Introduction
1.1. AYURVEDA

"Not for self, not for fulfillment of any earthly desire of gain, But solely for the good of suffering humanity should you treat the patients. Those who sell the treatment of disease as merchandise gather the dust and neglect the gold”,

Charaka

Ayurveda as an “alternative system of medicine” is finding increasing followers in the world mainly because the medicine described in ayurveda are said to have no side effects, with the recommended doses. The herbal medicine of ayurveda has thus attracted increasing attention not only of medical practitioners but also amongst researchers and pharmaceutical companies outside India.

Ayurveda is the “Veda or Knowledge” of Ayus or life. It has been classified as fifth Veda and it is a distinct one. It is even superior to the other Vedas because it gives life, which is the basis of all enjoyments, study, meditation and yoga sadhana.

The Ayurveda medicines deal with the causes, symptoms and treatment of diseases.

Ayurveda is based on ten fundamental considerations.

1. Dusyam (the seven Dhatus and Doshas)
2. Desam (Surroundings)
3. Balam (Strength)
4. Kalam (Season)
5. Analam (Fire of digestion, Agni)
6. Prakriti (Body)
7. Vayaha (Age)
8. Satvam (Mental state)
9. Satmayam (Compatibility)
10. Aharam (Dietary habits)
Introduction

Ayurveda has immense potentialities. Even today, it is used worldwide. Ayurvedic drugs are cheap, easy to obtain and administer and are also more effective.

Ayurveda has some uniqueness. One is Bhasmas which work in small doses can cure incurable diseases. Kaya kalpa is another marvel of Ayurvedic science. Nadi Vijnana is a third wonder. Diagnostic methods of Ayurveda are also simple and accurate. For example, “Til oil examination”, is a wonderful method of diagnosing diseases where no modern medical instruments are involved.

1.1.1. IMPORTANCE OF AYURVEDA

Ayurveda or the science of life is an upanga of aharvenaveda. It consist of 1,00,000 verses in 1000 chapters. It is divided into 8 parts.

- Shalya – surgery
- Shalaka-Treatment of disease of the eye, nose, mouth, ears etc.
- Kaya chikitsa-Treatment of general diseases affecting the whole body such as fever, diabetes etc.
- Bhoota vidya-Treatment of diseases caused by evil spirits.
- Kumara bhridya- Treatment of the infants and of puerpral state.
- Agada-Antidotes to poison.
- Rasayana-Treatment of medicine which promote health and longevity which preserve vigor, restore youth, improve memory, cure and prevent diseases in general.
- Vajikarma or Aphrodisiae-Describes the means of increasing the virile power, of giving tone to the weakened organs of generation.
1.1.2. NEED FOR RESEARCH IN AYURVEDA

In Ayurvedic system of medicine, materials from different sources, viz., plants, animals, metals and minerals, are used to prepare the formulations. While the processes of preparing metal-based Ayurvedic formulations are well specified, little is known about the form in which metal exists in the final product or its mechanism of action. Such knowledge is very much imperative not only for advancement of drug research but also for enhancing the effectiveness of Ayurvedic drugs and for improving their preparation. To derive benefit from such preparations, it is imperative to prepare the Bhasmas as per the classical methods and subject them to toxicity testing and thereby ensuring their efficacy and safety.

The world has now realized the limitation of the synthetic preparation and is in search of safe and effective answers. World wide, expenditure on health systems is growing rapidly with an estimated, 3 trillion dollars spent in the late 1990’s. Improvement in knowledge and technical advances, demographic transition, rapidly changing patterns of major public health problems such as SARS, HIV/AIDS and tuberculosis and bird flu are highlighted as where there exist more resources and researches. In recent years, drug regulatory authorities of several countries have allowed clinical evaluation of plants and herbo-mineral drugs for the various diseases.

In many countries consumers have embraced the use of botanical’s and other supplements as natural approach to their health care. Unfortunately misconceptions regarding safety and efficacy of the drugs are common and the fact that a substance can be called natural of course does not guarantee its safety. In fact, these products can be adulterated, misbranded or contaminated either intentionally or unintentionally in a variety of ways.

Ayurveda, the Traditional Indian medicine and Traditional Chinese medicine remain the most ancient yet living traditions. China has been successful in promoting its therapies through research and evidence based approaches whereas Ayurveda still needs more extensive and evidence base scientific researches.
1.1.3. **AYURVEDIC MEDICINE IN INDIA**

Ayurvedic medicine, as practiced in India, is one of the oldest systems of medicine in the world. Many Ayurvedic practices predate written records and were handed down by word of mouth. Two ancient books, written in Sanskrit more than 2,000 years ago, are considered the main texts on Ayurvedic medicine—*Caraka Samhita* and *Sushruta Samhita*. The texts describe eight branches of Ayurvedic medicine:

- Internal medicine
- Surgery
- Treatment of head and neck disease
- Gynecology, obstetrics, and pediatrics
- Toxicology
- Psychiatry
- Care of the elderly and rejuvenation
- Sexual vitality.

Ayurvedic medicine continues to be practiced in India, where nearly 80 percent of the population uses it exclusively or combined with conventional (Western) medicine. It is also practiced in Bangladesh, Sri Lanka, Nepal, and Pakistan.

Most major cities in India have an Ayurvedic college and hospital. The Indian government began systematic research on Ayurvedic practices in 1969, and that work continues.

