2  LITERATURE REVIEW

2.1 Bhasma

Nanomedicine are gaining popularity day by day owing to their various therapeutic applications with more efficacies and lesser side effects\(^1\). The popularity is due to their specific and selective pharmacological action. Bhasma, the ancient concept of nanomedicine, is used to treat various chronic ailments since 7\(^{th}\) century BC. It is very clear from the history of civilization traditional medicines were used to cure human ailments in every possible condition. In modern era we can use them over the synthetic molecules because they have fewer side effects \(^2\)-\(^4\). **Bhasma** is the calcination product of inorganic and organic substances. Bhasma as a medicine is a mystery due to severe side effect associated with metal when administered internally\(^5\). As per ayurvedic physician bhasma is nontoxic if metal is processed according to ancient ayurvedic literature. The rational pharmaceutical and therapeutically approach of Ayurveda in general and Rasa shastra in particular has transformed metal into medicinal form. The processes of shodhana (purification/potentiation) and marana (calcinations/detoxification – treatment with that quantum of energy which is needed for physico-chemical conversion of raw materials to Bhasma: a therapeutic form) which are very individualized in terms of material, media, method and absolute medicinal form\(^6\). The bhasmas are taken along with honey, milk, butter or ghee which makes these elements easily assimilable, eradicating their adverse effects and improving their biocompatibility\(^3\). Our ancient literature describes various method to ensure the quality of bhasma. In current few year tremendous work has been carried out to ensure the quality of bhasma. The present review deals with ancient as well as modern method of preparation of bhasma, therapeutic application of almost all bhasma and their method of characterisation by traditional method (as per ancient literature) and using modern analytical techniques.
2.2 Preparation of Bhasma

Bhasma can be prepared by putapaka method and kupipakwa method\(^5\,^7\). Summarised method of preparation is shown in Figure-2.1

**Figure 2.1: Methods of preparation of bhasma**

In recent time burning (Calcination) process is done in crucible at specific temperature as per nature of metal and the remaining procedure is kept same for preparation of bhasma.
2.3 Bhasma as nano medicine

Bhasma is considered as biologically produced nanomedicine as the size of individual particle is found in nano range. Heating of metal during sodhana may lead to increase in tension causing expansion of metal foil followed by cooling in liquid media lead to decrease in tension and increase in compression force. Repeated heating and cooling process may lead to brittleness, reduction in hardness and finally reduction in particle size. It is confirmed by various research carried out for characterisation of bhasma. The size of swarna bhasma and silver bhasma were found to be of 56 and 16 nm respectively\(^7\).

2.4 Chemical nature of bhasma

Bhasma is produced by the process of calcination of metal and minerals. Calcination of metal may lead to conversion of metal into its metallic oxide\(^8,9\). Major chemical composition of bhasma is reported in Table-2.1.

2.5 Evaluation of bhasma

The quality of bhasma can be evaluated by traditional method of evaluation (Bhasma pariksha). Evaluation can be done by physical and chemical test\(^10,11\).

2.5.1 Physical test

2.5.1.1 Nishachandratva

Bhasma can be observed under bright sunlight to detect the presence and absence of lustre. A good quality of bhasma should be free from metallic lustre indicating metal is completely converted in ash.

2.5.1.2 Rekha Purita

Small amount of bhasma is taken between the thumb and index finger and spread, it should be so fine as to get easily into the furrows of finger lines indicating that the size of bhasma is reduced to very fine size.

2.5.1.3 Varitara

When a small quantity of bhasma is spread on cold and distilled water, it should float on the surface. Properly calcined bhasma will float over surface of water. This test is performed to evaluate lightness and fineness of bhasma.
Chapter 2: Literature Review

2.5.1.4 Unama
It is further assessment of varitara test. In this test a rice grain is kept over floating bhamsa and observed either bhamsa float or sinks. If grain float over bhamsa, then it can be concluded that the bhamsa is excellently prepared.

2.5.1.5 Anjana sadrushi sukshmatva
When bhamsa is applied on eye lid as kajal should not cause any irritation indicating prepared bhamsa is of micro fine size.

2.5.1.6 Gatarastva
Very small quantity of bhamsa is placed over tongue for any specific taste. The good quality bhamsa should be tasteless.

2.5.1.7 Mridutva and slakshnatva
This test is performed to detect softness and fineness of bhamsa. A good quality of bhamsa should be very fine and soft in touch.

2.5.1.8 Avami
The bhamsa on oral administration should not produce nausea.

2.5.2 Chemical test

2.5.2.1 Nirdhumatva
Small amount of bhamsa is taken in spatula and subjected to heat treatment on flame directly. If no fumes are produced it indicates that bhamsa is free from organic impurity.

