CHAPTER 1

INTRODUCTION

1. Introduction

Haemorrhoids also known as anal cushions, are normal anatomical structures, made up of three types of tissues i.e., vascular tissue, smooth muscle and connective tissue aligned by epithelial cells of the anal canal. When there is a symptomatic enlargement and distal displacement of this anal cushion, then it becomes a pathological condition termed as the disease hemorrhoid [1]. Constipation and prolonged straining are widely believed to cause hemorrhoids because hard stool and increased intraabdominal pressure could cause obstruction of venous return, resulting in engorgement of the hemorrhoidal plexus [2]. Defecation of hard fecal material increases shearing force on the anal cushions. However, recent evidence questions the importance of constipation in the development of this common disorder. Many investigators have failed to demonstrate any significant association between hemorrhoids and constipation, whereas some reports suggested that diarrhea is a risk factor for the development of hemorrhoids. Increase in straining for defecation may precipitate the development of symptoms such as bleeding and prolapse in patients with a history of hemorrhoidal disease. Pregnancy can predispose to congestion of the anal cushion and symptomatic hemorrhoids, which will resolve spontaneously soon after birth. Many dietary factors including low fiber diet, spicy foods and alcohol intake have been implicated, but reported data are inconsistent [3].

The most common symptom of hemorrhoids is rectal bleeding associated with bowel movement. Evidence indicates that hemorrhoidal bleeding is arterial because of bright red color and arterial pH of the blood. Degeneration of supporting connective tissue within the anal cushion as well as abnormal dilatation and distortion of the vascular channel is a main

diagnostic finding of hemorrhoidal disease [2]. Certain inflammatory reactions are also involved in the pathogenesis of hemorrhoids [4].

Therapeutic treatment of hemorrhoids ranges from dietary and lifestyle modification to radical surgery, depending on degree and severity of symptoms [1]. Since shearing action of passing hard stool on the anal mucosa may cause damage to the anal cushions and lead to symptomatic hemorrhoids, increasing intake of fiber or providing added bulk in the diet might help eliminate straining during defecation. Lifestyle modification should also be advised to any patients with any degree of hemorrhoids as a part of treatment and as a preventive measure. While numerous topical treatments are available on the market in the forms of creams, ointments, and suppositories, there is no convincing evidence of their efficacy. These include various combinations of local anesthetics, corticosteroids, vasoconstrictors, antiseptics, and astringents. They may provide some short-term symptomatic relief but, particularly in the case of corticosteroid containing preparations, should not be used long term as they may be associated with thinning of the skin and ulceration. Again, evidence for these agents is poor. Phlebotonics (such as oral flavonoids) may have some role in the control of acute symptoms and may be useful in reducing symptoms after surgical treatment [5] although conclusive evidence is here, again, lacking.

From the time immemorial, herbs have been used in all cultures throughout history to cure diseases [6]. Our ancestors were completely dependent on nature for food, clothing, shelter, and medicine to cure myriads of ailments. Trial and error were the way to differentiate useful plants with beneficial effects from those that were toxic or inactive for Primitive people and by this they developed well-defined herbal pharmacopeias. The evidence of the use of herbal remedies some sixty thousand years ago by Neanderthal man was revealed in 1960 in a cave

in Northern Iraq [7]. As per WHO, 25% of modern medicines are derived from the herbal origin, and almost 80% of world's population is dependent on herbal medicine for their primary care treatment more abundantly in developing countries like India. Though, herbal drugs are of great interest still the complete systematic information of all the herbs used as medicine is not reported. Thus, herbal drugs lack wide acceptance.

Safety and effectiveness are the basic need for all medicines, whether they are synthetic or of plant origin [8-10]. The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by processes such as harvesting, drying, and storage [11]. Hence, the occurrence of variation is common. This variability may also be due to differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the assurance of quality, efficacy, safety, and reproducibility by prescribing a set of standards, constant parameters and different qualitative and quantitative tests [12].