1.1.4. **AYURVEDIC FORMULATIONS**

In Ayurvedic system of medicine, materials from different sources of medicines viz., plants, animals, metals and minerals are used to prepare the formulations. The various potent Ayurvedic drug forms are Asavas (infusions), Arishtas (decoctions), Tailas (various medicated oils), double Kashayams or Kvathas, Churna (powder), Lepa (ointment), Gutika or Kulikai (pills), Ghritams (medicated ghees), Bhasmas or Sindoor (metallic oxides), Rasa, Rasayanas, Lehyam (confections).
1.2. BHASMAS

The process of preparing metal based Ayurvedic formulations is called bhasmas. The Herbomineral Ayurvedic preparations essentially contain minerals and metals as integral part of the formulations. They are being used with an intention to give therapeutic efficacy to the product for a designated illness. We would like to add here that mere presence of a metal in any form is not going to create problems for human beings – it is the context of application and the due consideration given to all variables that determines the level of benefit or otherwise. In the practice of Ayurveda where the metallic drugs are used, first they are made bio-compatible in a particular chemical form, which has been designed in ancient Ayurveda classics through the specific processes of Shodhana (Purification) and Marana (Calcination). These Ayurveda detoxification processes remove the toxic potentials from metals and impart in them the therapeutic efficacy of a very high grade. The basic reason for their inclusion in to medicine was for a very potent efficacy in small dosage forms for specific disorders that were otherwise not curable by pure herbals alone. It is very clear from the long history of usage of herbomineral and metallic preparations in Ayurveda that properly processed Ayurveda medicines do not have any toxic potential. The history of Ayurvedic Rasa Shastra (the branch of Ayurveda mastering the art of preparation and usage of metals in medicines) dates back from around 800 AD from the era of Nagarjuna. These were required as new dosage form and their properties as fast acting medicines.

A considerable reduction in the particle size of the metals and metallic compounds on one hand and change in the structure of the metal after several processes of Shodhana and Marana render these preparations an element of safety, which is not expected in metals that have not undergone these processes. In metal based preparations of Ayurveda, the metal is not used as it is, but after subjecting it along with herbs, to an involved series of processing steps. The output of such a process is a fine powder, called Bhasma, which is either used as a drug or in combination with other herbs.
Though bhasma preparations are widely used in ayurveda, little knowledge is known as to what happens to the metal when it is subjected to bhavana with herbs and the subsequent calcination process. The traditional texts also do not throw any light on the changes undergone by a metal during the above processes.

It is interesting to note that same metal is processed (i.e. Given bhavana) with different sets of herbs, to be used for different indications. In this context, it is interesting to study as to what changes the metal undergoes during the different steps of bhavana and subsequently during the calcination process it acquires a non-toxic, therapeutically efficacious form. Bhasma are known to be effective in very small doses, as low as few milligrams. Shelf life of bhasma is believed to be infinite.

Existing technology is for analyzing the composition of drugs such as Atomic absorption spectroscopy and Flame photometry studies have their limitations, as they can reveal only the presence of the specific metal and quantity of the metal in the preparation. Earlier, there used to be a view that metal oxides/sulphates and other salts are formed during the process of Calcination.

With the advent of nanotechnology, the current belief is that during bhasmikaran (Process of Ashing) the metal acquires nano particle size, which is responsible for its enhanced bioavailability and activity and hence the dose is small. Even if it is so, it remains to be answered as to why the same metal is given bhavana (Grinding) with different set of herbs to be used in different disease conditions. There has to be much more to the elaborate process involved in the preparation of bhasma than simply imparting nano size to the metal particles. If we understand this we may be able to reduce the number of steps, especially for the satha puta (100 cycles) and sahasra puta (1000 cycles) using modern technologies and equipment available, including grinding machines (for bhavana) and muffle furnace to ash etc. It may also throw some light on the mechanism of action of bhasma and open new avenues for understanding diseases and cure using bhasma.
Table 1: IMPORTANT BHASMAS

<table>
<thead>
<tr>
<th>Name of Medicine</th>
<th>Active ingredients</th>
<th>Therapeutic use</th>
<th>Dose/Body wt. in Kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abhrak bhasma</td>
<td>Mica and juices of several indigenous drugs.</td>
<td>Tonic and haematinic, used in chronic asthma, phthisis, old age debility and gives strength to the body.</td>
<td>250 mg with honey</td>
</tr>
<tr>
<td>Godanti (Harithal) bhasma</td>
<td>Godanti and ghrit kumari rasa.</td>
<td>Emmenagogue, anti spasmodic, tonic, antacid. Used in influenza, cough head ache, malarial fever.</td>
<td>375 mg with honey</td>
</tr>
<tr>
<td>Lauh bhasma</td>
<td>Iron</td>
<td>Restorative, haematinic and astringent. Indicated in anemic conditions, disorders of liver and spleen jaundice, oedema and general debility. Increase hemoglobin content of blood.</td>
<td>125 mg with honey</td>
</tr>
<tr>
<td>Madhumandoor bhasma</td>
<td>Reduced iron</td>
<td>Alterative, Haematinic and diuretic. Used in anemia, oedema, chlorosis, rickets and jaundice.</td>
<td>125-750 mg with honey</td>
</tr>
<tr>
<td>Shankh Bhasma</td>
<td>Conch shell</td>
<td>Antiperiodic, carminative and analgesic. Used in colic, flatulence and tympanites.</td>
<td>250-750 mg with honey</td>
</tr>
<tr>
<td>Swarna Bhasma</td>
<td>Pure gold.</td>
<td>Nervine tonic, aphodisiac, emmenagogue and increase sexual power, stimulates activity of stomach, skin and kidney causing diaphoresis.</td>
<td>32-125 mg with honey</td>
</tr>
</tbody>
</table>
### Introduction