2.5.2.2 Apunarbhava
This test is applied to metallic bhamsa only. In this test bhamsa is mixed with equal quantity of mitra panchaka (seed of Abrus pecatorius, honey, ghee, borax and jagery) and sealed in earthen pot then similar grade of heat used for preparation of particular bhamsa is applied and on self-cooling product is observed. If any particle with lusture is found indicating bhamsa is not properly calcined.

2.5.2.3 Niruttha
In this test bhamsa is mixed with fixed weight of silver leaf and sealed in earthen pot then similar grade of heat used for preparation of particular bhamsa is applied and on
Chapter 2: Literature Review

self-cooling, weight of silver is taken. Increase in weight of bhasma indicate improper preparation of bhasma.

2.5.2.4 Amla pariksha
Small amount of prepared bhasma was mixed with a little quantity of curds in a petri dish and a little amount of lemon in a neat and clean test tube for any colour change. No colour change of curd and lemon was observed indicating metal is completely converted into ash.

2.6 Evaluation of bhasma by Modern analytical technique
Traditional method of evaluation of bhasma is self-satisfactory but not accepted by modern scientist. To consider this and to enhance the acceptance of bhasma as nano medicine it is necessary to characterise it using modern analytical technique.

2.6.1 Loss on drying
When bhasma gets exposed to atmosphere absorb moisture and particles of bhasma associates each other may lead to fail of varitara and unama test even the quality of bhasma is good. It can be understood by particle size analysis report by zeta sizer and SEM the particle size of copper oxide was found bigger in tamra bhasma when analysed with SEM obviously due to agglomeration of particles. Weigh accurate sample of bhasma. Transfer in tared weighed petriplate. Transfer the petri plate containing bhasma in hot air oven maintained at 105°C. Takeout the petri plate from hot air oven at certain interval and weigh on analytical balance. Repeat the procedure until constant weight.

\[
\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

2.6.2 Particle Size
As per literature the size of bhasma lies in nanorange so particle size was determined using a laser diffraction particle size analyzer (Zetasizer Nano ZS90). Bhasma was suspended in the chamber of the particle size analyzer containing distilled water, and subjected to dynamic light scattering (DLS) to analyse the particle size.
Chapter 2: Literature Review

2.6.3 Morphological characterisation and size distribution:
For morphological characterisation and size distribution of bhasma, scanning electron microscopy and transmission emission microscopy is generally used\(^9\).

2.6.4 Structural analysis
Fourier-transformed infrared spectroscopy (FT-IR) spectroscopy can be used for structural analysis using potassium bromide disc. The bhasma can be analysed by recording their spectra in the wavelength range 4000-400 cm\(^{-1}\). The bhasma should be free from any organic impurity. Presence of organic impurity indicates that bhasma is not processed in proper way or process of calcination is not complete\(^9\).

The prepared bhasma can be analysed for their crystallinity or amorphous behaviour using X-ray diffraction (XRD). Generally metal oxide are crystalline in nature\(^9\).

Chemical composition of bhasma:
The chemical composition of bhasma is analysed using energy dispersive x-ray analysis (EDX) attached to SEM\(^9\).

**Reported preclinical activity of Bhasma**
Preclinical activity about different bhasma is reported in Table-2.1.

**Table 2.1: Preclinical activity about different bhasma**

<table>
<thead>
<tr>
<th>Bhasma</th>
<th>Main chemical composition</th>
<th>Traditional Use</th>
<th>Reported Preclinical activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauha Bhasma</td>
<td>Ferrie oxide (Fe(_2)O(_3))</td>
<td>In treatment of Anaemia, Diabetes, tuberculosi</td>
<td>Antianaemic</td>
<td>12,13</td>
</tr>
<tr>
<td>Naga Bhasma</td>
<td>Lead oxide (Pb(_3)O(_4))</td>
<td>Appetizer, Imunomodulator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swarna Bhasma</td>
<td>Aurous oxide (Au(_2)O) and Auric oxide (Au(_2)O(_3))</td>
<td>Immunomodulator, Aphrodiasic, Cardiac stimulant</td>
<td>Analgesic, Arthritis, free-radical scavenging activity, Anti-cataleptic, anti-anxiety and anti-depressant activity, Immunomodulator, Antioxidant</td>
<td>14, 15,16,17,18,19,20</td>
</tr>
</tbody>
</table>
### Chapter 2: Literature Review

