BIOSYNTHESIS OF SILVER NANOPARTICLES USING ETHANOLIC FRUIT EXTRACT OF *Terminalia bellirica* FOR THEIR ANTIDIABETIC ACTIVITY IN STREPTOZOTOCIN INDUCED RATS

KAVIYARASI, P.

(17MPBCF001)

Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Philosophy in Biochemistry

January, 2019

CERTIFICATE

This is to certify that the thesis entitled **"Biosynthesis of silver nanoparticles using ethanolic extracts of fruits of *Terminalia bellirica* for their antidiabetic activity in streptozotocin - induced rats",** submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for the award of the degree of **Master of Philosophy in Biochemistry,** is a record of original research work done by **KAVIYARASI.P,** during the period of her study in the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or similar title to any candidate of any other University or Institute.

31/1/19

Signature of the
Head of the Department

31/1/19

Signature of the Guide

DECLARATION

I declare that the dissertation entitled" **Biosynthesis of silver nanoparticles using ethanolic extracts of fruits of *Terminalia bellirica* for their antidiabetic activity in streptozotocin - induced rats"** submitted by me for the degree of Master of Philosophy is the record of work carried out by me during the period 2017 to 2019 under guidance of Dr.S.Gayathri Devi and has not formed the basis for the award of any Degree, Associateship, Fellowship, Titles in this University or other similar institution of Higher Learning.

Kaviyarasi P

Signature of the candidate

ACKNOWLEDGEMENT

The author is grateful to **Dr.(Thiru) P. Krishna Kumar,** Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore**,** for permitting to carry out her work in this eminent Institution.

The author is thankful to **Dr.(Tmt). Premavathy Vijayan,** Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all facilities necessary for her thesis work.

With profound respect, she places her deep sense of gratitude **to Dr.(Tmt) S. Kowsalya,** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing adequate help required to carry out her work.

The author expresses her gratitude to **Dr. P. R. Padma, Dean, School of Biosciences, Professor and Head**, Department of Biochemistry, Biotechnology and Bioinformatics Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her constant support and tremendous care rendered during the course of study.

The author is highly indebted to her guide **Dr. S.Gayathri Devi,** Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her meticulous care, innovative ideas, constructive thoughts, motivation, patience and constant support throughout her thesis.

The author wishes to thank all the **staff members of the Department of Biochemistry, Biotechnology and Bioinformatics,** Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore who made a congenial atmosphere to work throughout her thesis.

The author is indebted to all her **friends Reena Joy.A, Rajakumari.M, Sneha Pravin, Research Scholars** and **Lab Assistants** for their selfless help, emotional support and constructive suggestions given during the various stages of her study.

The author records her sincere thanks to her most affectionate **father A.Ponnusamy, mother D.Rani , sister P.Kayalarasi, brother Karthick.P , relatives and all well wishers** without whom the thesis would not have seen the light of the day..

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1.0 INTRODUCTION

Science and technology research in nanotechnology promises breakthrough in the areas such as materials and manufacturing, nanoelectricals, medicine, health care, energy, biotechnology, information technology and national security. It has the possibility to revolutionize the pharmaceutical industry with the new tools for the molecular treatment of diseases and rapid disease detection. The application of nanotechnology in medicine is known as nanomedicine, which is concerned with the use of precisely engineered materials at this length scale to develop novel therapeutics and diagnostics modalities (Gandhi and Khan, 2016).

Nanotechnology can be defined as the formation, development, enhancement and exploration of nanosized materials having size range of 1-100nm. It is an enabling technology which has a strong potent to open up a new vision in the field of research and development. It aims the creation, manipulation and application of structure in the nanometer size range. It plays a vital role in the natural medicine and an impact on economy and society and works with the substances which have specific properties such as physical, chemical and biological. Nanotechnology works on the design and development of many novel formulations for the prevention, treatment and diagnosis of many critical diseases (Kumar, 2017).

From recent years, the nanoparticles have many biomedical applications such as antimicrobial, antioxidant, anti-inflammatory, antiviral, cytotoxic, anticancer and anti-HIV activities. The silver nanoparticles are extensively using for diagnosis and treatment of diseases and other purposes because they are eco-friendly (Govindappa *et al*., 2018). Because of their specific characteristics, silver nanoparticles (AgNP) may be used as catalysts. Silver nanoparticles prepared by green synthesis phenomena are effective in biological environmental systems (Garg *et al*., 2017**).**

Green nanotechnology is an area with significant focus at present on the important objective of facilitating the manufacture of nanotechnology based products that eco-friendly and safer for all beings, with sustainable commercial viability. Synthesis of nanoparticles in green chemistry has recently received wide spread attention among the physical and the chemical synthesis processes for its simple, speed, inexpensive, ecofriendly and size controlling approaches in the synthesis of the metal nanoparticles. As a result of its growing popularity, there is an increased need to produce metal nanoparticles (Pak *et al*., 2016).

The field of nanotechnology recently used plants as biosource for the reduction and stabilizing the metal nanoparticles. As the plants contains biological compounds that assists the reaction to be more compatible, green biosynthesis of nano materials is considered as non-toxic, environmental friendly methods analogize to chemical method. Nanotechnology-based methodologies hold significant potential for enhancing the compliance of patients with diabetes (Jamdagni *et al*., 2018).

Green synthesis also called sustainable chemistry is one of the methods for producing silver nanorods. It pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles. This method involves the use of plants which help in faster production of nanorods. Also, it seeks to reduce the negative impact of chemistry on the environment by preventing pollution at its source and using a fewer natural resources (Shanker and Mohan, 2017).

Nature serves as primary source for the cure of ailments. In many developing countries, the population is dependent on medicinal plants to meet primary health care needs. The use of the herbal medicine is increasing due to its safety, efficacy and therapeutic potential compared to synthetic products (Xavier *et al*., 2015). However, the potential of higher plants as a source of herbal medicine is yet to be fully explored. Till date, numerous medicinal plants have been reported to be effective in diabetes, still plenty of research is needed to be done. The increased admiration of herbal medicines for diabetes may be due to the sideeffects associated with the conventional antidiabetic drugs. Hypoglycemic activity of therapeutic plants is to restore the capacity of pancreatic tissues via bringing about an increment in insulin yield, hindering the intestinal ingestion of glucose or encouraging metabolites in insulin subordinate processes (Abolfazl *et al.,* 2014).

Diabetes mellitus is a global health crisis and it is characterized by loss of glucose homeostasis resulting from defects in insulin secretion or insulin insensitivity and impaired metabolism of other energy yielding fuels such as lipids and proteins (Amiri *et al*., 2018).

India is one of the leading countries for the number of people with diabetes mellitus. The World Health Organization (WHO) estimated that the number increased to more than 171 million in 2000 to over 366 million by 2030 and that large increases will occur in developing countries, which has been persistently affecting the humanity, irrespective of the socioeconomic profile and geographic location of the population. India is blessed with an enormous wealth of medicinal plants and it has been often

referred as the "Medicinal Garden of the World". But unfortunately, India has not been able to capitalize the herbal medicine due to lack of scientific input in herbal drugs. Therefore, it is necessary to identify plant-based product for the treatment of diseases for which no medicine or palliative therapy is available. Such herbal medicines will find speedy access into the world (Verma, 2016).

Medicinal plant biodiversity is the natural biological capital of the earth. Its conservation and sustainable management are of pivotal importance (Kumar *et al*., 2017). They have been used as natural medicines because of their indispensable sources of medicinal importance. This practice has been in existence since prehistoric times. There are different ways in which plants have been found useful in medicines such as crude extract of plants has been used directly because of the presence of natural chemical constituents. Generally fresh and dry parts of the plants are used for the preparation of medicine (Hoque *et al*., 2017). There are several species of medicinal plants popularly used in the treatment of diabetes mellitus (Aboonabi *et al*., 2014).

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world and there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents. In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important. More than 800 plant species have been found important sources for the discovery and development of new types of antidiabetic molecules. Thus, herbal remedies encircling anti diabetic potential may serve as a relevant and intact alternative or as an adjunct candidate in the management of diabetes. Therefore, there is a need for thorough literature search on some species to update the current state of knowledge and one such plant is *Terminalia bellirica.*.

Terminalia bellirica commonly known as Dhandrikkai belongs to the family Combretaceae. It is called bheetaki in Sanskrit which means "fearless", the fruit that takes away the fear of disease. In Ayurvedic system of medicine, it is used as "health – harmonizer" in combination with *Terminalia chebula* and *Emblica officnalis.* It is used to protect the liver, reduce high cholesterol and treat digestive as well as respiratory disorders (Kumari *et al*., 2017).

Nowadays, nanoparticles are being used in pharmacological studies for their exclusive properties such as small size, more surface area, biocompatibility and

enhanced solubility. Earlier studies conducted in our laboratory proves that the ehanolic extract of *Terminalia bellirica* found to posses strong antidiabetic activity against streptozotocin induced diabetic rats. Another study also proves that the silver nanoparticle synthesised ethanolic extract of *Terminalia bellirica* posses strong *in vitro* antidiabetic activity. Hence, in view of this, the present investigation aimed to study the antidiabetic activity of the ethanolic extracts of fruits of biosynthesized silver nanoparticles of *Terminalia bellirica* in streptozotocin induced rats" was done with the following objectives:

- To biosynthesize silver nanoparticles of fruits of ethanolic extract of *Terminalia bellirica*.
- To study the antidiabetic effect of biosynthesized silver nanoparticles of *Terminalia bellirica in s*treptozotocin induced diabetic rats.

2.0 REVIEW OF LITERATURE

Nanotechnology is a field of science that deals with the production, manipulation and use of materials in ranging in nanometers. Nanotechnology is the application of science to control matter at the molecular level. Entirely novel and enhanced characteristics such as size, distribution and morphology have been revealed by these particles in comparison to the larger particles of the mass material that they have been prepared (Sia, 2016).

The field of nanotechnology mainly encompasses with biology, physics, chemistry and material sciences and it develops novel therapeutic nanosized materials for biomedical, pharmaceutical applications and it is an emerging field in the area of interdisciplinary research, especially in biotechnology. With the advancement of technologies and improved scientific knowledge a way of research and development in the field of herbal and medicinal plant biology towards intersection of nanotechnology has been observed. The biological synthesis of nanoparticle are being carried out by different macro-microscopic organisms such as plant, bacteria, fungi, seaweeds and microalgae. The biosynthesized nanomaterials have been effectively controlling the various endemic diseases with less adverse effect (Temps, 2016).

To the possible extent sincere efforts have been made to collect the relevant literature of the study. After thorough reviewing of all possible sources, it was observed that no studies on the antidiabetic role of silver nanoparticles synthesized from the fruit extract of *Terminalia bellirica* in streptozotocin induced rats have been conducted. Hence, the review of literature pertaining to the present research entitled "Biosynthesis of silver nanoparticles using ethanolic extracts of fruits of *Terminalia bellirica* for their antidiabetic activity in streptozotocin induced rats" is appropriately presented under the following headings.

