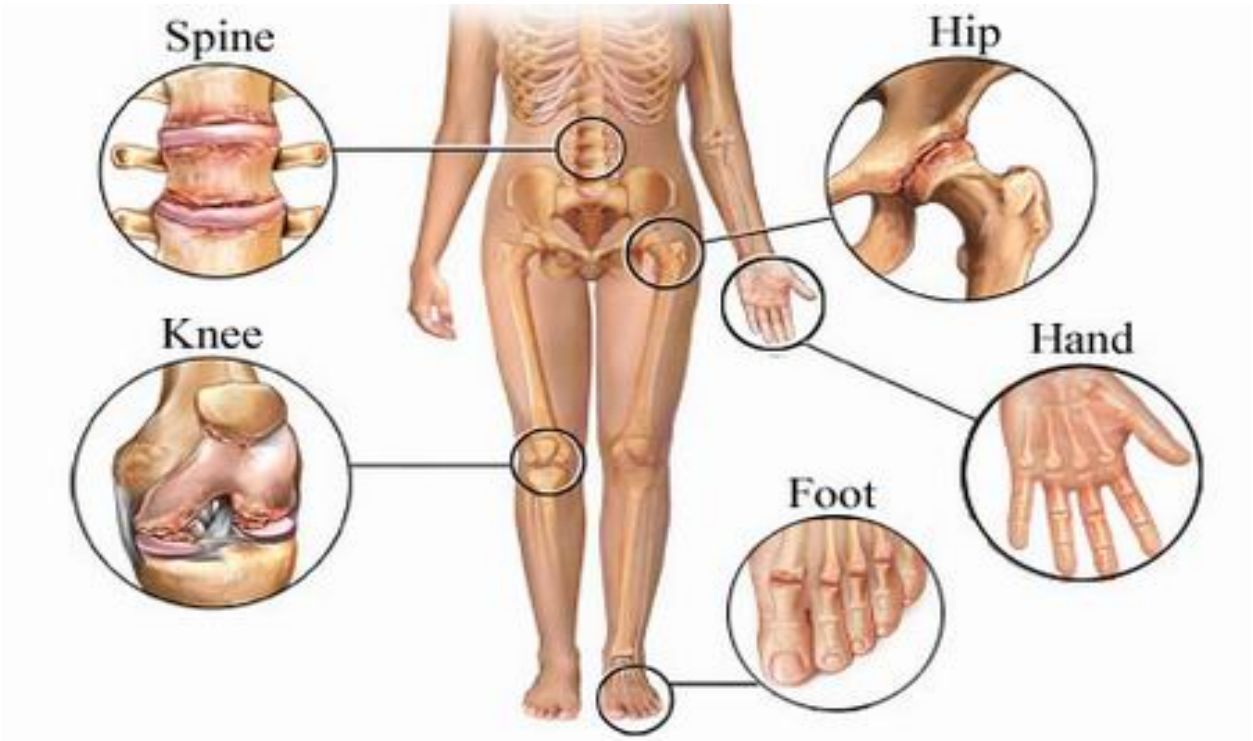

Fig. 1.2: Major accumulation sites of MSU crystals

Uric acid is the end product of purine metabolism as depicted in Fig. 1.3 and the hyperuricemia condition arises after the deposition of uric acid beyond its solubility point of 6.8 mg/dL (Gaffo and Saag 2008; Stamp, Zhu et al. 2011; Punzi, Scanu et al. 2012).