<table>
<thead>
<tr>
<th>Bhasma</th>
<th>Metal</th>
<th>Properties</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamra Bhasma</td>
<td>Copper</td>
<td>Astringent, sedative, antispasmodic, antiseptic. Used in cough, phthisis, asthma and liver disorders.</td>
<td>65-130 mg with honey</td>
</tr>
<tr>
<td>Vanga Bhasma</td>
<td>Tin</td>
<td>Diuretic and urinary antiseptic. Used in urinary disorders and general debility.</td>
<td>125-250 mg</td>
</tr>
<tr>
<td>Yasada Bhasma</td>
<td>Zinc</td>
<td>Alterative, diuretic and hypoglycemic and astringent. Used in internal haemorrhage, diabetes, urinary disorders and night sweats in tuberculosis.</td>
<td>125-250 mg with honey</td>
</tr>
</tbody>
</table>

### 1.2.1. PREPARATION OF BHASMAS

Bhasmas are prepared and purified from raw minerals and metals after purifying with specific plants and animal products in the following steps:

**I Sodhana – Purification Process**

a. Samanya Sodhana – Common Methods

b. Visesha Sodhana – Specific methods

(i) Bhavana - Grinding

(ii) Svedhana – Heating Employing “Dolayantra”

(iii) Nirvapana - Immersing

(iv) Mardana - Triturating

**II Jarana – Roasting & Trituration Process**

**III Marana – Calcination Process**
1.2.1.1. PROCESS OF PURIFICATION

In Ayurveda, the process of purification is called Shodhana. Chemical purification is different from medicinal purification. In chemical purification it is only elimination of foreign particles. In medicinal purification the objects aimed at are

a) Elimination of harmful matter from the drug.

b) Modification of undesirable physical property of the drug.

c) Conversion of some of the characteristics of the drugs.

d) The enhancement of the therapeutic action, thereby potentizing the drug.

Herbomineral Preparation (Yasada bhasma) is subjected to medicinal purifications.

Shodhana is of two kinds

(i) *Samanya sodhana* - Bhasma is purified using this method. This is applicable to large number of metals or minerals and involves heating thin sheet of the metals and immersing them in Taila (Gingelly Oil), Takra (Curd), Gomutra (Cow Urine), Khadi (Rice Gruel), Kulatta (Horse Gram Extract) etc.

(ii) *Visesha Sodhana* - This is applicable only to certain drugs of certain preparation.

1.2.1.2. PROCESS OF TRITURATION

The purified metals are melted in an iron pan while they are melting, a powder of suitable herbs is sprinkled in small quantities and stirred with Loha darvi (iron spatula). This process is continued till the melted purified metals are reduced to powder form.

1.2.1.3. PROCESS OF CALCINATION

The second stage is the preparation of bhasma. The purified drug is put in to a khalva (stone mortar and pestle) and ground with juice of the specified plants or kasayas of drugs mentioned for the method. It is ground for the specified period of time then made into small cakes (pellets) (*Cakrikas*). The size and thickness of the cakes depend on the heaviness of the drug. The heavier the drug, the thinner are the cakes. These cakes are dried well under sunlight and placed in one single layer in a shallow earthen plate (*Sarava*) and closed with another plate. The edge is sealed with clay-smeared cloth in seven consecutive layers and dried.
A pit is dug in an open place. The diameter and depth of the pit depends on the metal (Zinc) that is to be calcined. Half of the pit is filled with cow dung cakes. The sealed earthen container is placed in it and the remaining space is filled with more cow dung cakes. Ignited on all four sides and in the middle of the pit. When the burning is over, it is allowed to cool completely. The earthen container is removed and seal is opened and the contents are taken out. The medicine is ground into a fine powder in a Khalva. The process of triturating with the juice, making Cakrikas and calcination process is repeated seven times as prescribed in the standard Ayurvedic procedures or till the proper fineness and quality of Bhasma are obtained.

The Putas are described under different names to indicate the size of the pit and the number of cow dung cakes to be used in the preparation of Bhasmas. They also indicate the amount of heat required and the period of burning. The metal is subjected to gaja puta seven times, till a faint reddish yellow color bhasma appears. Thus Yasada bhasma is processed, and prepared.

1.2.2. CHARACTERISTIC FEATURES OF BHASMAS

The properly prepared bhasmas should have the following characteristic features.

1. There should be no metallic lustre (Cadrika).
2. When taken between the index finger and thumb and spread, it should be so fine as to get easily into the finger lines (rekha purita).
3. When a small quantity is spread on cold water, it should float on the surface (varitaram).
4. The bhasma should not revert to the original state (apunarbhava).

The present Dissertation is aimed to prepare an Ayurvedic Herbomineral formulation (Yasada Bhasma) from zinc metal by subjecting it into various purification processes, as per standard Ayurvedic textual procedures.
1.2.3. HERBOMINERAL PREPARATION (YASADA BHASMA)

It is specially processed zinc and is known as zinc calx. It is used as an alterative, diuretic, hypoglycemic and astringent, used in internal haemorrhages, diabetes, urinary disorders and night sweat in tuberculosis. Yasada bhasma is prepared from pure zinc metal.