2.1 Concept of Nanotechnology
2.2 Impact of Nanobiotechnology
2.3 Nanoparticles
2.4 Synthesis of Nanoparticles
2.5 Types of Nanoparticles
2.6 Plant Mediated Nanomaterials
2.7 Diabetes Mellitus
2.8 Herbal Medicine

2.1 Concept of Nanotechnology

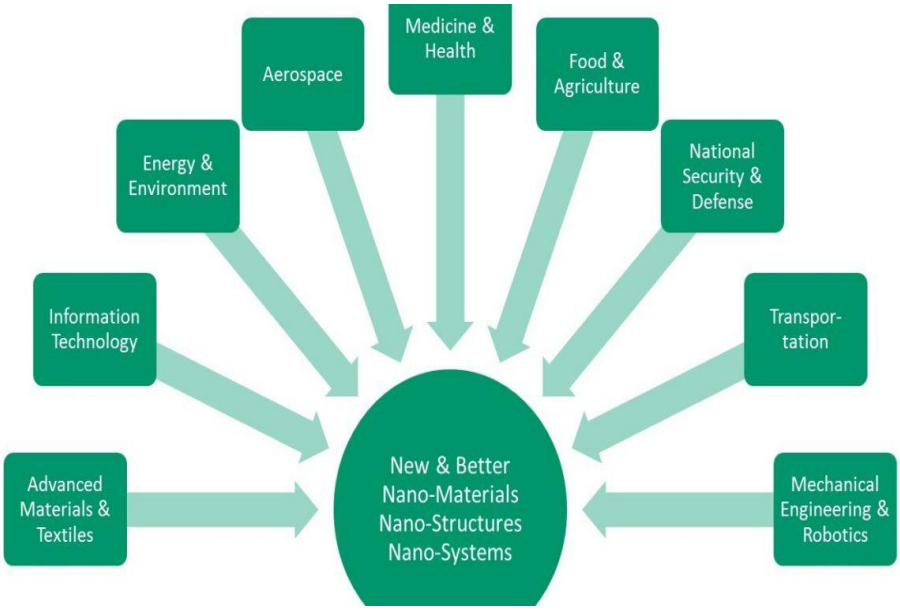
Nanotechnology is the emerging area of research and it is one of the rapid growing fields in science and technology. It has been applied to other fields of science such as electronics, engineering, physical sciences, material sciences, biomedical sciences and many others. Nanotechnology has given a greatest impact towards society and simultaneously applied in the health risks of nano-objects. Recently, many types of nanoparticles have been identified and utilised in several areas. The nanoparticles are used to discover various aspects. Thereby, scientific areas exhibits advantages in the development of nanotechnology mainly in the areas of forensic science, health sciences and various areas of automotive engineering as well. The growing demand of nanotechnology today have been enabled most of the scientist and analyst to go in the efficient strategic objectives by combining the most advanced chemical and physical technologies with the needs of modern applications of research (Chauhan *et al*., 2017).

2.2 Impact of nanobiotechnology

Nanobiotechnology has the most powerful impact of research on plants and different plant products and widely used in the synthesis of nanoparticles (NPs). In recent years, synthesis of metal nanoparticles with the help of plants has become an interesting subject of nanoscience and nanobiotechnology. However, there is a growing attention for biosynthesis of nanoparticles using metal accumulating organisms. Among these biological entities, plant extract is supremely suitable for large-scale biosynthesis of nanoparticles. Nanoparticles produced by plant extracts were stable and the rate of synthesis is much faster compared to microbe mediated synthesis (Meenakshi *et al*., 2017**).** Figure 1 represents the impact of nanotechnology .

FIGURE 1

IMPACT OF NANOTECHNOLGY



(http://www.wraltechwire.com/2018/02/05/nanotechnology)

2.3 Nanoparticles

Nanoparticles can be defined as objects ranging in size from 1-100 nm, their size may differ due to the material. Different metallic nanoparticles have been produced using copper, zinc, titanium, magnesium, gold, alginate and silver. The nanoparticles can be synthesized chemically or biologically. The chemical synthesis methods have many adverse effects due to the presence of some highly toxic chemicals which is absorbed on the surface. Biological way of synthesis of nanoparticles is an alternative to the physical and chemical method by using microorganisms, enzymes, fungus, plants or plant extracts. The development of these eco friendly synthesis of nanoparticles is becoming an important branch in nanotechnology mainly the silver nanoparticles, which may have many applications. The biological way of synthesis of the nanoparticle is a green and ecofriendly technology (Rajaram *et al*., 2015).

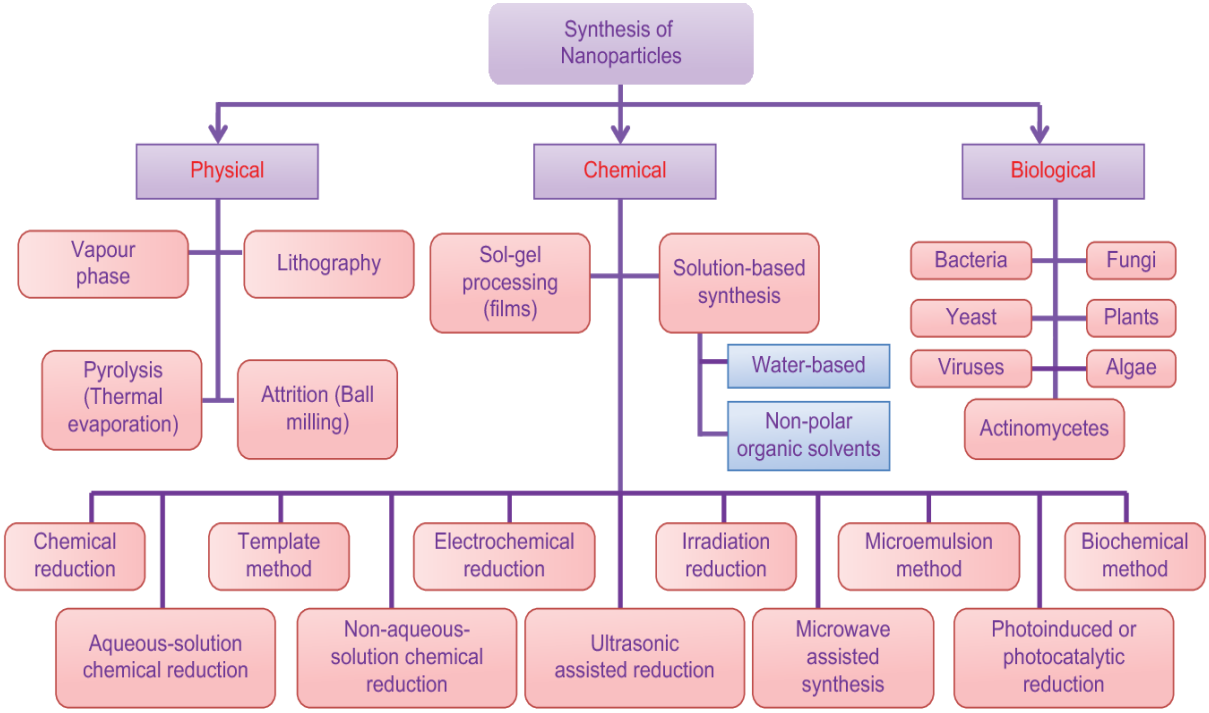
Both prokaryotes and eukaryotes were used in the synthesis of the metallic nanoparticles. The metallic nanoparticles such as the silver, gold, platinum, zirconium, palladium, iron,cadmium and metal oxides such as the titanium oxide and zinc oxide. Among these, a silver nanoparticle plays an important role in the field of nanotechnology and also in nanomedicine. Nanomaterial is an upcoming focus towards the scientific research not only by its application and also by the way of synthesis (Khan *et al*., 2017).

2.4 Synthesis of Nanoparticles

Nanoparticles have multifunctional properties and it has very interesting applications in various fields such as medicine, nutrition and energy. Additionally it has broad application in agriculture, industry and in plant sciences. Silver metal is used to control bodily infection, prevent food spoilage, wound healer agents and in ulcer treatment. Now a days the colloidal silver nanoparticles are used as antimicrobial agent, wound dressing material, bone and tooth cement and in water purifier. The different methods which are used for the synthesis of the nanoparticles such as the physical, chemical and biological are illustrated in Figure 2

FIGURE 2

METHODS OF SYNTHESIS OF NANOPARTICLES



(Ayesha, 2017)

2.4.1 Physical Method

In physical method, the metal nanoparticles were generally synthesized by evaporation – condensation that could be carried out by atmospheric pressure. Nanoparticles of various materials such as Ag, Au, PbS and fullerene have been produced using evaporation-condensation technique. However the generation of the silver nanoparticles have several drawbacks because it occupies a large space consumes a great deal of energy while raising the environmental temperature around the source material and requires a lot of time to achieve a thermal stability (Haider and Kang, 2015).

The physical methods include, plasma arcing, ball milling, thermal evaporate, spray pyrolysis, ultra thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of nanoparticles. The physical method can be useful as a nanoparticle generator for long term experiments for inhalation toxicity studies and as a calibration device for nanoparticle measurement equipment (Reddy, 2015).

2.4.2 Chemical Method

The chemical methods have been used to synthesize the nanoparticles by electro-deposition, sol-gel process, chemical solution deposition, chemical vapour deposition, soft chemical method, Langmuir Blodgett method, catalytic route, hydrolysis, co-precipitation method and wet chemical method. Chemical methods provide an easy way to synthesize silver nanoparticles in solution. Each method has its own advantages and disadvantages with common problems like costs, scalability, particle sizes and size distribution. The silver nanoparticles are synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinyl pyrrolidone (PVP), this process is called polyol process. In this case, ethylene glycol served as both reluctant and solvent. It showed the presence of PVP and its molar ratio relative to silver nitrate both played important role in determining the geometric shape and size of the product. The metallic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable (Trans *et al*., 2013).

Generally the chemical synthesis of the silver nanoparticles in solution employs the following three main components as metal precursors, reducing agents and stabilizing or capping agents. The formation of colloidal solutions from the reduction of silver salts involves two salts namely the nucleation and subsequent growth. It is also revealed that the size and the shape of the synthesized silver nanoparticles are strongly dependent on these stages. Furthermore, for the synthesis of the silver nanoparticles with uniform size distribution, all nuclei are required to form at the same time. Thus all the nuclei are likely to form at the same or similar size and then they will have the same subsequent growth. The initial nucleation and the subsequent growth of the initial nuclei can be controlled by adjusting the reaction parameters such as the reaction temperature, pH, precursors, reducing agents and stabilizing agents.

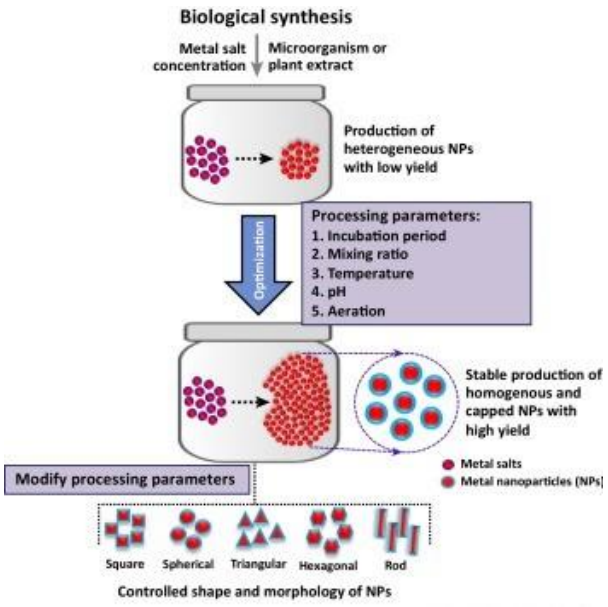
2.4.3 Biological Method

The biological methods uses microorganisms and enzymes that had been suggested as possible ecofriendly alternatives. It was found to be more pharmacologically active than

physicochemical synthesized nanoparticles. The biological synthesis of nanoparticles is a single step bio reduction method and less energy is used to synthesize ecofriendly nanoparticles. These methods use resources such as plant extracts, bacteria, fungi, micro algae such as cynobacteria, diatom macro algae and enzymes. The development of the biologically inspired experimental processes for the synthesis of the nanoparticles is evolving into an important branch of nanotechnology. Figure 3 explains the biological synthesis of nanoparticles.