<table>
<thead>
<tr>
<th>Bhasma</th>
<th>Main chemical composition</th>
<th>Traditional Use</th>
<th>Reported Preclinical activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raupya Bhasma</td>
<td>Argentous and Argentic oxide (Ago, Ag20)</td>
<td>Aphrodisiac, Immunomodulator, Anti-ageing</td>
<td>Hypolipidimic, Anticataleptic, Analgesic</td>
<td>21,22,23,24</td>
</tr>
<tr>
<td>Tamra Bhasma</td>
<td>Cupric oxide (CuO)</td>
<td>Wound healer, purgative, wound healer</td>
<td>Hepatoprotective</td>
<td>24</td>
</tr>
<tr>
<td>Yasada bhasma</td>
<td>Zinc oxide (ZnO)</td>
<td>Ophthalmic nourisher, Immunomodulator</td>
<td>In arrest of myopia</td>
<td>25</td>
</tr>
<tr>
<td>Praval Bhasma</td>
<td>Calcium carbonate</td>
<td>Antacid</td>
<td>Treatment of bone metabolic disorder (Osteoporosis) anti-inflammatory</td>
<td>26,27</td>
</tr>
<tr>
<td>Mukta shouktic bhasma</td>
<td>Calcium oxide</td>
<td>Antacid</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Mandur bhasma</td>
<td>Ferric oxide</td>
<td>Immunomodulator, Anaemia</td>
<td>Hepatopurative, Anti anaemic</td>
<td>29</td>
</tr>
</tbody>
</table>

#### 2.7 Toxicity of Bhasma

Accumulated toxicity report on the hazardous effects of heavy metals have made health scientists apprehensive to use metal as medicine. However, Ayurvedic metal based medicine (bhasma) is used in treatment of various disease. Other than Ayurveda, the traditional system of medicine of China and Egypt have described about ample use of metals. Heavy metals are toxic, but their oxides are usually not. FDA has approved Arsenic trioxide to be used in acute leukemia. If the bhasma is not prepared in correct manner, it would be toxic to human. Preparation of bhasma involves several calcination cycles, which lead to conversion of a metal into mixed oxides. Transformations of metal in to oxide may lead to conversion of the zero valent metal state into a form with higher oxidation state and the most important aspect of this synthesis is that the toxic nature (i.e. systemic toxicity causing nausea, vomiting, stomach pain, etc.) of the resulting metal oxide is completely destroyed while inducing the medicinal properties into it.⁸,⁹
2.8 Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles

Metalllic nanoparticles, including zinc, silver, iron, gold and metal oxide nanoparticles, have made knowncountlesspotential in biomedical application. Due to their large surface area to volume ratio such properties are observed\textsuperscript{30,31}. SNPs or nanosilver (NS) are emerging as one of the fastest growing product in the field of nanotechnology. In daily life NS is used in room spray, wall paints, water purifier and laundry detergent. SNPs are also incorporated in textiles for manufacturing of cloth, vests, underwear and socks. It is estimated that all nano materials in medical and healthcare sector, NS application has higher degree of commercialization. A wide category of product is already available in market. In medical sector they are used in wound dressing, contraceptive devices, surgical instrument and bone prostheses. SNPs or NS are being used increasingly in catheters, wound dressings and various household products due to their antimicrobial activity\textsuperscript{31}. The antibacterial property of silver has been known for thousands of years with the ancient Greeks cooking from silver pot. The antimicrobial activities of silver were in practice to keep water safe as early as 1000 BC. In recent year it is proved that antimicrobial properties of silver is due to released Ag\textsuperscript{+} ions. The first documented therapeutic use of silver goes back to 8th century\textsuperscript{32}. Treatment of ulcers with the use of Silver nitrate was a common practice during 17th and 18th century. Now a days, the silver is in practice in medical sector as a biocide to prevent traumatic wounds infection in burns, and diabetic ulcers\textsuperscript{33}. Interaction of silver with structural proteins and preferentially binding with DNA bases causes inhibition of replication. Additionally, bactericidal properties of silver has also been recognised to inactivation of the enzyme (phosphomannose isomerase)\textsuperscript{34}. Presently silver is considered a non-essential accumulative element. Silver is widely distributed in human body fluid and tissues including heart, lungs, aorta, blood, erythrocytes, plasma, bones, brain, breast, caecum, oesophagus, colon, diaphragm, duodenum, hair, ileum, larynx, kidney, urinary bladder, urine, liver, pancreas, adrenal gland, thyroid gland, lymph nodes, muscles, nails ovary, prostate gland, rectum, serum, skin, spleen, testes, teeth (dentine and enamel), trachea, uterus etc. Such wide distribution in the human body suggests that this metal could have some specific functions which are not clear at present\textsuperscript{35}. 