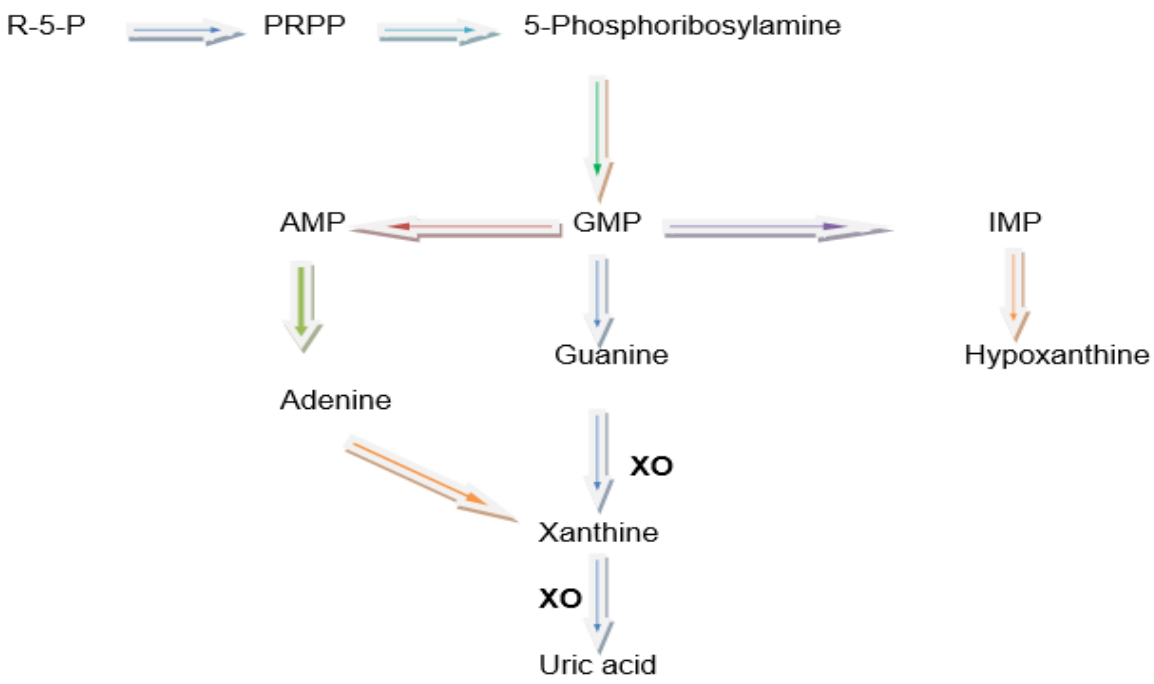

Fig. 1.3: Purine metabolism pathway (Nuki 2006; Maiuolo, Oppedisano et al. 2016)

However, the low concentration of uric acid acts as an antioxidant which scavenges serum free radicals and protects against cancer (Kuo, Luo et al. 2012; Kumar, Misra et al. 2014). The concentration of uric acid is regulated by the endogenous metabolism, reabsorption and excretion rate by the kidney (Dehghan, Köttgen et al.). The most common cause of hyperaccumulation of uric acid is the reduced renal clearance (Pande 2006). This result in the hypertension and an increase in the kidney related disorders. The mechanism of uric acid clearance and its hyperaccumulation is depicted in Fig. 1.4.