FIGURE 3.0

BIOLOGICAL SYNTHESIS OF NANOPARTICLES



(Singh *et al.*, 2016)

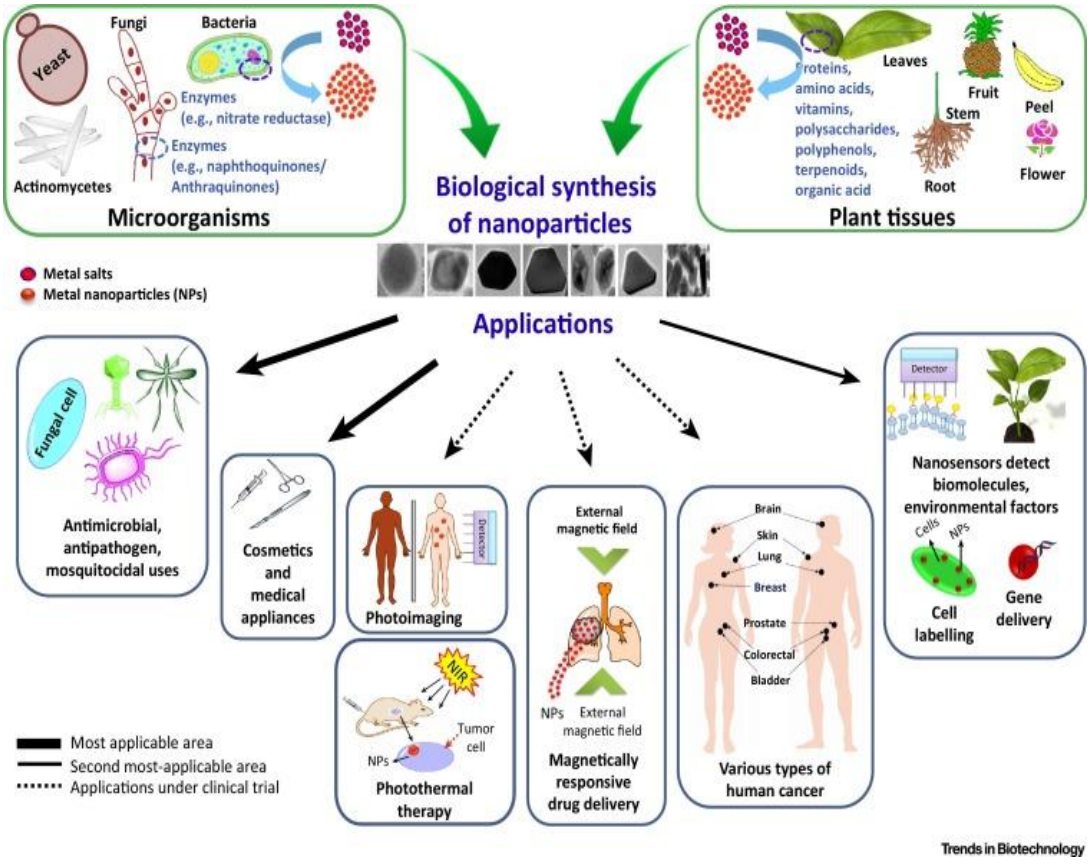
Nanoparticles can be synthesized from a wide variety of biological entities. These entities differ from the degree of the biochemical processing abilities that can be effectively used to synthesize particular metallic or metallic oxide nanoparticles. Not all biological entities can synthesize nanoparticles due to their enzyme activities and intrinsic metabolic processes. So a careful selection of the appropriate biological entity is necessary to produce nanoparticles with well defined properties such as size and morphology (Malik *et al*., 2014).

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology have been received increased attention due to growing need to develop environmentally benign technologies in material synthesis. A great deal of effort have been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants (Khalil *et al*., 2014).

It had emerged as an attractive to traditional synthesis methods for producing nanoparticles. The biological synthesis of the nanoparticles offers a clean, nontoxic and good method of synthesizing nanoparticles with a wide range of sizes, shapes, compositions and physicochemical properties. It has the ability to act as a template in the synthesis, assembly and organization of nanometer scale materials to fabricate well defined micro and macro scale structures. These properties acts as efficient biological factories capable of significantly reducing the environmental pollution and reclaiming metals from the industrial waste (Shah *et al*., 2015). Figure 4 illustrates the applications of biosynthesis of nanoparticles.

FIGURE 4

APPLICATIONS OF BIOSYNTHESIS OF NANOPARTICLES



(Singh *et al.*, 2016)

The synthesis of the nanoparticles by physical and chemical methods can cause considerable environmental defect, technically laborious and economically expensive. In general, the physical and chemical methods involves the use of hazardous chemicals or costly physical methods. However the biological methods make their ways in between and proving their advantages over them. Recently, the biological synthesis of the nanoparticles using the plant extracts appears to be an attractive alternative to conventional chemical synthesis.

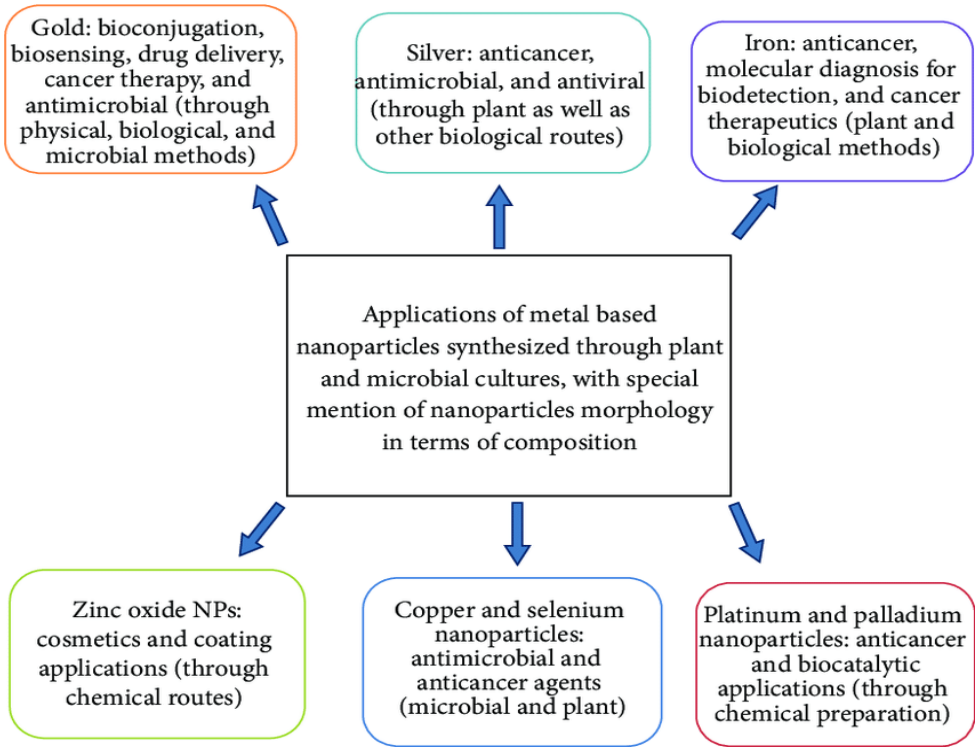
More over the combination of molecule found in plant extract perform as both reducing and stabilizing agents during the nanoparticle synthesis (Kulkarni and Muddapur, 2014).

2.5 Types of Nanoparticles

The metallic nanoparticles such as copper, titanium, magnesium, zinc, gold and alginate have a strong bactericidal potential owing to their large surface area to volume ratio. Nanoparticles are generally classified into two types namely organic and inorganic nanoparticles. Organic nanoparticles which include carbon nanoparticles while some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles and semi conductor nanoparticles (titanium oxide and zinc oxide). There is an emerging interesting inorganic nanoparticle like noble metal nanoparticles (gold and silver) as they provide superior material properties with functional versatility (Hasan, 2015). The various type of metal nanoparticles and its applications are illustrated in Figure 5.

FIGURE 5

METAL NANOPARTICLES AND ITS APPLICATIONS



(https://www.researchgate.net/figure/Applications-of-different-kinds-nanoparticles)

2.5.1 Silver Nanoparticles

Silver nanoparticle has been proved to be most effective because of its good antimicrobial efficacy. Silver nanoparticle is the particles of silver, size between 1 to 100 nm. They are incorporated into the wide range of medical devices. The ionic silver in right amount is suitable in treating wounds. In fact silver nanoparticles are now replacing silver

sulfadiazine as an effective agent in the treatment of wounds. Due to their effective physiochemical properties these nanomaterial has been received considerable attention (Abdelghany *et al*., 2018).

Nanoparticles cover a broad area of interest including cosmetics, food industry, electronics, medicine and environmental applications. Surface modification of the nanomaterials have strong effect on the interaction of these nanomaterials with cells in addition to this it also helps to convert toxic nanomaterials to the less toxic to more toxic nanomaterials. The metallic property of the metal nonoparticles exhibit the photo electric effect (Khan *et al*., 2014).

Silver is well known from the ancient times mainly due to its medicinal and preservative properties and it is one of the basic elements that make up the planet. It has efficient antimicrobial agents when compare to the other salts because of its large surface area. Silver nanoparticles find use in many fields and the major applications include their use as catalyst, as optical sensors, in textile engineering and most importantly in the medical field as a bactericidal and as a therapeutic agent. In the coating industry, silver nanoparticles are employed for spectrally selective coating for solar energy. Wide use of silver nanoparticles are found in pharmaceutical industries and medicine, inkjet printing application, biological labelling, nanodevice fabrication, biosensing, photonics and optoelectronics (Jawaad *et al*., 2014) .

The silver nanoparticles are widely used as food additives and plastics mainly to eliminate the presence of the microorganisms. Many types of silver based compounds have been identified. They were reported to show better healing capacity and scar less healing when tested using an animal model. It has its unique role in various fields because of its distinctive properties,such as good conductivity, chemical stability, catalytic and antibacterial activities. An important method of biosynthesis of nanoparticles is by the application of plant extract to the biosynthesis reaction. Synthesis of silver nanoparticles had been studied with the help of chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important branch of nanotechnology (Logeswari *et al*., 2015).