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32
Chapter 2: Literature Review

There has been a resurgence of promotion of colloidal silver as an alternative medicinesince 1990’s. It was claimed with colloidal silver that it can treat various diseases being an essential mineral supplement\(^{36,37}\). Even though the products of colloidal silver are legally accessible as health supplements, still it is illegal in USA to make claim of medical effectiveness of silver in colloidal state. The marketable product stated to as colloidal silver comprises solutions that contain various concentrations of compound of ionic silver. Unlike other modern medicine, the manufacturing of colloidal silver is not subjected to quality control and colloidal silver of various concentrations and particle sizes are available in market. In recent time, there are no fact-based medical uses for colloidal silver through oral route. The national center of USA for complementary and alternative medicine has issued an advisory showing that the therapeutic claims made about colloidal silver are not supported scientifically\(^ {36}\).

2.9 Method of preparation of SNPs
SNPs can be prepared by traditional Ayurvedic literature, physico-chemical method and biological method.

2.9.1 Traditional Ayurvedic method

*Bhasma* is the calcination product of inorganic or organic substances and claimed to be biologically produced nanoparticles. Silver nanomedicine of ancient Ayurveda is known as *raupya bhasma*. It is prepared by methods described an Ayurvedic text in *Rasendrasara Samagraha*. Equal amount of pure silver and sulphur (by weight) are mixed together with half amount of arsenic trisulphite, then it is soaked in lemon juice and subjected to calcination. The process is repeated 14 times to obtain raupya bhasma\(^ {38}\).

2.9.2 Physicochemical syntheses of SNPs

Physical and chemical methods are mainly used for preparation of nanoparticles. SNPs can be prepared by “top down” and “botton-up” methods. Mechanical grinding methods are mainly used as a top down method and the reduction of metal by electrochemical method is used in botton up method\(^ {39,40}\). SNPs synthesized by different physicochemical method is shown in Table 2.2.
Chapter 2: Literature Review

Table 2.2: SNPs synthesized by different physicochemical method

<table>
<thead>
<tr>
<th>S.no</th>
<th>Methods</th>
<th>Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemical method of reduction of the metal salt AgBF$_4$ by NaBH$_4$ in water.</td>
<td>3-40 nm</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Electrochemical method which involves the electro reduction of AgNO$_3$ in aqueous solution in the presence of polyethylene glycol</td>
<td>10 nm</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Sonodecomposition of an aqueous silver nitrate solution in an atmosphere of argon-hydrogen</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Electrostatically complexing silver ions with an anionic surfactant aerosol in extremely stable liquid foam. The foam is then drained off and reduced by introducing sodium borohydride. These silver nanoparticles are very stable in solution, suggesting that the aerosol stabilizes them.</td>
<td>5-40 nm</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Reduction of silver nanoparticles using variable frequency microwave radiation.</td>
<td>15-25</td>
<td>16</td>
</tr>
</tbody>
</table>

2.9.3 Biological synthesis of SNPs

It is also possible to synthesise SNPs by biological methods. Different methods are reported using bacteria, fungi and plant extracts by different researchers.$^{46,47}$

2.9.3.1 Synthesis of SNPs using bacteria

The mechanism behind synthesis of SNPs is the existence of the nitrate reductase enzyme which causes conversion of nitrate into nitrite.$^{48}$ The bacteria involve in synthesis of silver nanoparticles is shown in Table 2.3.

Table 2.3: Different bacteria for synthesis of SNPs

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. licheniformis</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>46.9</td>
<td>50</td>
</tr>
<tr>
<td>Bravibacterium casei</td>
<td>50</td>
<td>51</td>
</tr>
</tbody>
</table>
2.9.3.2 Synthesis of SNPs using Fungi

It is believed that the mechanism involve in synthesis of SNPs by fungi (Table 2.4) is trapping of Silver ion (Ag+) in the exterior of the fungal cells and the succeeding reduction of the silver ions by the enzymes present in the fungal system \(^{59-61}\).