However, the majority of pharmaceutical companies are not paying sufficient attention in this area due to some assorted limitations, such as lack of information about the uses of herbal drugs in modern diseases like cancer, viral infection, multidrug-resistant microbial infections, diabetes, obesity and cardiovascular disorders [13]; lack of reproducibility in formulation contents owing to effects like geographical variation, biodiversity, and seasonal variation on net contents of essential herbal drugs [14]; relatively higher cost of drug discovery from natural sources [15]; loss of synergistic effect during isolation of active constituents [16]; low aqueous or lipid solubility of major bioactive phytoconstituents [17]; inadequate permeability across the bio-membrane, and poor bioavailability of active phytoconstituents, etc. [12]

The concept of nanomedicine is as old as Ayurveda, where they use nanoparticles of metals like Bhasma in therapeutics [18]. The Bhasma (ash) preparation process is known as

"Bhasmikarana", where the metal ions and herbal drugs are converted into a higher oxidative state called metal ion-nanoparticles. These metal nanoparticles have superior properties like better absorption, improved stability, and compatibility for the human body. Metals like silver (Ag, Rajata), mercury (Hg, Parada), zinc (Zn, Yasada), iron (Fe, Loha/Aayasa), tin (Sn, Vanga), lead (Pb, Naga/Sisaka), copper (Cu, Tamra) and gold (Au, Swarna) are commonly used to prepare the Dhatu Bhasma [19].

Herbal nanomedicines are nano-sized medicines containing herbal drugs like extracts, enriched fractions or biomarker constituents. The nanoformulation has several unique features like smaller particle size and high surface area; dual nature, i.e., hydrophilic and lipophilic; and bioavalability.

In recent times, synthesis of nanoparticles by means of green technology using single step-one pot method [20] has become popular as it omits the limitations of other physical and chemical methods [21]. Green synthesis of nanoparticles using plants are cost effective, least dangerous, ecofriendly and easy to scale up for large production [22, 23] and allows the coating of secondary metabolites on NPs surface and sometimes entrapment in the metal core [21, 24]. There is a wide range of phytoconstituents involved during the reduction process such as amino acids, enzymes, vitamins, polysaccharides, alkaloids, phenolic compounds, tannins, saponins, steroids and proteins etc. [25, 26]. Generally, plant mediated biosynthesis of NPs involve mixing of extract and $AgNO_3$ solution in an appropriate ratio and in optimized conditions [27]. Whole plant extracts possess less amount of phytoconstituents as compared to enriched fractions, which may affect the size, shape and yield of NPs during biosynthesis [28].

Blumea lacera is an aromatic annual herb, widely distributed throughout the plains of Northwest India, upto 2,000 m height above sea level [29]. This plant possesses several

medicinal and therapeutic properties [30]. The aqueous extract of the plant leaves is reported to have antifungal activity [31] antimicrobial activity [32] and wound healing activities [33]. Traditionally, *Blumea lacera* has been used to treat fever [34], cuts & bleeding wounds [35, 36], diarrhoea and other GI disorders [37-39], piles [40-42] and also as anti-inflammatory agent [43]. Several phytoconstituents, especially essential oils like fenchone, geneol, coniferyl alcohol derivatives have been reported from *Blumea lacera* along with phytosterols such as, campesterol, lupeol, hentriacontane, hentriacontanol, alpha-amyrin, stigmasterol, beta-sitosterol and flavonoids [44]. Apart from this, a few glycosides and flavonoids have been isolated from *Blumea lacera* [45, 46]. Phytosterols, such as sitosterol and campesterol present in *Blumea lacera* are reported to have potent anti-inflammatory agents [47-50].

Therefore, the current study intends to validate the traditional claims of *Blumea lacera* being used as an anti-haemorrhoids agent. For this, the ethanolic extract of *Blumea lacera* was prepared and the extraction process was optimized using Box Behnken design then the anti-haemorrhoid potential was evaluated. Further, five different fractions from the obtained extract were prepared and evaluated for the presence of various phytoconstituents, *in vitro* antioxidant and anti-inflammatory activity. The best amongst all fractions (ethyl acetate fraction) was then selected for the development of the development of phytosterol loaded silver nanoparticles. These nanoparticles were then evaluated for *in vitro* antioxidant and anti-inflammatory activity along with anti-bacterial activty. Finally, these nanoparticles were evaluated for their anti-haemorrhoid potential.