2.5.2 Gold Nanoparticles

Gold nanoparticles have been used in the immunochemical studies for the identification of protein interactions. It is used in the DNA fingerprinting process mainly to detect the presence of DNA in a sample. They are also used for the detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. The gold nanorods are used to detect the cancer stem cells, beneficial for cancer diagnosis and identification of different

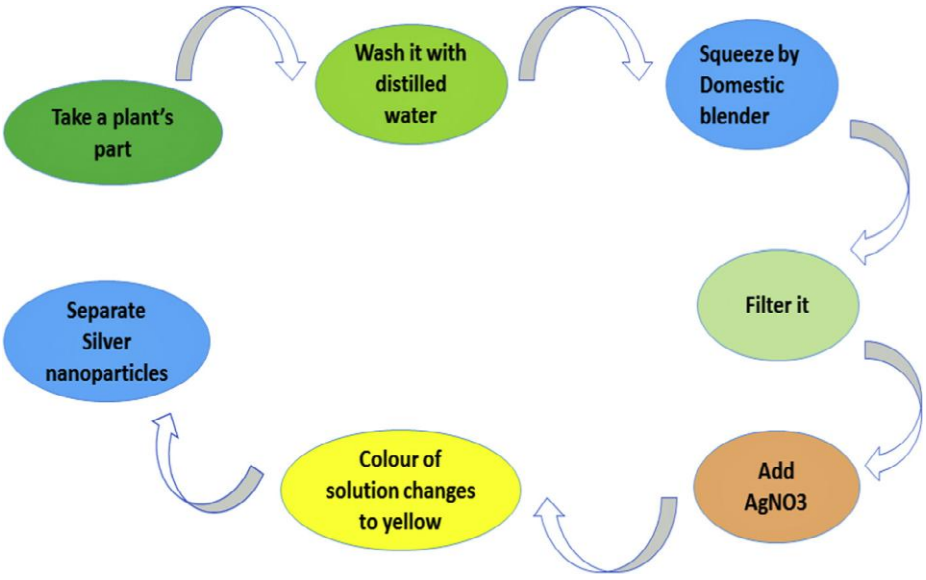
classes of bacteria. It is widely used in the field of biotechnology and biomedical because of their large surface area and high electron conductivity. The modification of these nanometers is conducted to enhance the interaction of these nanoparticles with biological cells. It is proved to be the safest and much less toxic agents for drug delivery. Fabrication of gold nanoparticles can perform to have a different size from 1nm to 150 nm. Their structural design enables the coating of the surfaces with various targeting agents. They have physical, chemical and photo properties of the gold nanoparticles can be the innovative ways to control the transported pharmaceutical compounds. Among various properties the important one are non toxic and biocompatible (Alaqad and Saleh, 2016).

2.6 Plant mediated nanomaterials

Recently, the plant mediated nanomaterial has been given more attention due to its wide application in many fields due to their physico-chemical properties. Different metallic nanoparticles such as gold, silver, platinum, zinc, copper, titanium oxide, magnetite and nickel are synthesized from natural resources and that has been studied exclusively. The different parts of plant such as stem, root, fruit, seed, callus, peel, leaves and flower were used to synthesize the metallic nanoparticles in different shapes and sizes by their biological approaches. With the help of the metal concentration and the amount of the plant extract which is utilised in the reaction medium has the power to alter the biosynthetic reactions easily transform the shapes and size of the nanoparticles. Figure 6.0 represents the green synthesis of silver nanoparticles.

FIGURE 6.0

GREEN SYNTHESIS OF SILVER NANOPARTICLES



(Rajaram *et al*., 2015)

Now a day's plants are widely used for the production of silver nanoparticles due to its non-pathogenic and economical protocol. It is widely used for many applications such as antimicrobial, antioxidant, anticancer and also used in industries. This medicinal plant was reported for antioxidant and antidiabetic activity. Green synthesis of nanoparticles had been given more advantage over other methods because they are simple, one step, cost-effective, and relatively reproducible mostly results in more stable materials. Regarding the wide potential of plants as sources, the green technique for the synthesis of silver nanoparticles act as an alternative (Ahmed *et al*., 2016).

2.7 Diabetes Mellitus

Diabetes mellitus is identified by hyperglycemia or by metabolic dysfunction in which the person have uncontrolled sugar level in blood or relatively deficient in the insulin levels. The diabetes mellitus was classified into two types, such as Type I diabetes mellitus and Type II diabetes mellitus and other classification such as the prediabetes and gestational diabetes. Type I diabetes mellitus leads to destruction of β-cell leads to absolute insulin deficiency that is mediated by immune mechanism. Type II diabetes mellitus due to progressive insulin deficiency and cause various other metabolic syndrome. In gestational diabetes mellitus the glucose tolerance was diagnosed during pregnancy. Other specific type of diabetes mellitus is caused by genetic defects or by drug or chemical induced (Nentich and Ulbig, 2015).

Even though insulin therapy is widely used for management of diabetes mellitus, its side effects such as, insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment makes it a risky proposition. Certain foods and balanced diet has been given to prevent the diabetes at certain levels, but the complete treatment for diabetes mellitus is a biggest challenge now a days. Therefore, extensive research is necessary to find safer and effective agents (American Diabetes Association, 2014).

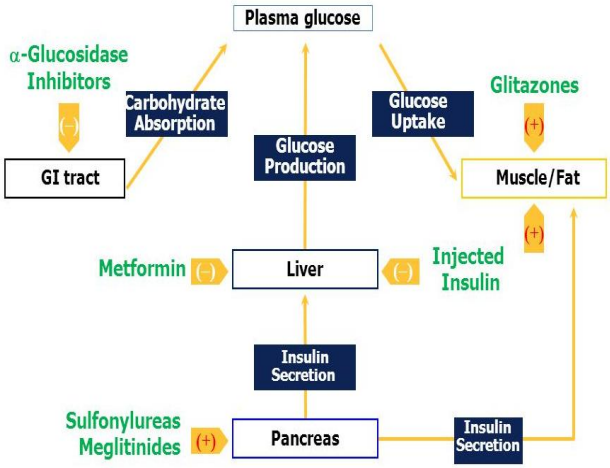
Diabetics are prone to many complications due to the nature of the diseases. Long standing diabetes can lead to macro and micro vascular complications, heart, kidney, circulation problems and stroke. It is a group of metabolic diseases associated with the high level of blood sugar over a prolonged period. Diabetes is fast gaining potential epidemic in India with more than 62 million diabetic individuals where diagnosed with the disease. India currently faces an uncertain potential burden that diabetes may impose upon the country (Ullah *et al*., 2016).

Many influences affect the prevalence of disease throughout a country. It is generally a heterogenous disturbance of metabolism that is either impaired with the insulin secretion or impaired insulin action. Ocular complications with diabetes mellitus are progressive and rapidly becoming the world's most significant cause of morbidity. The main five complications associated with diabetes mellitus are diabetic retinopathy, papillopathy, cataract, glaucoma and ocular diseases (Sayin *et al*., 2015).

Treatment of diabetes includes improvement of the activity of insulin at the objective tissues, with the utilization of the sensitizers (biguanides, thiozolidinediones), incitement of endogenous insulin discharge with the utilization of sulfonylureas (gilbenclamide, glimepiride) and decrease of the interest for insulin utilizing the particular enzyme inhibitors. Metformin, is considered as a initial pharmacologic agent for the treatment of type 2 diabetes Figure 7 represents the treatment of diabetes mellitus.

FIGURE 7

TREATMENT OF DIABETES MELLITUS



(https://goo.gl/images/XQSmEc)

2.8 HERBAL MEDICINE

In traditional world, nutrition and health care have connectivity for which many plants are consumed as food in order to benefit health. Motivation of people towards the herbal medicines is increasing to avoid side effects of drugs prepared from the synthetic materials. Medicinal plants are considered to be an important source of antidiabetic compounds and the therapeutic benefit of many medicinal plants is often attributed to their hypoglycaemic activity (Ezuruike and Prieto, 2014).

Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years. Medicinal properties of plants are mainly due to the active chemical constituents present in different parts of the plant. Herbal medicines are safer than the modern synthetic drugs because they are naturally existing (Kakati *et al*., 2016).

Hypoglycemic activity of the therapeutic plants is because of their capacity to restore the capacity of the pancreatic tissues that brings an increment in insulin yield, hindering the intestinal ingestion of glucose or encouraging metabolites in insulin subordinate processes. Plants have been a commendable wellspring of medications that have been got specific way from them. Around 800 plants are hostible to diabetic potential (Ramprasad and Madhusudhan, 2016).

The genus *Terminalia* is widely distributed and is known as a rich source of secondary metabolites, flavanones and chalcones. *Terminalia bellirica* belonging to the family –Combretaceae, commonly known as myrobalan, is a deciduous tree found throughout the Indian forest and plains. It is known as Bahera in India and has been used for centuries in Ayurveda. The tree is about 30-40 m in height and 2-3 in girth. The stem is straight and the leaves are brodly elliptic clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 cm in diameter, drupe of grey to brown in colour. Bark is brownish grey in colour with shallow longitudinal fissures. Fruit extract used as astringent, antiseptic, rejuvenation, brain tonic, expectoant and laxative. It is used in coughs and sore throat. Its pulp used in dysentry, diarrhoea and liver disorders. It exhibits several pharmacological effects including antibacterial, antidiabetic, antimalarial, antifungal, anti HIV, antioxidant and antimutagenic effects (Chanda *et al*., 2013).

3.0 EXPERIMENTAL PROCEDURE

Nanotechnology provides a broad knowledge of applied science and technology to control the matter on the atomic and molecular scale. It is an important and emerging technical tool for development of eco-friendly and reliable methodology for synthesis of nanoscale materials using biological sources. In modern nano science and technology, the interaction between inorganic nanoparticle and biological structures are one of the most exciting areas of research. However, discovery of new molecules and manipulating those available naturally in nanosize could be appealing for their greater potential to improve health care. Many of its applications are very much important for the developing countries (Elobeid, 2016).

Diabetes mellitus is not a single disease but a group of several metabolic disorders that have in common hyperglycemia and dyslipidemia and lead to serious complications causing damage to many organs, especially the eyes, kidneys, nerves and heart making diabetes mellitus the seventh cause of death in developed countries. It is one of the common metabolic disorders worldwide and is a major public health problem (Barde *et al*., 2016).

Medicinal plants and green synthesis of silver nanoparticles have proven to be good sources of agents effective in the treatment of diabetes mellitus. Recent studies with nanostructures show a promising progress in the diagnosis and treatment of diseases. To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is proposed as a cost-effective and environmental friendly alternative. Plant extracts have reportedly been used in the preparation of silver nanoparticles. Green approach is a technique for the controllable synthesis of nanoparticles of well-defined size and shape (Jain and Mehata, 2017).

Terminalia bellirica is a native plant of India belonging to Combrataceae family. It is a large deciduous tree of 50 m tall and a diameter of 3m. Extracts of various parts of *Terminalia bellirica* (*T.bellirica*) have been found to contain constituents such as glucoside, gallo-tannic acid, colouring matter, resins, gallic acid, lignans, tannins, glucose, fructose and rhamnose. These compounds are believed to be responsible for the pharmacological activities (Deb *et al*., 2016).

3.1 Characterization of the Selected Medicinal Plant

3.1.2 Collection of *T.bellirica*

The fruits of *T.bellirica* was collected from Velliangiri hills Coimbatore, Tamil Nadu, India. The sample was identified and authenticated by botanical survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2014-2015/Tech 510. The fruits were washed thoroughly in tap water, shade dried and pulverized. The powder was weighed, packed in airtight containers and stored at 4ºC until use. Plate 1 represents *Terminalia bellirica* and its classification.

PLATE I

***TERMINALIA BELLIRICA* AND ITS CLASSIFICATION**

FRUIT

SCIENTIFIC CLASSIFICATION

Kingdom	**Plantae**
Class	**Magnoliopsida**
Order	**Myrtales**
Family	**Combrataceae**
Genus	***Terminalia***
Species	***T.bellirica***

3.2 Preparation of Ethanolic Extract

The collected sample was washed thoroughly and shade dried at room temperature. The dried sample was powdered.100ml of ethanol was added to 10g of the powdered sample, which was kept in the mild shaker for seven days. It was then filtered by Whatmann no.1 filter paper and the filtrate obtained was used for further studies.