Table 2.4: Different fungi for synthesis of SNPs

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus clavatus</em></td>
<td>10 to 25</td>
<td>62</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>7 to 10</td>
<td>63</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>5 to 25</td>
<td>64</td>
</tr>
<tr>
<td><em>Coriolus versicolor</em></td>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>20 to 50</td>
<td>66</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>5 to 35</td>
<td>67</td>
</tr>
<tr>
<td><em>Phanerochaete chrysosporium</em></td>
<td>100</td>
<td>68</td>
</tr>
<tr>
<td><em>Phoma sp.</em> 3.2883*</td>
<td>70</td>
<td>69</td>
</tr>
</tbody>
</table>

2.9.3.3 Synthesis of SNPs using Plant

The major mechanism for synthesis of SNPs using plant (table 2.5) involves the reduction of the ions. The main phytochemicals which participates are flavones, amides, terpenoids, ketones, aldehydes, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the instant reduction of the ions \(^{70}\).
Chapter 2: Literature Review

Table 2.5: Different Plant used in synthesis of SNPs

<table>
<thead>
<tr>
<th>Plant</th>
<th>Size (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera</td>
<td>15 to 20</td>
<td>71</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>50</td>
<td>72</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>15</td>
<td>73</td>
</tr>
<tr>
<td>Cinnamomum camphora leaf</td>
<td>55 to 80</td>
<td>74</td>
</tr>
<tr>
<td>Cinnamomum zeylanicum bark</td>
<td>50 to 100</td>
<td>75</td>
</tr>
<tr>
<td>Coriandrum sativum leaf</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>Desmodium triflorum</td>
<td>5 to 20</td>
<td>77</td>
</tr>
<tr>
<td>Jatropha curcas</td>
<td>10 to 20</td>
<td>78</td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>2 to 20</td>
<td>79</td>
</tr>
<tr>
<td>Piper betle leaf</td>
<td>3 to 37</td>
<td>80</td>
</tr>
</tbody>
</table>

2.10 Pharmacological Aspects

Different researches independently reported several pharmacological activities of Silver Nano Particles. The activities reported includes antibacterial, antifungal, antiplatelet, antiproliferative, antiangiogenic, anti-inflammatory, analgesic, cytotoxic, genotoxic. Recently reported activity of Silver NPs include against ulcerative colitis, colon cancer and HIV-I. 35,81-93

2.11 Toxicity

In minute concentration, silver is considered to be non-toxic in normal use. One of the most important side effect reported for silver product is argyria. Argyria is irreversible grey to black colouration of skin due to deposition of silver in sub dermal layer. Argyria is just a cosmetic problem it do not cause any physical harm31. On the other hand raupya bhasma (Ancient silver nanomedicine) was consider as safe at due to their use from ancient time therapeutic doses. Recently it is proved scientifically by Inder D et al (2011) who had carried out toxicity of raupya bhasma on mice and concluded that minimum toxic dose of raupya bhasma was 1.5 g/kg and and LD50 was 2.0 g/kg. However the therapeutic dose for human being is 125 mg only.
Chapter 2: Literature Review

2.12 Probiotics

Pharmaceuticals have not been able to completely control the global morbidity and mortality in case of both acute and chronic diseases. Hence, search for the other alternatives has always been there. The old age quote of Hippocrates becomes most pertinent in the current health scenario i.e. “let food be thy medicine and medicine be thy food”. In the late 90’s, microbiologists identified the difference between the micro flora of the diseased human beings and those of normal human beings. The beneficial micro flora were termed as “probiotics”. There are billions of bacteria present in human Gastro Intestinal Tract (GIT) forming about 1 kg of the human weight, which includes both harmful as well as beneficial bacteria. Together they are called as gut flora. Delicate balance between the harmful and the beneficial bacteria is responsible for maintenance of health. When this balance is disturbed, the person becomes diseased. One of the ways to regain this balance is the external administration of probiotics (beneficial bacteria) into the body of the diseased person. Probiotics include a large number of different types of bacteria that are normal inhabitants of human GIT. The most common among them are various species of *Lactobacilli* and *Bifidobacteria*. They reside in small intestine and colon. Probiotics have been able to attract the maximum attention among several food supplements as they have additional benefits beyond their nutritional value. In 1965, Stillwell and Lilly introduced the term “probiotics”. The term is made up of two words Latin preposition pro means "for" and the Greek adjective βιοτικός means “biotic”. Hence, it means “for favour of life”. According to World health organisation (WHO) and Food and Agriculture Organization (FAO) it is defined as “living microorganism intended for administration into the host body in adequate amount so as to confer health benefits”.

2.12.1 History

It has been known since long that there are benefits of using fermented milk products and poultices of bread moulds. But Elie Metchnikoff started the probiotic therapy via fermented milk products in 1907. In 1915, the therapy was used for the treatment of urogenital infections. However, in the intertwining period of 7-8 decades less study is reported on probiotics due to an increased interest in antibiotics. These were labelled as “alternative medicines”. Recently there has been a resurgence of probiotics due to
Chapter 2: Literature Review

demand of consumers for better treatment. This resurgence can also be attributed to development of resistance against antibiotics\textsuperscript{99}.