1.2.3.1. CONSTITUENTS OF HMF SELECTED FOR THE STUDY

Table 2: Ingredients used in the Preparation of HMP (Yasada bhasma)^3

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the ingredients</th>
<th>Botanical/Animal/ Mineral source</th>
<th>Products/Parts used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yasada</td>
<td>Zinc metal</td>
<td>-</td>
<td>Q.S.</td>
</tr>
<tr>
<td>2</td>
<td>Taila</td>
<td>Sesamum indicum Linn.</td>
<td>Seed oil</td>
<td>Q.S.</td>
</tr>
<tr>
<td>3</td>
<td>Takra</td>
<td>Bos taurus Linn.</td>
<td>Curd</td>
<td>Q.S.</td>
</tr>
<tr>
<td>4</td>
<td>Gomutra</td>
<td>Bos taurus Linn.</td>
<td>Urine</td>
<td>Q.S.</td>
</tr>
<tr>
<td>5</td>
<td>Kanjika</td>
<td>Oryza sativus Linn.</td>
<td>Rice gruel</td>
<td>Q.S.</td>
</tr>
<tr>
<td>6</td>
<td>Kulattha kasaya</td>
<td>Dolichus biflorus Linn.</td>
<td>Seed extract</td>
<td>Q.S.</td>
</tr>
<tr>
<td>7</td>
<td>Apamarga</td>
<td>Achyranthes aspera. Linn.</td>
<td>Whole plant extract</td>
<td>Q.S.</td>
</tr>
<tr>
<td>8</td>
<td>Kumari svarasa</td>
<td>Aloe vera Burm.f.</td>
<td>Aloe juice</td>
<td>Q.S.</td>
</tr>
</tbody>
</table>

Q.S - Quantity sufficient

The present investigation is focused towards the standard preparation as well as Scientific Validation studies on Herbomineral preparation (Yasada Bhasma).

1.3. USES OF HMP UNDER STUDY IN FREE RADICAL MEDIATED DISEASES

This selected traditional Herbomineral preparation is used in controlling/managing free radical mediated diseases such as Diabetes Mellitus, Kidney disorders and Liver disorders.

Before carrying out the scientific evaluation studies on this selected traditional preparation, it is essential to know about the diseases & disorders, where this preparation finds use which will enable easy understanding of mechanism of action.
1.3.1. DIABETES MELLITUS

Diabetes is a syndrome characterized by disordered metabolism and inappropriately high blood sugar (hyperglycemia) resulting from either low levels of the hormone insulin or from abnormal resistance to insulin's effects coupled with inadequate levels of insulin secretion to compensate. The characteristic symptoms are excessive urine production (polyuria), excessive thirst and increased fluid intake (polydipsia), and blurred vision; these symptoms are likely to be absent if the blood sugar is only mildly elevated.

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications producing cardiovascular diseases, retinopathy, nephropathy and neuropathy. Hyperglycemia, a condition characterized by an abnormal excess of sugar in the blood, has been linked to the onset of type 2 diabetes mellitus and associated cardiovascular complications including hypertension.

**Fig 1: Structure of Pancreas**
**Introduction**

The pancreas is a gland organ in the digestive and endocrine system of vertebrates. It is both an endocrine gland (producing several important hormones, including insulin, glucagon, and somatostatin), as well as an exocrine gland, secreting pancreatic juice containing digestive enzymes that pass to the small intestine. These enzymes help in the further breakdown of the carbohydrates, protein, and fat in the chyme.

**TABLE 3: FUNCTIONS OF PANCREAS**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Appearance</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Islets of Langerhans</td>
<td>Lightly staining, large, spherical clusters</td>
<td>Hormone production and secretion (endocrine pancreas)</td>
</tr>
<tr>
<td>Pancreatic acini</td>
<td>Darker staining, small, berry-like clusters</td>
<td>Digestive enzyme production and secretion (exocrine pancreas)</td>
</tr>
</tbody>
</table>

**GRAPH 1a: EPIDEMIOLOGY OF DIABETES MELLITUS**
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GRAPH 1b: GROWTH RATE OF DIABETES MELLITUS

GRAPH 1c: DIABETES MELLITUS IN DIFFERENT AGE GROUPS

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Graph 1 depicts estimated prevalence rates for diabetes, by sex, combined across the three provinces. The prevalence rate increases smoothly among both males and females until age 75 to 79 years, after which it shows a modest decline. The prevalence rate among females is slightly higher in the 20 to 40 age group, but this is likely an artifact of how gestational diabetes was handled within the pilot. Thereafter, the prevalence rates are higher among males than females.

1.3.1.1. ACUTE SYMPTOMS

Acute symptoms of diabetes are due to severe hyperglycemia and include polyuria, polydipsia, polyphagia, weight loss and blurred vision. Patients may exhibit impaired growth and increased susceptibility to infections (e.g. recurrent vaginal candidiasis or urinary tract infections). Acute marked hyperglycemia may lead to diabetic ketoacidosis (DKA) in type 1 diabetes or to the hyperglycemic hyperosmolar nonketotic syndrome (HHNS) in type 2 diabetes.