3.3 Synthesis of Silver Nanoparticles

Synthesis of silver nanoparticles was carried out by the method explained by Harbone (1998). Ninety ml of 1mM silver nitrate solution was prepared using deionized water. It was then added to 10ml of ethanolic extract. The mixture of ethanolic extract of fruits of *T.bellirica* with silver nitrate was exposed to sunlight for the duration of 20 minutes. The synthesized silver nanoparticles of the ethanolic extract of fruits of *T.bellirica* (AgNPsEFTB) sample was centrifuged for 20 minutes under refrigerated centrifugation at 13000 rpm and washed 3 times with deionised water. The residue of silver nanoparticles was obtained by freeze drying.

3.4 *In vivo* Antidiabetic Activities of Synthesised Silver Nanoparticles of *T.bellirica*

In vivo studies were carried out by intraperitoneal administration of ethanolic extract of fruits of *T.bellirica* to determine their antidiabetic activities by using Male albino rats.

3.4.1 Maintenance of Experimental Animals

Male albino rats of 8-10 weeks old, weighing 100 – 200 g used for this study were procured from KMCH College of Pharmacy, Coimbatore, India. They were acclimatized to animal house conditions and were housed in polypropylene cages at room temperature (25° - 30° C) and at 45-55% relative humidity for 12h, each of dark and light cycle. They had free access to drinking water and were fed with standard pellet diet. All animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Approval to conduct the animal experiments Proposal number (IAEC/17-18/08) and Approval number (AIW: IAEC.2017: BC: 02) was obtained from the Institutional Animal Ethics Committee of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-43.

3.4.2 Induction of Diabetes Mellitus in Experimental Rats

The experimental rats were made diabetic by a single intraperitoneal injection of streptozotocin (60mg/kg i.p.) in sterile saline. Three days after streptozotocin injection, rats with blood glucose level > 180mg/dl were separated and used for the study.

3.4.3 Experimental Design for Antidiabetic Activity of Ethanolic Extract of *T.bellirica*

The male albino rats were divided into five groups of six rats for each for treatment period. Table 1 represents the experimental design.

TABLE 1
EXPERIMENTAL DESIGN

Group	Designation	Treatment (Extract/Drug Dose)
I	Control	-
II	Ctrl+ AgNPs EFTB	Control rats + Silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* (10mg / kg b.w)
III	STZ	Streptozotocin induced rats (60mg/kg b.w)
IV	STZ+ AgNPs EFTB	Streptozotocin induced rats + Silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* (10mg / kg b.w)
V	STZ+ Glib	Streptozotocin induced rats + Glibenclamide (10mg / kg b.w)

EFTB – Ethanol extract of fruit of *Terminalia bellirica*

The experiments were carried for 45 days. At the end of the study period, the animals were sacrificed after an overnight fasting. The blood samples were collected from the left ventricle and used for selected biochemical analysis. The liver, pancreas and kidney were dissected out and stored in buffered formalin (10%) for histopathological examination.

3.4.4 Body Weight of Experimental Rats

The body weight (g) of the experimental rats was recorded on the day one and then every week for 45 days using digital weighing balance.

3.4.5 Haematological and Biochemical Parameters

The haematological parameters namely haemoglobin, red blood corpuscles(RBC) count and differential counts of white blood corpuscles (WBC), polymorphs, lymphocytes and monocytes were estimated in both the experimental and control rats. Table 2 represents the details of haematological and biochemical parameters in experimental rats.

TABLE 2

HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS ASSESSED IN EXPERIMENTAL RATS

Parameters	Method	Appendix
Haemoglobin	Drabkin and Austin (1932)	I
Red Blood Corpuscles(RBC)	Sanderson and Phillips (1981)	II
White Blood Corpuscles(WBC)	Sanderson and Phillips (1981)	III
Polymorphs	Automated cell counter-CD 1700	-
Lymphocytes	Automated cell counter-CD 1700	-
Monocytes	Automated cell counter-CD 1700	-
Blood Glucose	Hjelm and Verdier, (1963)	IV
Plasma Insulin	Hales and Randle, (1963)	V
Total Cholesterol	Abell *et al*. (1952)	VI
Triglycerides	Van Handel and Zilversmit, (1957)	VII
Aspartate Transaminase (AST)	Reitman and Frankel, 1957	VIII
Alanine Transaminase (ALT)	King, (1965)	IX
Alanine Phosphatase (ALP)	Reitman and Frankel, (1957)	X
Glucokinase	Brandstrup *et al*. (1957)	XI
Glucose-6-phosphatase	Koida and Oda, (1959)	XII
Fructose-1,6 bisphosphatase	Gancedo and Gancedo , (1971)	XIII

3.5 Histological Examination

Liver, kidney and pancreas of experimental animals were dissected out and transferred to containers with 10% formalin solution for histopathological observation (Culling, 1979) and the procedure is given in Appendix XIV.

3.6 Statistical Analysis

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group.

4.0 RESULTS AND DISCUSSION

The medicinal plants are a rich source of medicines that is used as herbal remedies and involved in the development of the modern pharmaceuticals. It has gained increased attention in solving the health care problems of the world. The research on plants of the medicinal importance is growing tremendously and has revealed that many plants are synthesized and accumulate the natural constituents that have active physiological and psychological effects on the human body. India is a country with a vast reverse of the natural resources. A number of the plant extracts which have been used as medicines over hundreds of years contain numerous biologically active compounds which are helpful in improving the life and used for treating several diseases, pain and other afflicitions (Mamedov *et al*., 2015).

Phytochemicals are natural constituents and non nutritive bioactive compounds produced by the plants that act as protective agents. The plants or plant extract that is involved in the nanoparticle synthesis have great advantageous over other biological processes and can also be used for large scale nanoparticle synthesis. The plants are the rich source of the micro and macro elements. Secondary metabolite is crucial for plant defences that have enabled plants to survive. Moreover the plant mediated nanoparticle synthesis is widely preferred because it is cost effective, environmentally friendly and a single step method for biosynthesis process and safe for human therapeutic use (Sadeghi and Gholamhoseinpoor, 2015).

Nanotechnology is a promising field of interdisciplinary research. It opens up a wide array of opportunities in various fields like medicine, pharmaceuticals, electronics and agriculture. Nanotechnology is now well-established, it is applied to many unrelated products and technologies. Nowadays it is gaining profoundness owing to its increase of vital role in most dynamic areas of research in modern materials science. Novel applications of nanoparticles and nanomaterials are growing, rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. Green chemistry principles focuses on easy, safe, efficient, cost effective, ecofriendly process and significantly reduce the toxicity of synthesized nanoparticles (Gupta, 2017).

Earlier studies in our laboratory was carried out using the ethanolic extract of fruits of *Terminalia bellirica* found to possess a strong antidiabetic activity against streptozotocin induced diabetic rats. It was found that the extracts did not pose any threat to the animals tested and the LD_{50} value was found to be greater than 5000mg/kg b.w. As a model of human diseases, the rat offers many advantages over the mouse and other organism. For diabetes, the

rat model behaves more like the human diseases in many important ways, including the ability of environmental agents to modify disease (Iannaccone and Jacon, 2009).

The silver nanoparticle synthesis of ethanolic extract of *Terminalia bellirica* possess strong *in vitro* antidiabetic activity. All the physiochemical characterization like UV-Visible spectrophotometer, FT-IR, SEM and EDX,TEM, XRD and Zeta potential analysis carried out in the earlier study in our laboratory confirmed the nature of silver nanoparticles synthesized from fruit extracts of *Terminalia bellirica.* Many herbal extract for the antidiabetic activity was used for the treatment of diabetes. The results of the *in vitro* inhibition of alpha amylase and alpha glucosidase activities of ethanolic extract of synthesized silver nanoparticles of fruits of *Terminalia bellirica* also can act as a good source of natural inhibitors for the enzymes. As per the results of the earlier study, it is evident that the silver nanoparticles synthesized from the fruit extracts of *Terminalia bellirica* via green route will also possess effective antidiabetic activity using animal models.

Hence the present study is aimed to find out the *in vivo* antidiabetic activity of ethanolic extract of fruits of biosynthesized silver nanoparticles of *Terminalia bellirica* in streptozotocin-induced rats. The results obtained are presented and discussed below.

4.1 *In vivo* antidiabetic activity of synthesized silver nanoparticles of ethanolic extract of fruits of extract of *Terminalia bellirica*

4.1.1 Synthesis of silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica (AgNPs EFTB)*

The silver nanoparticles were synthesized by exposure to sunlight. The synthesis of the silver nanoparticles was noticed by a color change. The color of the reaction mixture changed exponentially with reaction time as aggregation proceeds due to excitation of surface Plasmon vibration confirming the formation of silver nanoparticles (Ahmad and Sharma, 2012). Silver nanoparticles exhibited yellowish brown to dark brown color. Therefore for the present study the silver nanoparticles was synthesized using the sunlight.

According to Srikar *et al*. (2016), plant extract of *Prunus amygdalus* when added to $AgNo_3$ the color of the solution change from pale yellow to dark brown which indicates the formation of AgNPs. The colour formation occurs due to excitation of the surface plasmon resonance effect and the reduction of $AgNO_3$.

According to Sundararajan and Kumari (2014), the silver nanoparticles are formed as the colour of the solution changes from green to reddish brown colour, 0.5mM silver nitrate ($AgNO_3$) was added to the leaf extract of *L.speciosa*. It was well known that silver nanoparticles exhibit reddish brown colour in aqueous solution.

Moosa *et al*. (2015) confirmed the reduction of silver ions which is carried out by green synthesis method in which about 90ml of 1mM silver nitrate solution was added to the 15ml of *Aloe vera* plant extract and the mixture was exposed to sunlight. A colour change occurs from pale green to reddish brown due to surface Plasmon resonance which is denoting the formation of AgNPs.

4.2 Body weight of the experimental rats

The body weight of the experimental rats of the different groups are recorded initially and also by the end of the 45^{th} day. The change in body weight of the control and the experimental rats are given in Table 3

TABLE 3
BODY WEIGHT OF THE EXPERIMENTAL RATS

Groups	Initial	Final
I	151.5±1.784	153±2.683
II	152.7±1.333	153.7±1.978
III	155.7±1.978z	99.67±31.74
IV	154.8±1.641	165.7±2.092
V	155±1.915	136.7±27.66

Values are expressed in mean ± S.E.M (n=6)

Group I - Control Group II-Ctrl+ AgNPs EFTB

Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB

Group V - STZ induced rats + glib

It is clear from the above table that the untreated control animals and the rats treated with the synthesized silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* of 10 mg/kg body weight did not produce any weight loss but gained weight ($p<0.05$) and there was no significant differences in the increases in the body weight between the controls and the rats treated with AgNPs synthesized EFTB. A decrease in body weight ($p<0.05$) was noted in steptozotocin-induced diabetic rats. When the diabetic rats were treated with the synthesized silver nanoparticles of ethanolic extract of *Terminalia bellirica*, decrease in the body weight was suppressed.

Similar results for the body weight of the control and the diabetic rats treated with the extracts of *Trigonella foenumgraecum L.seeds*, *Calophyllum brasiliense* and *Rumex hymensosepalus* were reported by Virk (2018), Carvalho *et al*. (2016) and Ortiz *et al.*(2015).