2.12.2 Probiotic criteria

An organism must fulfil the following criteria in order to be considered as probiotics: There should be high cell viability, and should be able to survive in low pH. Even if strain cannot colonize in gut, it should have the ability to persist. They should have the ability to adhere to the epithelium of GIT so as to overcome the flushing effect due to peristalsis. They should have the ability to interact or to send signals to the immune cells associated with GIT, capable of being isolated from humans, processing resistance, non-pathogenic and positive influence on local metabolic activities.

A dose of five billion colony forming units are generally recommended for adequate health benefits. Probiotics should be Generally Recognized as Safe (GRAS). Probiotics preparations involve the use of both single as well as mixture of microorganisms\textsuperscript{99}.

2.12.3 Mechanism of action of probiotics

To explain the effects of probiotics several mechanisms have been proposed. The effects can be attributed to a number of activities and their action is proposed to be multipronged. Probiotics stimulate the intestinal lactase activity. They partially digest the lactose and can be used in the case of lactose intolerance and in certain types of diarrhoea\textsuperscript{100}. Various fermented milk industries use lactobacilli in order to decrease the lactose concentration in the dairy products which ultimately affects the severity of osmotic diarrhoea\textsuperscript{100}. Lactic acid bacteria inhibit the growth of various pathogenic microorganisms present in dairy products by producing various metabolites such as free fatty acids, bacteriocins and hydrogen peroxide etc\textsuperscript{101}.

Probiotics also cause modification in the toxin receptors and thus hinder the toxin receptor mediated pathology of disease\textsuperscript{102}. They also offer competitive inhibition during colonization to the pathogenic bacteria\textsuperscript{103}. The other mechanisms involve lowering of pH, releasing the gut protective metabolites, production of mucous and regulation of gut motility\textsuperscript{104}. Gastrointestinal mucosa acts as an interface between the body’s immune system and the external environment. Whenever there is decrease in gut
flora the antigen transportation increases. This clearly depicts that gut flora maintain the gut defences (Figure 2.2)\textsuperscript{104,105}.

The interaction between the gut epithelial and immune cells with non-pathogenic probiotic micro-organisms may lead to generation of immunological signals. This interaction occurs in the Peyer’s patches\textsuperscript{106}. Probiotics also modulate the immunoglobulin (Ig) production. They increase the production of IgA, a secretory immunoglobulin which plays an important role in mucosal immunity and thus act as a barrier against the various pathogenic microorganisms and viruses\textsuperscript{106,107}. It has also been demonstrated that probiotics also causes induction of T independent IgA\textsuperscript{108}. Probiotics also increase the production of certain types of cytokines tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukins-10 (IL-10). The up and down regulation of immune response is also affected by probiotics so as to maintain the intestinal homeostasis\textsuperscript{109}.

\[\text{Figure 2.2: Barrier to antigen absorption in intestine.}\]
2.12.4 Probiotics in health

The overall health of the person depends upon his/her eating habits and life style. In ancient time humans used to take enormous live bacteria but as the concept of hygiene developed, there has been a decrease in intake of live bacteria along with the food. The dietary habits in the western world are a cause of development of certain diseases like ulcerative colitis. Their diet lacks fruits, vegetables and omega-3 fatty acids. Due to which they have more chances of development of diseases such as heart diseases and cancer. The increase in allergic and inflammatory conditions, obesity, heart diseases and cancer has been found to be proportional to the decrease in probiotic content in the diet\(^{110}\).

2.12.5 Probiotics in specific diseases

2.12.5.1 Allergies /Eczema

Probiotics are very effective in treatment of food allergy especially in case of infants suffering from atopic eczema or cow’s milk allergy. With the use of *Lactobacillus GG*, there occurs a significant clinical improvement among the people suffering from atopic dermatitis. The clinical improvement is accompanied by reduction in inflammatory marker\(^{111,112}\).

2.12.5.2 Antioxidant activity

*Bacillus coagulans* RK-02 has been reported to produce extracellular polysaccharide having four heteromonosaccharides as its constituents. This has shown a significant antioxidant and free radical scavenging activity\(^{113}\). The powerful antioxidant activity is also shown by *Streptococcus thermophilus*. It protects the body from many dangerous free radicals that develop in the body due to aging, sugar, stress, antibiotics other toxins and chemicals\(^{114}\). Significant antioxidant activity is also shown by *Bifidobacterium bifidum* due to which it has been reported to produce protection to the intestinal lining from the lipid peroxidation in iron over loaded mice\(^{115}\).