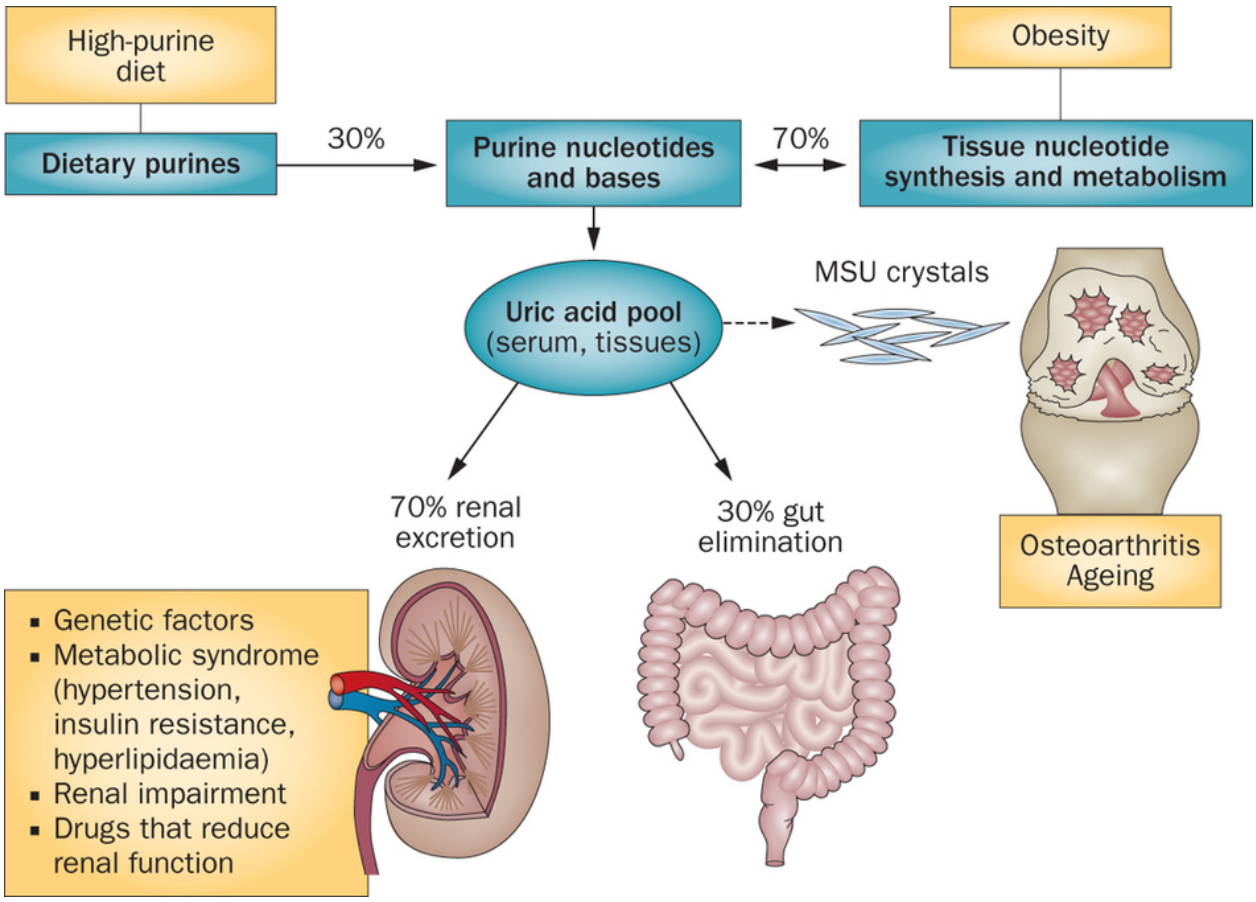

Fig. 1.4: Mechanism of uric acid and risk factors for gout (Rees, Hui et al. 2014)

Table 1.1 shows the variability of concentration of uric acid with respect to age and sex. After attainment of menopause, the female uric acid concentration becomes nearly equal to the matured male. Hence the chances of MSU crystal formation are more in the females exceeding age 50.

Table 1.1: Concentration of uric acid with age and sex

Sr. No.	Person	Concentration of Uric acid (mg/dL) (Pennes and Martel 1986)
1	Child	3.6
2	Mature male	7.3
3	Mature female	5.9

The current research on animals and humans conclude that the accumulation of monosodium urate crystals results in the stimulation of inflammatory response which indeed activates the nitric oxide, prostaglandins, and proinflammatory cytokines as IL-1, IL-1β, which are produced by macrophages, dendritic cells, monocytes and inflammasome complex (Pinto, Mora et al. 2013; Taylor 2013).

Anti-inflammatory therapies including colchicines, non-steroidal anti-inflammatory drugs, glucocorticoids are commonly used decades ago for the treatment (Keenan, O'Brien et al. 2011).

There are two possible approaches for the regulation of uric acid concentration in the body as

1] More than three decades ago allopurinol was considered as a first line drug which regulates uric acid synthesis by inhibiting xanthine oxidase (XO) enzyme (Jennings, Mackenzie et al. 2014; Kachroo and Schwarzschild 2014; Williams, McGill et al. 2014). In addition to allopurinol, Febuxostat is also used to reduce the synthesis of uric acid by inhibiting the XO enzyme and is approved by the European Medical

Agency in 2008 and US FDA in 2009 (Faruque, Ehteshami-Afshar et al. 2013). In contrast to allopurinol, it acts on both reduced as well as an oxidized form of XO (Ye, Yang et al. 2013). Partial relief from inflammation can be obtained by administration of hydroxychloroquine and non-steroidal anti-inflammatory drugs (Le Goff, Berthelot et al. 2008). However, all these drugs are prone to have enormous side effects, including severe kidney and heart-related disorders.

2] So in order to regulate the uric acid level in serum or soft tissues, enzymatic treatment is considered to be the better way without any side effects. The enzyme uricase is well known for the treatment of gout disease. Uricase (urate oxidase) EC 1.7.3.3 is a tetramer therapeutic enzyme which oxidatively opens the purine ring of uric acid and forms allantoin, CO_2 and H_2O_2 (Lotfy 2008). Allantoin is a highly water-soluble compound compared to the uric acid. Uric acid is poorly water-soluble as 6.8 mg/dL compared to the product allantoin which has solubility ~147 mg/dL. Hence allantoin is easily excreted through the urine. Uricase is also acting as the diagnostic enzyme used to determine the concentration of urate in serum and urine (Huang, Shih et al. 2004; Lotfy 2008). In addition to the uricase, peroxidase can also be alternatively used to estimate the uric acid concentration (Kumar, Misra et al. 2014). Uricase enzyme is highly conserved present in mammals, plants, fungi, bacteria, and yeasts but is absent in humans due to evolutionary mutations in uricase gene (Huang, Shih et al. 2004).

Aim and Objectives:

The present study focuses on

- Selection of uricase producing microorganisms by agar plate assay method

- Media optimization for uricase production using Taguchi DOE methodology under submerged fermentations.
- Purification of uricase enzyme.
- Identification and molecular characterization of purified protein
- *In vivo* applications of uricase in hyperuricemia treatment in the uricase deficient Swiss albino mice