Balamurugan *et al*., (2014) also reported a significant increase in body weight in diabetic rats administered with *Melastoma malabathricum.*

Prabhu *et al*. (2018) reported that treatment with 100µg/kg leaf extract of *Pouteria sapota* and 10 mg/kg silver nanoparticles increased the body weight of the rats as compared with both the normal control and untreated diabetic groups.

Thus the result reveals that the synthesized silver nanoparticles of ethanolic fruit extract of *Terminalia bellirica* has increased the body weight and do not cause any adverse effect on rats.

4.3 Haematological parameters in experimental rats

Analysis of haematological parameters reveals the deleterious effect of foreign compounds including the plant extracts on the blood constituents of an individual. They also used to determine possible alteration in the levels of biomolecules as such enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs (Oyedemi *et al.,* 2011). The haematological parameters are associated with health and diagnostic significance in the clinical evalution of health. The parameters such as haemoglobin, RBC, WBC, polymorphs, lymphocytes and monocytes. The results of haematological parameters are presented in Table 4

TABLE 4

HAEMATALOGICAL PARAMETERS IN EXPERIMENTAL RATS

Groups	Total Haemoglobin(g/dl)	RBC	WBC	Polymorphs (%)	Lymphocytes (%)	Monocytes (%)
I	14.0±0.86	8.1±0.21	9.1±0.22	14.0±0.86	47.9±0.52	8.5±0.36
II	11.5±0.51	7.3±0.39	10.70±0.44	12.1±0.35	46.4±0.47	8.3±0.43
III	6.9±0.50	5.5±0.50	8.5±0.35	7.9±0.50	29.7±0.46	8.0±0.35
IV	12.1±0.35	7.9±0.36	11.00±0.60	12.97±0.80	45.8±0.30	8.8±0.50
V	11.3±0.26	6.5±0.50	10.03±0.78	11.27±0.40	40.8±0.35	7.9±0.53

Values are expressed in mean ± S.E.M (n=6)

Group I - Control Group II-Ctrl+ AgNPs EFTB

Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB

Group V - STZ induced rats + glib

The above table clearly indicates that the oral administration of the synthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica* AgNPs EFTB and

standard drug glibenclamide to the diabetic rats for 45 days showed significant reduction ($p<0.05$) in RBC, haemoglobin, polymorphs, lymphocytes, WBC and monocytes. The decreased levels of haemoglobin in the diabetic rats may be attributed due to hyperglycemia.

Similar observations were also reported with methanolic seed extract of *Hunteria umbellate* on some haematological parameters in diabetic rats (Olufunmilayo *et al*., 2015). The results of the present study were similar to the observation made by Mahmoud (2013), who had recorded significant increase in WBC and significant decrease in lymphocytes and monocytes in the diabetic rats. The study findings of Pankaj and Varma (2013), showed that the powdered *Spirulina plantensis* has increased the RBC count.

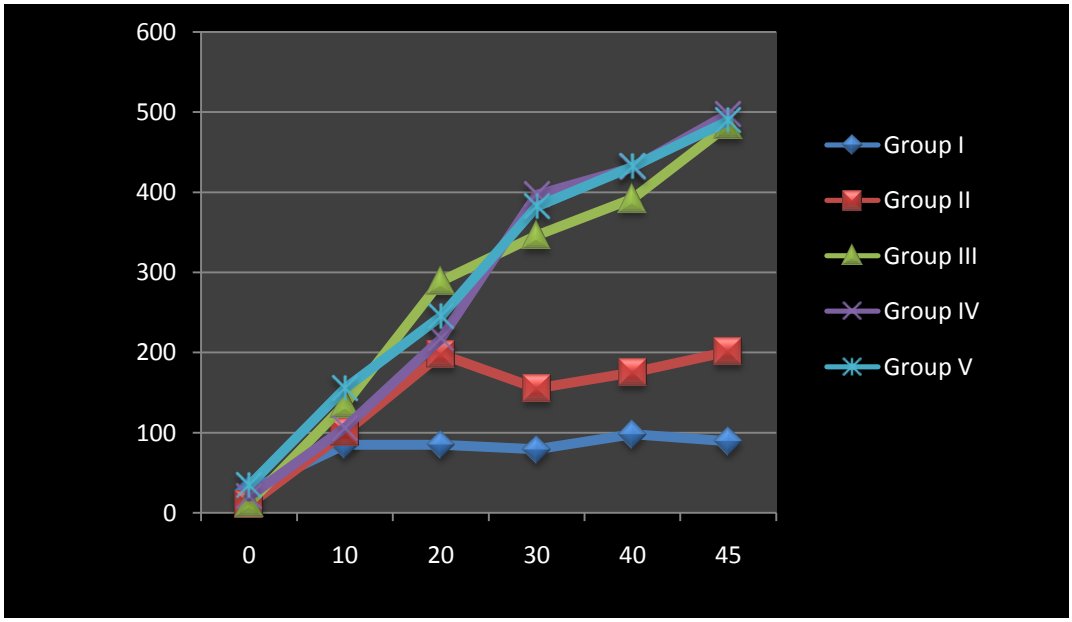
Changes in haematological profile are very common in the diabetic state. The observed reduction in the concentrations of haemoglobin, RBC as well as increased concentrations of WBC and lymphocytes in the diabetic rats indicated impairment in haematological function. Administration of synthesized silver nanoparticles of ethanolic extract of fruits of *T.bellirica* reversed these abnormal situations and restored normalcy.

4.4 Blood Glucose in experimental rats

Blood glucose level was recorded in the control and diabetic rats for 45 days and the results are given in figure 8

FIGURE 8

BLOOD GLUCOSE LEVEL IN EXPERIMENTAL RATS



Values are expressed in mean ± S.E.M (n=6)

Group I - Control Group II-Ctrl+ AgNPs EFTB

Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB

Group V - STZ induced rats + glib

Administration of biosynthesized of silver nanoparticles of the ethanolic extracts of fruits of *Terminalia bellirica* was found to reduce the levels of blood glucose in diabetic rats. Maximum reduction in the levels of blood glucose was noted from 20th day. Control rats treated with the synthesized silver nanoparticles of the ethanolic extracts of fruits of *Terminalia bellirica* did not exhibit any significant change in the blood glucose level. Also the levels were found to be within the normal range. The glucose lowering effect of synthesized silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* was found to be effective than that of standard drug when treated with streptozotocin induced diabetic rats.

Earlier studies reported that the ethanolic extract of leaves, fruits and bark of *Terminalia bellirica* showed the maximum *in vitro* hypoglycaemic activity mediated by increasing glucose absorption by increasing glucose transport across the cell membrane (Das and Devi, 2015).Administration of the ethanolic extract of leaves of *Boerhavia diffusa* and glibenclamide to the diabetic rats restored the levels of blood glucose to the normal levels which may be due to increased secretion of insulin from the existing beta cells or from regenerated beta cells providing the insuliogenic activity of the plant extract (Vasundhara and Devi, 2018).

Similar findings were reported by Prabhu *et al*.(2018), that the blood glucose level of diabetic rats was found to be significantly decreased in diabetic rats after the administration of *Pouteria sapota*. Anusooriya *et al*. (2014) have reported a significant decrease in the blood glucose level of the diabetic rats after the administration of fruit extract of *Passiflora ligularis*. Singh *et al*. (2018), also explained that the level of blood glucose was found to be decreased after the administration of the seed of *Terminalia chebula*. Our results are similar to that of Singh *et al.* (2004), who has stated that there was a signifinant decrese in the blood glucose level in the diabetic rats treated with the *Momordica charantia* juice.

Improper management of diabetes may lead to the development of many chronic complications. The pancreas is the primary sensing organ of energy states, it detects glucose concentration in the blood and in response to elevated blood glucose concentrations, insulin will be secreted. In diabetic condition, the mechanism is impaired (Prabhu *et al*.2018). Hyperglycemia is considered as a main factor in the development and progression of the complications of diabetes mellitus. The significant elevation in the level of glucose in diabetic rats could be due to the destruction of Pancreatic β-cells by Streptozotocin (Maiti *et al*.2014).The hypoglycaemic effect and consequent decrease in urine sugar excretion is one of the essential characteristics of antidiabetic agents (Pradeepa *et al.,* 2013).

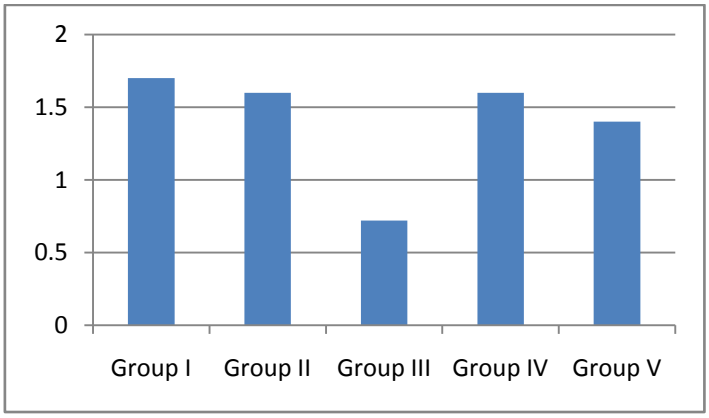
Hence the results indicated that the synthesized silver nanoparticles of the ethanolic extract of fruits of *Terminalia bellirica* was found to be effective than that of the standard drug.

4.5 Plasma Insulin in Experimental Rats

Insulin is a hormone secreted by the pancreas that metabolizes and stores carbohydrates, proteins and fats. Insulin transport glucose from blood into different cells of the body. When the pancreas produce low level of insulin or improperly work, the glucose stays in the blood which makes the blood sugar level high (Kasali *et al.,* 2016). It is a stimulator of glycogenesis and inhibitor of glycogenolysis, therefore the glycogen content decreases due to lack of insulin in the diabetic state (Choudhari *et al.,* 2017). Figure 3.0 represents the levels of insulin in experimental rats.

FIGURE 9

LEVELS OF INSULIN



Values are expressed in mean ± S.E.M (n=6)

Group I - Control Group II-Ctrl+ AgNPs EFTB

Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB

Group V - STZ induced rats +glib

Biosynthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica* and standard drug glibenclamide for 45 days showed significant increase reduction ($p<0.05$) in the levels of insulin in treated diabetic rats whereas diabetic rats have shown decreased levels of insulin. The increased insulin level may be due to the activation of beta cells of islets of langerhans and the results are in accordance with Gondi and Rao (2015), whose results reveals that the diabetic rats treated with ethanol extract of mango (*Mangifera indica L.*) peel showed increased levels of the insulin level. Kyu *et al.* (2015) who have reported that the Yam supplemented (*Dioscorea batatas*) to the diabetic rats showed increased levels of the insulin. Pradeepa *et al.* (2013), also mentioned in her studies that

administration of *Pithecellobium dulce* fruit significantly increases the levels of plasma insulin in diabetic rats.