2.12.5.3 Antibacterial activities

Multiple probiotics via *in vitro* studies have been found to be effective against many of the pathogenic microorganisms including *Listeria monocytogenes*, *Salmonella typhimurium*, *E.coli* and *H.pylori*. Therefore, prototypic antimicrobial substances can
be obtained from probiotic agents. It may prove to be useful for the pharmaceutical companies to develop new antibiotics\textsuperscript{116,117}.

**2.12.5.4 Diarrhoea**
Probiotics are used for prevention as well as for the cure of various types of diarrhoea. The activity of dietary probiotics against various types of diarrhoea successfully investigated. e.g. *Lactobacillus rhamnosus* GG, strains of *L. Casei*, strains of *L. Acidophilus, L. Reuteri, Escherichia coli* strain, Bifidobacteria and Enterococci, and Probiotic yeast *Saccharomyces boulardii*\textsuperscript{118}.

**2.12.5.5 Rota virus diarrhoea**
Both preventive as well as curative probiotic treatment is available which has been proven with the help of randomized, double blind and placebo studies. *Bifidobacterium lactis* BB-12 and *Lactobacillus rhamnosus* GG are used for prevention whereas *Lactobacillus reuteri* SD 2222 is used for the treatment in acute cases\textsuperscript{119-121}.

**2.12.5.6 Antibiotic associated diarrhoea**
Although broad spectrum newer antibiotics have been developed with fewer side effects but they are liable to cause antibiotic associated diarrhoea (AAD). The chances of incidence ranges from 3.2 to 29/100 patients admitted to the hospital. The complications of AAD involve: electrolyte imbalance, dehydration, pseudo membrane colitis. Antibiotics which are used against anaerobic bacteria are supposed to cause more AAD. *Saccharomyces boulardii* can be used in the treatment of AAD\textsuperscript{122}.

**2.12.5.7 Radiation induced diarrhoea**
The patients who are receiving radiation therapy during cancer usually develop diarrhoea. A study of high potency probiotics preparation was done on such patients (double blind and placebo) taking VSL\#3 as a preparation. It has been shown that probiotic preparation decreases the bowel movements and daily incidences of diarrhoea. From the study, a conclusion was withdrawn that lactic acid bacteria can be a safe, efficient and easy approach to treat radiation associated diarrhoea in cancer patients\textsuperscript{123}.
Chapter 2: Literature Review

2.12.5.8 Traveller’s diarrhoea
It is the diarrhoea associated with the travellers. The chance of incidence ranges from 5% to 15% depending on destination\textsuperscript{31} A mixture of *Saccharomyces boulardii, Lactobacillus acidophilus* and *Bifidobacterium bifidum* is found to have high efficacy in this regard\textsuperscript{124}.

2.12.5.9 Hyperlipidemia
Probiotic strains can be used to lower the body cholesterol level (especially the lactic acid bacteria). Two strains found in yogurt have been found to have significant cholesterol lowering effect. These include *Lactobacillus acidophilus* and *Bifidobacterium lactis*. When *Lactobacillus sporogenes* was given to hyperlipidemic patients over a period of three months a reduction of 32% total cholesterol level and 35% reduction in low density lipoprotein (LDL). The mechanism behind this effect is the inhibition of production of HMG CoA reductase. *L. plantarum* has also been shown to possess cholesterol lowering activities\textsuperscript{125,126}.

2.12.5.10 Hepatic diseases
A report was published which demonstrates the role of multicultural probiotics in the treatment of liver cirrhosis. The study included first one month probiotic treatment followed by 1 month wash out period, followed again by the second probiotic treatment. During the study, blood pressure of hepatic portal vein was measured, which usually was found to lower in case of liver cirrhosis. During the first probiotic therapy period there was an increase in portal vein pressure followed by decrease in that at the end of wash out period which again rise during second period of probiotic therapy. The microorganisms present in formulation included *Streptococcus thermophilus, Bifidobacteria, Lactobacillus plantarum, L. acidophilus, L. casei, L. debrueckii bulgaricus* and *Streptococcus faecum*\textsuperscript{127}.

2.12.5.11 Hypertension
Blood pressure (BP) decrease with the consumption of fermented milk products with species of *Lactobacilli*. The antihypertensive effect of probiotic is attributed to the bacterial cell wall components. Moreover the bacteria are known to generate peptides which have angiotensin converting enzyme inhibiting property\textsuperscript{128}.
Chapter 2: Literature Review

2.12.5.12 Hemolytic uremic syndrome
This syndrome is usually develops in children taking antibiotic therapy for E.coli\(^{129}\). In this syndrome, epithelial injury occurs due to drop in transepithelial electrical resistance. Such injury can be prevented by pre-treatment of intestinal (T84) cells with lactic acid producing bacteria. E.coli produces vero cytotoxin which causes haemorrhagic colitis and haemolytic uremic syndrome in humans. This can be inhibited by the use of probiotic containing Bifidobacterium longum, which produces substances that can bind to vero cytotoxins\(^{130}\). Bifidobacterium longum also offers protection against Salmonella typhimurium\(^{131}\).