1.3.1.2. CHRONIC SYMPTOMS

Chronic symptoms of diabetes are due to vascular damage from persistent hyperglycemia. Vascular damage leads to end-organ damage. Other conditions associated with diabetes, such as hypertension, dyslipidemia (as well as smoking) accelerate the development of vascular damage and the chronic complications of diabetes, which are the following:

- retinopathy with potential loss of vision
- nephropathy leading to kidney failure
- peripheral neuropathy leading to pain, foot ulcers, and limb amputation
- autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction
- coronary heart disease which is the major cause of death for patients with diabetes
- peripheral vascular disease
- cerebrovascular disease
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Unfortunately, many patients with diabetes remain asymptomatic for long periods, so that the first presentation of the disease is frequently a chronic complication. Indeed, about 50% of newly diagnosed type 2 diabetes would have already developed a vascular complication.

1.3.1.3. TYPE 1 DIABETES MELLITUS

Type 1 diabetes (DM-1) was previously known as IDDM (insulin dependent diabetes mellitus) or juvenile-onset diabetes. About 5-10% of patients with diabetes have DM-1. Type 1 diabetes affects 3 in 1000 children and its incidence is increasing worldwide both in low and high prevalence populations.

1.3.1.4. PATHOGENESIS OF TYPE 1 DIABETES

Type 1 diabetes is primarily a disease of the young given its peak incidence at the age of 10 to 12 years for girls and 12 to 14 years for boys; however, the disease can occur at any age, but most patients are diagnosed before age 20. Type 1 diabetes refers to cell-mediated autoimmune destruction of pancreatic beta islet cells, which leads to absolute insulin deficiency and predisposes individuals to diabetic ketoacidosis (DKA). The etiology is most often autoimmune in origin, but idiopathic destruction of beta islet cells without evidence of autoimmunity is also classified under this group. Although the presentation and progression is variable, all patients with DM-1 require insulin for survival.

The autoimmune nature of DM-1 has been intensively investigated, and it has long been assumed that the pathogenesis of the disease can be explained by interplay between genetics and environment. The pathogenesis can be summarized as follows: in a genetically predisposed individual, (currently not well-defined) environmental factors trigger an autoimmune process (activation of T lymphocytes reactive to islet cell antigens) that leads to destruction of islet cells and insulin deficiency.
Based on epidemiologic and genetic studies, it is well accepted that there is a strong genetic component for development of DM-1, although 90% of affected patients do not have a close relative with the disease. Multiple chromosomal loci associated with the disease have been identified; however, few true genes have been described. Loci associated with the development of DM-1 are found within the MHC-HLA class II region. These loci are known to harbor genes associated with presentation of antigens to T lymphocytes. There are also MHC haplotypes that provide protection and non-HLA loci that further contribute to the genetic variability of the disease.

The rising incidence of DM-1 suggests an important role of the environment in its pathogenesis. However, understanding of the influence of the environment toward the pathogenesis of DM-1 is incomplete. It has long been thought that environmental triggers of the disease exist. These triggers may include infections agents (e.g. viruses- congenital rubella, enteroviruses), early life factors (early exposure to cow’s milk, vitamin D deficiency, rapid growth), toxins, vaccinations, stress and climatic influences. However, more recently, it is thought that environmental agents do not serve as triggers but as modifiers of genetic susceptibility to autoimmunity.
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Immune deregulation, caused by genetic susceptibility and environmental modifiers, leads to development of auto antibodies against various islet cell components, including glutamic acid decarboxylase antibodies (GAD-65), islet cell antibodies (ICA512/IA-2) and insulin antibodies (IAA). These antibodies serve as markers for DM-1. Indeed, the best predictor for future development of DM-1 is the expression of multiple auto antibodies. Beta cell destruction is thought to be primarily a T-cell mediated process, as evidenced by the presence of intense insulitis in newly diagnosed patients. Beta cell destruction is variable being more rapid in younger individuals and slower in older individuals. Type 1 diabetes is associated with other autoimmune disorders including Graves' disease, Addison's disease and autoimmune polyendocrine syndromes.

1.3.1.5. CLINICAL FINDINGS

Diabetic ketoacidosis may be presenting clinical picture for DM-1 but more often, DM-1 presents classic symptoms of diabetes such as polyuria, polydypsia, polyphagia, blurry vision and unexplained weight loss. Upon presentation, DM-1 patients may also exhibit diabetic dyslipidemia, characterized by low HDL and high TG rich particles (such as VLDL, chylomicrons). Insulin deficiency, in type 1 diabetes, will lead to release of FFA from adipose tissue and transport to the liver where they are re-esterified to VLDL and secreted back in the circulation. Additionally, in the absence of insulin, LPL will not function appropriately, and clearance of TG-rich particles will be deficient. Increased (hepatic) production and decreased clearance of TG-rich particles, therefore leads to the characteristic dyslipidemia of diabetes. In type 1 diabetes, the dyslipidemia is fully corrected with adequate insulin therapy.