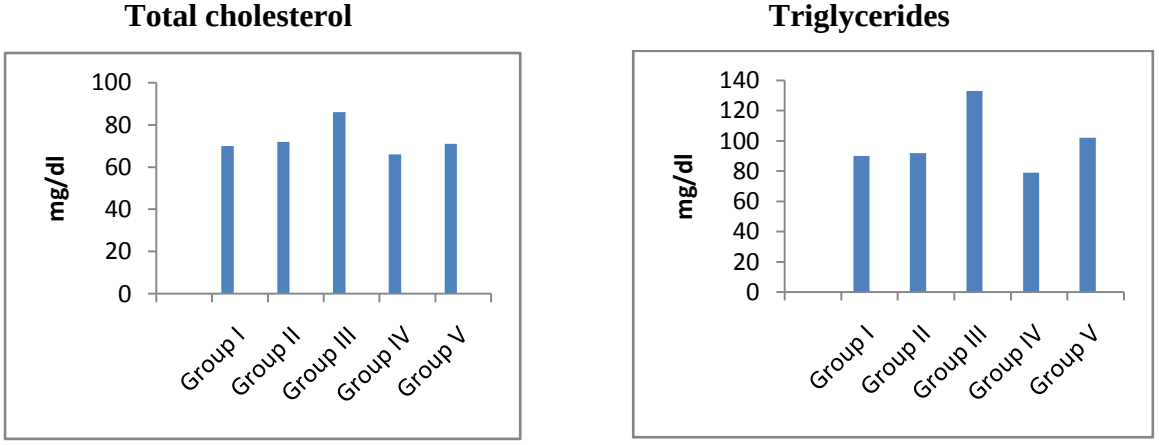
4.6 Total Cholesterol (TC) and Triglycerides (TG) in Experimental Rats:

The inadequate glucose control in diabetes leads to various acute and chronic complications including atherosclerosis. Alteration in the serum lipid profile was known to occur in diabetes and thus it increases the risk of heart disease. Diabetes induced hyperlipidemia was attributed to excess mobilization of fat from the adipose tissue owing to the under utilization of glucose. In diabetic condition the increased levels of Total Cholesterol and Triglycerides are reported. Lowering of blood glucose and plasma lipid levels is associated with the risk of vascular disease (Choudari *et al.*, 2017).

The administration of biosynthesized silver nanoparticles of ethanolic extracts of fruits of *T.bellirica* on Total cholesterol and Triglycerides of experimental rats was carried out and the results are tabulated in Figure 10

FIGURE 10

TOTAL CHOLESTEROL AND TRIGLYCERIDES LEVELS OF THE EXPERIMENTAL RATS



Values are expressed in mean ± S.E.M (n=6)

Group I - Control

Group II- Ctrl+ AgNPs EFTB

Group III – STZ induced rats

Group IV- STZ induced rats + AgNPs EFTB

Group V - STZ induced rats + glib

The levels of total cholesterol and triglycerides in serum of the experimental rats clearly indicates that the mean levels were significantly higher ($p<0.05$) in streptozotocin induced diabetic rats than the control rats.

The above figure clearly indicates that the biosynthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica* and standard drugs for 45 days to STZ

induced rats showed ($p<0.05$) decreased levels of total cholesterol and triglycerides when compared to their respective controls.

Zhou *et al*. (2015) that the elevated total cholesterol and triglycerides were compared in control, diabetic mice and streptozotocin induced diabetic mice. The streptozotocin induced diabetic mice exhibited significantly increased triglycerides and total cholesterol levels. Hassan *et al*. (2015) have reported a significant decreases in the levels of triglycerides and total cholesterol after the administration of the *Caesalpinia ferrea martius* leaf extract in streptozotocin induced diabetic rats. Abad *et al*. (2014) who explained that after induction of diabetes *Capparis spinosa* L. root extract in diabetic rats showed a ($p<0.05$) decrease significantly in the levels of total cholesterol and triglycerides when compared to their respective controls. Ahmed *et al*. (2015) that the administration of the ethanolic extracts of *Euryale ferox salisb.* and glibenclamide reduced the total cholesterol and triglycerides level in the STZ-induced diabetic rats.

4.7 Liver marker enzymes in experimental rats

The liver is the vital organ responsible for the metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. Reliable markers of liver function include AST (Amino transaminase), ALT (Alanine Aminotransaminase) and ALP (Alkaline phosphatase). It is the most important organ which plays a vital role in regulating various physiological processes in the body .The activities of AST,ALT and ALP was carried out and the results are are recorded in Table 5

TABLE 5

LIVER MARKER ENZYMES IN EXPERIMENTAL RATS

GROUP	Group I	Group II	Group III	Group IV	Group V
AST(U/L)	148.2±8.685	145±6.128	402.1±21.48	139.1±16.34	146±46.52
ALT (U/L)	35.27±1.821	35.6±3.58	37.2±2.227	30±2.663	35±1.914
ALP (U/L)	53.5±3.983	50±2.71	128.1±4.05	95±2.43	111±4.53

Group I - Control Group II-Ctrl+ AgNPs EFTB
Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB
Group V - STZ induced rats + glib

AST Units: µmoles oxaloacetate liberated/min/L
ALT Units: µmoles pyruvate liberated/min/L
ALP Units: mm p-nitrophenol liberated/ min/L

A significant elevation in the activities of AST, ALP, ALT levels ($p<0.05$) were observed in the serum of the diabetic rats when compared to the control rats. The continous oral administration of the biosynthesized silver nanoparticles of the ethanolic extract of fruits

of *Terminalia bellirica* and the standard drug to the diabetic for 45 days was able to restore all the functions of the liver marker enzymes back to the normal.

Similar findings were reported by Hamden *et al., (*2012) the administration of Trigonelline in diabetic rats in terms of ALT, ALP and AST have been significantly increased. Prabhu *et al*.(2018) explained that the elevated activities of AST,ALP,ALT were found in the diabetic rats have been significantly decreased in diabetic rats after the administration of *Pouteria sapota* in streptozotocin induced diabetic rats. The results are supported by Kasali *et al.* (2016), who have reported that the administration of *Physalis perviana L.Leaves* had prevented a significant increase in the levels of ALT,AST and ALP in the STZ-induced rats and reduced the levels of liver enzyme after the administration of extract in the diabetic rats.

Sangeetha *et al*. (2015) who explained the continous administration of the ethanolic extract of *Cyclea peltata* to streptozotocin induced diabetic rats caused a significant reduction in the activities of AST, ALP and ALT. The results are supported by Aswar and Kuchekar (2012) the oral administration of fruit extract of *Memordica charantia* was found to reduce the level of the total cholesterol and triglycerides.

Anusooriya *et al.* (2014) have reported a significant decrease in the activities of liver marker enzymes AST, ALP and ALT in diabetic rat after the administration of fruit extract of *Passiflora ligularis*. Choudhari *et al.*,(2014) showed a significant decrease in the serum cholesterol and triglycerides of the diabetic rats. Hence results indicated that the synthesized silver nanoparticles of the ethanolic extract of fruits of *Terminalia bellirica* have been significantly decrease treated when compared with untreated diabetic rats.in diabetes rats.

4.8 Activities of Carbohydrate Metabolism Enzyme in Experimental Rats

One of the prime enzymes in the glycolytic pathway is glucokinase,which is insulin dependent and plays an important role in the maintanence of glucose homeostatis and the cells that metabolized glucose by ATP to produce glucose-6-phosphate..The activities of carbohydrate metabolism in experimental rats was resulted in Table 4.0

TABLE 6
ACTIVITES OF CABOHYDRATE METABOLISM ENZYME IN EXPERMENTAL RATS

Groups	Group I	Group II	Group III	Group IV	Group V
Glucokinase	0.975±0.03	0.866±0.03	0.7933±0.03	0.837±0.09	0.666±0.06
Glucose-6-phosphatase	0.180±0.03	0.178±0.07	0.107±0.04	0.158±0.05	0.142±0.07
Fructose-1,6-bis-phosphatase	0.503±0.04	0.491±0.11	0.454±0.05	0.474±0.06	0.460±0.03

Group I - Control Group II-Ctrl+ AgNPs EFTB
Group III – STZ induced rats Group IV- STZ induced rats + AgNPs EFTB
Group V - STZ induced rats + glib

A significant reduction was observed in the levels of Glucokinase activities of ($p<0.05$) were observed in the diabetic rats when compared to the control rats. The continous oral administration of the biosynthesized silver nanoparticles of the ethanolic extract of fruits of *Terminalia bellirica* have been increased the levels of metabolism.

Similar studies was also observed that fenugreek leaves supplementation increased the activity of glucokinase and decreased the activities of glucose 6-phosphatase and fructose-1,6 bisphosphatase in diabetic liver and kidney. The increased activity of glucokinase can cause increased glycolysis and increased utilization of glucose for energy production. The observed increase in plasma insulin in STZ-induced diabetic rats may be a consequence of the decreased activities of these gluconeogenic enzymes. The increased activity of glucokinase and decreased activities of glucose 6-phosphatase and fructose-1,6-bisphosphatase can result in a reduction of blood glucose. Thus this study reveals that fenugreek leaves possess an antidiabetic action in STZ-induced diabetic rats. This study also shows that fenugreek leaves control the increase in the levels of glucose by increasing glycolysis and by decreasing gluconeogenesis. This is possible as it controls the activities of the key enzymes of glycolysis (Devi *et al*., 2003).

A significant decrease in glucokinase activity was observed in the livers of diabetic rats, whereas the activity of hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase was significantly increased compared with normal rats. Oral administration of CFEt () and glibenclamide to diabetic rats resulted in a significant increase in glucokinase activity and a decrease in gluconeogenic enzyme activity in the livers of diabetic rats. Oral administration of CFEt and glibenclamide to normal rats resulted in a significant decrease in hepatic

gluconeogenic enzymes, whereas the activity of glucokinase was not altered significantly compared with normal rats (Latha *et al.*, 2003)

4.9 Histopathological studies in experimental rats

Histopathological examination of controls and the experimental rats are studied in liver, pancreas and kidney. The results are recorded and represented in Table 7,Plate 2,3 and 4

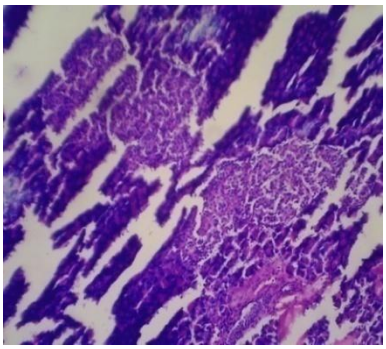
TABLE 7

HISTOPATHOLOGY STUDIES IN EXPERIMENTAL RATS

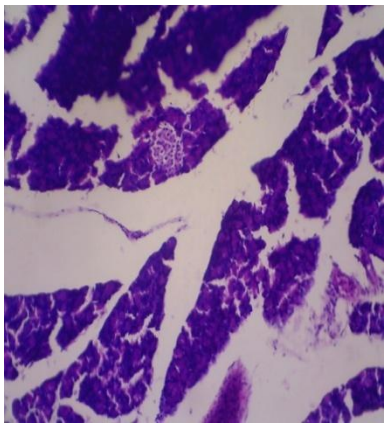
Groups	Treatment	Liver	Pancreas	Kidney
I	Control	Normal histology	Normal histology	Normal histology
II	Ctrl+ AgNPs EFTB	Normal histology	Normal histology	Normal histology
III	STZ induced rats	Necrosis	Islets are reduced in number and size	Inflammation in Tubules
IV	STZ induced rats + AgNPs EFTB	Hydrophobic lesions	Partial destruction of islets	Inflammation in tubules
V	STZ induced rats + glib	Normal histology	Partial destruction of islets	Normal histology