2.12.5.13 Inflammation /Arthritis
Probiotics produce both direct as well as indirect effects. The direct effects produce locally with in the GIT includes vitamin production etc. The indirect effects which are produced outside the GIT occur in joints, skin and lungs. Amongst the indirect effects it exerts its influence on immunity and alters the level of inflammatory mediators. Modulation of inflammatory response can be localized within the GIT or it may be systemic. It is postulated that inflammation associated with rheumatoid arthritis can be modulated with the help of probiotics. In chronic juvenile arthritis, there is a disturbance in the gut defence mechanism and an alteration in the permeability of GIT which may account for the inflammation associated with arthritis. The effects of Lactobacillus GG administration to the patients for two weeks shows remarkable improvement\(^{132}\).

2.12.5.14 Inflammatory Bowel Disease (IBD)
IBD involves two chronic diseases: Ulcerative colitis and Crohn’s disease
The available clinical data shows the role of intestinal micro biota in the pathogenesis of IBD and there by provides an evidence that alteration in the intestinal micro biota with the help of probiotics can be helpful in the treatment of disease. E.g. Bifidobacteria infantis has been found to reduce the inflammatory response of the gut lining by inhibiting the bacteroides. Lactobacillus plantarum has also been reported to be used in IBD. A probiotic formulation containing no of microbes (VSL#3) used in case of ulcerative colitis although its clinical efficacy is not certain. E. coli has also been used in case of ulcerative colitis but its clinical efficacy depends upon its dose\(^{133-137}\).
2.12.5.15 Kidney stones

Kidney stones develop as the result of increased concentration of oxalate in the urine. *Oxalobacter formigenes* is responsible for degrade oxalate with the help of enzyme oxalyl-CoA. People with renal stone are found to have less *Oxalobacter formigenes* which ultimately leading to increased oxalate in the urine (hyperoxaluria). *Bifidobacterium breve* has also been reported to be beneficial in case of kidney stones as it also exhibits oxalate degrading property\textsuperscript{138,139}.

2.12.5.16 Neonatal enterocolitis

Caplan and Jilling reported that supplementation with probiotics can be helpful in preventing the neonatal enterocolitis. They developed murine model explaining different characters of neonatal enterocolitis that are clinically and pathologically important. *Bifidobacterium infantis* was found to be effective in this case\textsuperscript{140}.

2.12.5.17 Cancer

There are several hypotheses which explain the mechanism of action of probiotics in treatment of various types of cancers. These include: Detoxification of ingested carcinogens. Inhibition of bacterial growth and/or production of their metabolites those are carcinogenic in nature. Inhibition of tumour cells growth via production of specific compounds. Immune system stimulation against tumour cells. Metabolite production (e.g. butyrate) which improve apoptosis\textsuperscript{141}. *Lactobacilli* have been reported to prevent establishment and growth of tumour cell and their metastasis\textsuperscript{142}. With increase in consumption of products containing *Lactobacilli* or *Bifidobacterium* the chances of breast and colon cancer reduces\textsuperscript{143,144}. The recurrence of superficial bladder cancer has been found to be significantly reduced with the consumption of *Lactobacillus casei*\textsuperscript{145}.

2.12.5.18 Peptic ulcers

According to a report *Lactobacillus salivarius* has been found to be useful in *H. pylori* induced peptic ulcers as it produces lactic acid which can completely inhibit *H. pylori* growth, this in turn, inhibits the inflammatory response associated with it. The first bacteriocin (natural antibiotic substance) was isolated from *Lactobacillus salivarius*.*L. casei* (strain Shirota) can also be used in case of *H. pylori* induced peptic ulcer\textsuperscript{146,147}. 

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44
Chapter 2: Literature Review

2.12.5.19 Lactose intolerance
Probiotics relieve the symptoms associated with intolerance as well as they decrease oro-cecal transit. Those individuals who have such intolerance can tolerate 12 to 15g of lactose when probiotic therapy is given. Yogurt is used in case of such patients as it contains less lactose as compare to milk. Moreover, it contain lactase enzyme and delay gastric emptying time\textsuperscript{148-151}.

2.13 References

45
Chapter 2: Literature Review


Chapter 2: Literature Review

Chapter 2: Literature Review


Chapter 2: Literature Review


Chapter 2: Literature Review


Chapter 2: Literature Review

Chapter 2: Literature Review