1.3.1.6. TYPE 2 DIABETES MELLITUS

Type 2 diabetes (DM-2), previously known as NIDDM or adult-onset diabetes, is the most prevalent form of diabetes, accounting for over 90% of all cases of diabetes. Type 2 diabetes is characterized by varying degrees of insulin resistance and insulin deficiency. It is thought that the earliest defect in the pathogenesis of DM-2 is impaired insulin action or insulin resistance. Resistance to the action of insulin will result in
impaired insulin mediated glucose uptake in the periphery (by muscle and fat), incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance (and therefore prevent abnormal fuel metabolism and maintain normal glucose and lipid levels), beta islet cells will increase the amount of insulin secreted. Higher circulating insulin levels will overcome the impedance to the action of insulin. This state of high insulin levels with euglycemia persists for many years. Abnormal fuel metabolism (hyperglycemia and dyslipidemia) occurs when there is a mismatch between insulin requirements, as dictated by insulin resistance, and insulin supply, as dictated by beta cell function. Therefore, for DM-2 to develop, two defects are necessary: insulin resistance and insulin deficiency relative to the resistance (figure 4).

The dual defect of insulin deficiency and insulin resistance in DM-2 is caused by interplay between genetic and environmental.
Introduction

Fig 4 MECHANISM OF ACTION OF INSULIN

Pancreas

β-cell dysfunction

Diabetes genes
Adipokines
Inflammation
Hyperglycaemia
Free fatty acids
Other factors

Insulin

Lipolysis
Glucose production

Fat
Liver
Muscle

Glucose uptake

Fatty acids
Blood glucose

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Fig 4: INSULIN SECRETION AND ITS ACTION
1.3.1.7. MECHANISM OF INSULIN RELEASE IN NORMAL PANCREATIC BETA CELLS

Insulin secretion does not depend on blood glucose levels but releasing of stored insulin depends on blood glucose levels. Since insulin is the principal hormone that regulates uptake of glucose into most cells from the blood (primarily muscle and fat cells, but not central nervous system cells), deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

Most of the carbohydrate in food is converted within the first two hours to the monosaccharide glucose, the principal carbohydrate in blood. Insulin is produced by beta cells (β-cells) in the pancreas in response to rising levels of glucose in the blood (e.g. after a meal). Insulin enables most body cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose (the basic sugar used for fuel) to glycogen for storage in liver and muscle cells. Lower insulin levels result in the reverse conversion of glycogen to glucose when glucose levels fall, although only glucose produced this way by the liver re-enters the bloodstream.

Higher insulin levels increase many anabolic ("building up") processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa.

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, glucose is not handled properly by body cells (about ⅔ require it) or stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis.
1.3.2. KIDNEY AND KIDNEY DISEASES

Kidney is a paired organ, whose functions include removing waste products from the blood and regulating the amount of fluid in the body. The basic units of the kidneys are microscopically thin structures called nephrons, which filter the blood and cause wastes to be removed in the form of urine. The body’s kidneys are dark red in color and have a shape in which one side is convex, or rounded, and the other is concave, or indented.

The kidneys lie against the rear wall of the abdomen, on either side of the spine. They are situated below the middle of the back, beneath the liver on the right and the spleen on the left. Each kidney is encased in a transparent, fibrous membrane called a renal capsule, which helps to protect it against trauma and infection. The concave part of the kidney attaches to two of the body’s crucial blood vessels—the renal artery and the renal vein—and the ureter, a tube-like structure that carries urine to the bladder.

1.3.2.1. STRUCTURE OF KIDNEY

The outermost layer of the kidney is called the cortex. Beneath the cortex lies the medulla, an area that contains between 8 and 18 cone-shaped sections known as pyramids, which are formed almost entirely of bundles of microscopic tubules. The tips of these pyramids point toward the center of the kidney. The cortex extends into the spaces between the pyramids, forming structures called renal columns. The center of the kidney is a cavity called the renal pelvis.

The task of cleaning, or filtering, the blood is performed by millions of nephrons, remarkable structures that extend between the cortex and the medulla. Under magnification, nephrons look like tangles of tiny vessels or tubules, but each Nephron actually has an orderly arrangement that makes possible filtration of wastes from the blood. The primary structure in this filtering system is the glomerulus, a network of extremely thin blood vessels called capillaries. The glomerulus is contained in a cuplike structure called Bowman’s capsule, from which extends a narrow vessel, called the renal
Fig 5 CROSS SECTION OF KIDNEY

Capsule
Cortex

medulla

Renal papilla

Fat in renal sinus

Renal sinus

Minor Calyx
Major Calyx

Renal artery

Renal Pelvis

Renal Vein

Renal pyramid in renal medulla

Ureter

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tubule. This tube twists and turns until it drains into a collecting tubule that carries urine toward the renal pelvis. Part of the renal tubule, called the loop of Henle, becomes extremely narrow, extending down away from Bowman’s capsule and then back up again in a U shape. Surrounding the loop of Henle and the other parts of the renal tubule is a network of capillaries, which are formed from a small blood vessel that branches out from the glomerulus.

Fig 6: STRUCTURE OF NEPHRON

1.3.2.2. KIDNEY FUNCTIONS

Kidneys play a critical role in the survival, and following are few important functions of kidney.

1. To excrete Toxins.
2. To excrete excess drugs.
3. To maintain fluid balance.
4. To maintain electrolyte balance.
5. To maintain acid-base balance.
6. To monitor blood pressure.
7. To produce Vitamin D and Erythropoietin.
1.3.2.3. COMMONLY RECORDED KIDNEY DISEASES ARE

1. Hereditary Disorders like Alports syndrome, hereditary nephritis.
2. Congenital disease like Genitourinary tract malformation, kidney tissue destruction.
3. Acquired Kidney Diseases such as Nephritis-Glomerulo nephritis.
5. Nephritic syndrome.
6. Long-standing High Blood Pressure (Hypertension) leading to Kidney disorders.
7. Kidney damages due to toxins and drugs.

Graph 2a: EPIDEMIOLOGY OF KIDNEY DISEASES
1.3.2.4. SYMPTOMS OF KIDNEY DISEASES

Although many forms of kidney disease do not produce symptoms until late in the course of the disease. But there are six signs that may indicate kidney diseases:

- Burning or difficulty during urination
- An increase in the frequency of urination
- Passage of blood in the urine
- Puffiness around the eyes, swelling of the hands and feet
- Pain in the small of the back just below the ribs
- High blood pressure

1.3.2.5. ACUTE PHOSPHATE NEPHROPATHY

Acute phosphate nephropathy (APN), a clinicopathological entity consists of damage to the kidneys caused by the formation of phosphate crystals within the renal tubules, damaging the nephron, and can cause acute renal failure.¹

Acute phosphate nephropathy (also called acute nephrocalcinosis) presents as acute renal failure with minimal proteinuria and bland urine sediment in patients recently exposed to OSP. Renal biopsy reveals acute and/or chronic renal tubular injury.
(depending on time to diagnosis) with calcium-phosphate crystal deposition in the distal tubules and collecting ducts and no other pattern of histological injury.

1.3.2.6. INCREASED RISKS FOR ACUTE PHOSPHATE NEPHROPATHY

Age, renal failure, and the ongoing use of medications, such as angiotensin-converting enzyme inhibitors and/or angiotensin-receptor blockers, are now recognized as risk factors for the development of phosphate nephropathy.¹⁰

1.3.2.7. NEPHROCALCINOSIS

Phosphate induced Nephrocalcinosis refers to the deposition of calcium (Ca) salts in the cortico medullary junction of the kidney. Kidney calcification may occur within a few weeks upon weaning. It could be suggested that an enlarged, calcified kidney causes stretching of the renal capsule, which in turn may give rise to discomfort in the animal.¹¹

This renal calcification may occur at a molecular, microscopic or macroscopic level leading to progressive amounts of renal damage. The major causes include those associated with an increase in urinary levels of calcium, oxalate and phosphate. Under these conditions, urine concentration and supersaturation leads to calcium crystal precipitation, which may be an intratubular event or initiated within the renal interstitium.¹²

CAUSES OF NEPHROCALCINOSIS

Nephrocalcinosis may be caused by a number of conditions:

- Excess excretion of calcium by the kidney
- Renal tubular acidosis
- Medullary sponge kidney
- Hypercalcemia (high calcium levels in the blood)
- Renal cortical necrosis
- Tuberculosis
- High intake of Phosphate
Fig 7: NEPHROCALCINOSIS
1.3.3. LIVER

The liver is a vital organ in the human body and is present in vertebrates. The liver is necessary for survival; a human can survive up to 24 hours without liver function. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. The liver is also the largest gland in the human body. It lies below the diaphragm in the thoracic region of the abdomen. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues.

1.3.3.1. PHYSIOLOGY OF LIVER

The various functions of the liver are carried out by the liver cells or hepatocytes.

- The liver produces and excretes bile (a greenish liquid) required for emulsifying fats. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder.

- The liver performs several roles in carbohydrate metabolism:
  - Glycogenolysis (the breakdown of glycogen into glucose) (muscle tissues can also do this)
  - Glycogenesis (the formation of glycogen from glucose)
  - The breakdown of insulin and other hormones

- The liver is responsible for the mainstay of protein metabolism. For instance, the liver can convert lactic acid to alanine.

- The liver also performs several roles in lipid metabolism:
  - Cholesterol synthesis
  - Lipogenesis, the production of triglycerides (fats).
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Fig 8a: STRUCTURE OF LIVER

Fig 8b: INTERNAL STRUCTURE OF LIVER
• The liver produces coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.
• The liver breaks down hemoglobin, creating metabolites that are added to bile as pigment (bilirubin and biliverdin).
• The liver breaks down toxic substances and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor.
• The liver converts ammonia to urea.
• The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin B12, iron, and copper.
• In the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow will completely take over that task.
• The liver is responsible for immunological effects- the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.
• The liver produces albumin, the major osmolar component of blood serum.

1.3.3.2. HEPATOCYTES

Hepatocytes make up 70-80% of the cytoplasmic mass of the liver. These cells are involved in protein synthesis, protein storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids, and detoxification, modification and excretion of exogenous and endogenous substances. The hepatocyte also initiates the formation and secretion of bile.

1.3.3.3. HEPATOCYTE HISTOLOGY

Hepatocytes display an eosinophilic cytoplasm, reflecting numerous mitochondria, and basophilic stippling due to large amounts of rough endoplasmic reticulum and free ribosomes. Brown lipofuscin granules are also observed (with increasing age) together with irregular unstained areas of cytoplasm; these correspond to cytoplasmic glycogen and lipid stores removed during histological preparation. The average life span of the hepatocyte is 5 months; they are able to regenerate.
Hepatocyte nuclei are round with dispersed chromatin and prominent nucleoli. Anisokaryosis is common and reflects tetraploidy & polyploidy, a normal feature of over 50% of hepatocytes. Binucleate cells are also common. Hepatocytes are organised into plates separated by vascular channels (sinusoids), an arrangement supported by a reticulin (collagen type III) network. The hepatocyte plates are one cell thick in mammals and two cells thick in the chicken. Sinusoids display a discontinuous, fenestrated endothelial cell lining. The endothelial cells have no basement membrane and are separated from the hepatocytes by the space of Disse which drains lymph into the portal tract lymphatics. Kupffer cells are scattered between endothelial cells; they are part of the reticuloendothelial system and phagocytose spent erythrocytes. Stellate (Ito) cells store vitamin A and produce extracellular matrix and collagen; they are also distributed amongst endothelial cells but are difficult to visualise by light microscopy.

Hepatocytes are an important physiological example for evaluation of both biological and metabolic effects of xenobiotics. They do not proliferate in culture. Hepatocytes are intensely sensitive to damage during the cycles of cryopreservation including freezing and thawing.

1.3.3.4. DISEASES OF THE LIVER

Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the hemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile. There are also many pediatric liver diseases, including biliary atresia, alpha-1 antitrypsin deficiency, alagille syndrome, progressive familial intrahepatic cholestasis, and Langerhans cell histiocytosis to name but a few.

Fatty changes in the liver are closely associated with metabolic impairments and hyperglycemia and dislipidemia. Liver cirrhosis is always accompanied by increased levels of triglycerides and cholesterol; this was attributed to decreased hepatic lipase activity and low lecithin: cholesteryl ester transfer activity.
Graph 3a: EPIDEMIOLOGY OF LIVER DISEASES

Graph 3b: EPIDEMIOLOGY OF LIVER DISEASES
1.4. EXISTING TREATMENT AND MANAGEMENT OF DISEASES / DISORDERS

Diabetes is a chronic disease, and emphasis is on managing short-term as well as long-term diabetes-related problems. There is an important role for patient education, nutritional support, self glucose monitoring, as well as long-term glycemic control. A scrupulous control is needed to help reduce the risk of long-term complications. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications must be implemented to control blood pressure and cholesterol by exercising more, smoking cessation, and consuming an appropriate diet.

In countries with a general practitioner system, such as the United Kingdom, care may be extended mainly in the community, with hospital-based specialist input only in case of complications, difficult blood sugar control, or participation in research. In other circumstances, general practitioners and specialists may be sharing the care of a patient.

The general treatment for kidney diseases include diet/life style changes, blood pressure control, usage of ACE inhibitors and immunosuppressive drugs, dialysis and surgery.

At present several Synthetic drugs, many Herbs & Herbal based formulations are recommended to manage this disorder.

Traditional Indian medicines have long used plants and herbal extracts as anti-diabetic agents. These plants are typically rich in phenolic compounds, which are known to interact with protein and can inhibit enzymatic activity. A number of medicinal plants and herbal extracts have been found to inhibit the enzymatic activity of α-glucosidase and α-amylase, and there in may have potentials as dietary anti-diabetic agents which can improve and control post-prandial hyperglycemia.
Of late it is found that patients have started developing resistance to synthetic drugs, besides they are also known to cause serious side effects. Herbs and Herbal formulations are safe and efficacious but difficulties and problems are encountered so procuring authentic herbs is a problem. Whereas, minerals and metals are easily procurable and identification is not a problem. Hence in the present dissertation, Zinc based HMP is selected and evaluated for its antidiabetic, hepatoprotective and nephroprotective potentials as it can cure or manage these free radical mediated diseases / disorders in a minimal dose.

1.5. NANOTECHNOLOGY

Nanotechnology deals with the field of applied science and technology whose theme is the control of matter on the atomic and molecular scale, to a size of 100 nanometers or smaller, and the fabrication of devices or materials that lie within that size range.

1.5.1. NANOMATERIALS

Nanomaterials are materials with morphological features smaller than a micron in at least one dimension. Despite the fact that there is no consensus upon the minimum or maximum size of nanomaterials, some authors restricting their size from 1 to 100 nm, a logical definition would situate the nanoscale between microscale (1 micron) and atomic/molecular scale (about 0.2 nanometers).

1.5.2. NANOPARTICLES

Nanoparticles or nanocrystals made of metals, semiconductors, or oxides are of interest for their mechanical, electrical, magnetic, optical, chemical and other properties. Nanoparticles have been used as quantum dots and as chemical catalysts.
Fig 9: NANOTECHNOLOGY – AN OVERVIEW

Introduction
Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale this is often not the case. Size-dependent properties are observed such as quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials.

Nanoparticles exhibit a number of special properties relative to bulk material. For example, the bending of bulk copper (wire, ribbon, etc.) occurs with movement of copper atoms/clusters at about the 50 nm scale. Copper nanoparticles smaller than 50 nm are considered super hard materials that do not exhibit the same malleability and ductility as bulk copper. The change in properties is not always desirable. Ferroelectric materials smaller than 10 nm can switch their magnetisation direction using room temperature thermal energy, thus making them useless for memory storage. Suspensions of nanoparticles are possible because the interaction of the particle surface with the solvent is strong enough to overcome differences in density, which usually result in a material either sinking or floating in a liquid. Nanoparticles often have unexpected visible properties because they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep red to black in solution.

Nanoparticles have a very high surface area to volume ratio. This provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles. This theoretically does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. The surface effects of nanoparticles also reduce the incipient melting temperature.

In the present study, attempts are made to prepare and standardize the Herbo mineral preparation from Zinc metal from nanotechnological point of view as bhasma or calx are suppose to contain nano particles.