PLATE 2

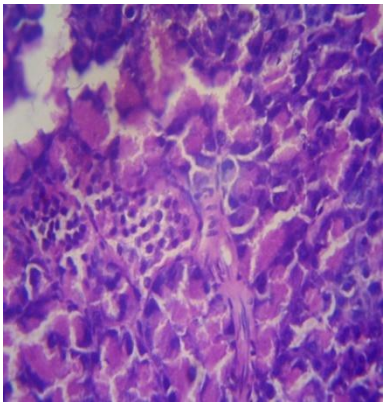
HISTOPATHALOGY OF LIVER IN EXPERIMENTAL RATS



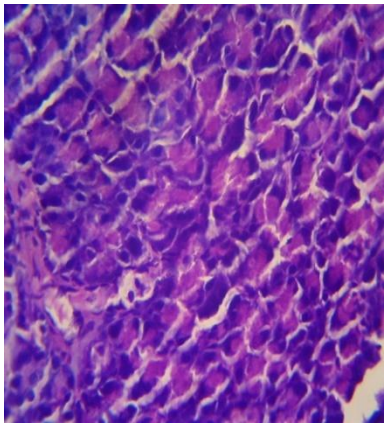
Group I



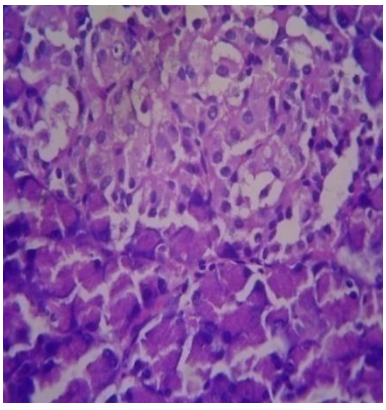
Group II



Group III



Group IV

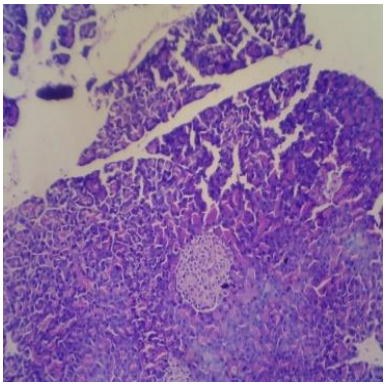


Group V

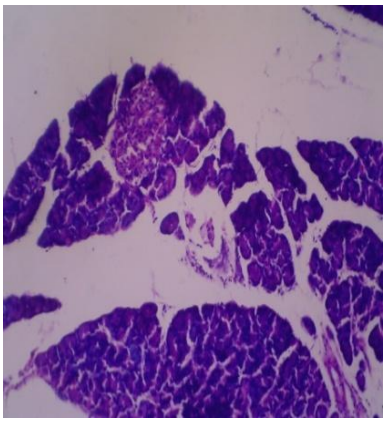
Group I - Control
Group II-Ctrl+ AgNPs EFTB
Group III – STZ induced rats
Group IV- STZ induced rats +AgNPs EFTB
Group V - STZ induced rats + glib

PLATE 3

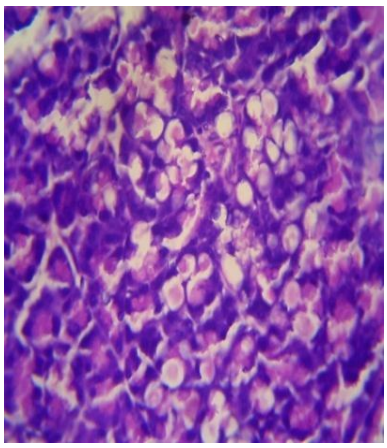
HISTOPATHOLOGY OF PANCREAS IN EXPERIMENTAL RAT



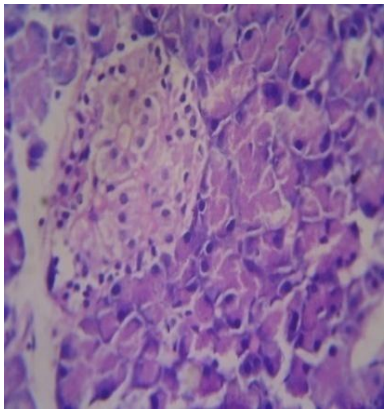
Group I



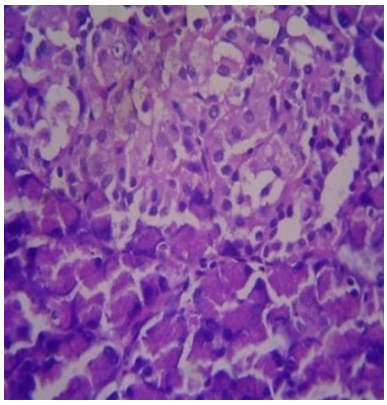
Group II



Group III



Group IV



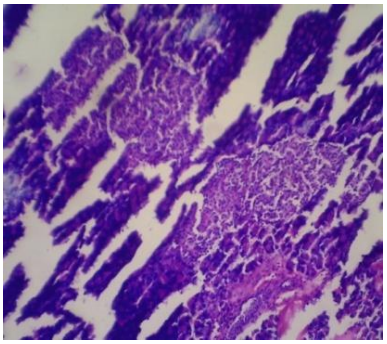
Group V

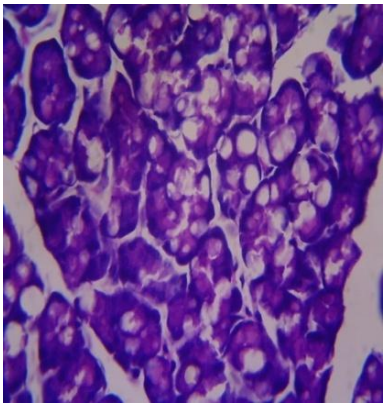
Group I - Control
Group II-Ctrl+ AgNPs EFTB
Group III – STZ induced rats
Group IV- STZ induced rats + AgNPs EFTB
Group V - STZ induced rats + glib

PLATE 3

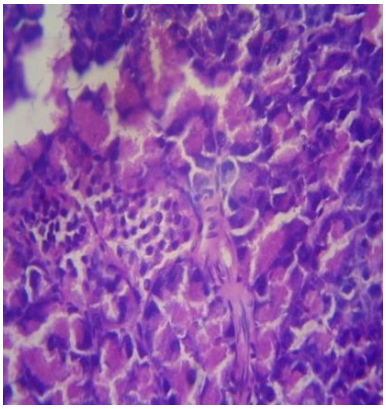
HISTOPATHOLOGY OF KIDNEY IN EXPERIMENTAL RATS

Group I

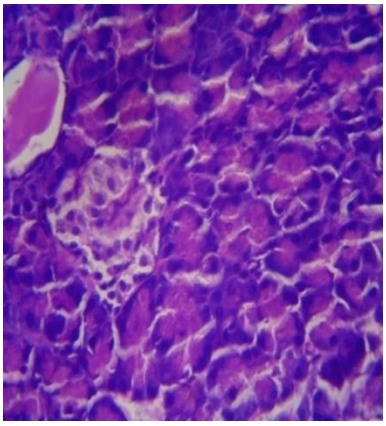




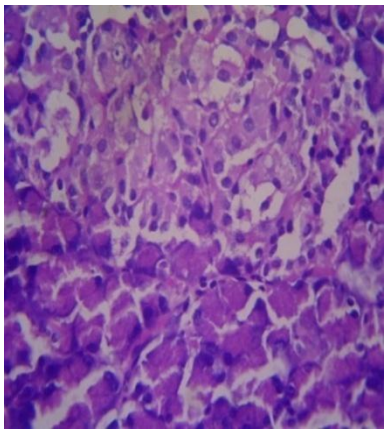
Group II



Group III



Group IV



Group V

Group I - Control
Group II-Ctrl+ AgNPs EFTB
Group III – STZ induced rats
Group IV- STZ induced rats + AgNPs EFTB
Group V - STZ induced rats + glib

The biochemical effect observed in diabetic rats showed a pathological changes in the liver, pancreas and kidney, whereas the rats treated with ethanolic extract of fruits of *T.bellirica* and standard drugs that occurred due to diabetes. Moderate destruction in the

β-cells was observed in the diabetic control rats. No pathological observation were made when the diabetic animals were treated with ethanolic extract of fruits of *Terminalia chebula*.(Kannan et al.,2012)

Arunachalam and Parimelazhagan (2014), have reported the result of histopathological study of diabetic rats induced by Streptozotocin showing degeneration of liver and pancreas and the damage reversed to normal in the diabetic rat treated with *Ficus talboti* bark for 21 days.Ahmed et al. (2015) have reported the protective effect of extract of *Euryale ferox salisb*. On the liver, pancreas and kidney in STZ induced diabetic rats when administered for 45 days.

Vasundhara and Devi (2018), have reported the result of histopathological study of diabetic rats in *Boerhavia diffusa* induced by streptozotocin , in which the pathological changes was observed in the STZ induced rats.

5.0 SUMMARY AND CONCLUSION

Nanobiotechnology is an upcoming branch of nanotechnology which has been playing an important role in the field of medicinal science. Biomedical nanotechnology is an evolving field having enormous potential to positively impact the health care systems. Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against the metabolic disorders.

Traditional system of medicine has become a topic of global importance. Current estimated suggest that in many developing countries a large proportion of the population uses the traditional practitioners and medicinal plants to meet the primary health care needs. Herbal medicines have gained more popularity for its historical and cultural reasons than the modern medicine. A few plants species have been significantly evaluated for their possible medical application.

Diabetes mellitus is an endocrine and metabolic disorder indicated by chronic hyperglycemia that produces biochemical changes and tissue destruction. Several obstacles of biochemical changes and tissue destruction like atherosclerosis, neuropathy, etc., having chronically elevated glucose levels due to glycosylation and metabolic disorders. It is a debilitating disease affecting millions of people worldwide. Since the disease has no known modern allopathic cure, it requires lifelong health. In fact, modern medicine merely attempts to control the symptoms of diabetes like increased blood sugar level and tries to mitigate the various other complicated problems that can arise out of diabetes.

Literature for synthetic drugs for diabetes mellitus tells that most of these drugs have many side effects. So, scientists are in search of safe, natural antidiabetic agents that can cure the diabetes without causing harm and the world health organisation has also recommended the development of herbal medicine in this concern. Streptozotocin is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cell.

The new era in diabetes mellitus treatment facilitates the rapid green synthesis of multifunctional, biocompatible and ecofriendly metal colloidal nanoparticles, as effective nanomedicine against the emerging threat of diabetes mellitus. Green synthesis of nanoparticles is an upcoming novel method of nanoscience and nanobiotechnology. Since this method is safer, stable and inexpensive, it is widely used to treat various diseases especially diabetes. Plant based nanoparticle synthesis have attracted more attention due to

growing interest in environmentally conscious products. Hence the present study framed to synthesize the silver nanoparticles from the ethanolic extracts of fruits of *Terminalia bellirica* and to evaluate its antidiabetic activity.Current global excitement in the use of eco-friendly and cost effective resources drives the application of highly hailed medicinal plants to the green synthesis of nanoparticles that acquire diverse pharmacological properties. In the present study, diabetes mellitus was induced in rats through Streptozotocin (STZ) injection that cause the destruction of β-cells of islets of langerhans, as proposed by many others.

This effect was represented in the current study through the elevation of blood glucose and decrease of insulin levels in diabetic control rats. The glucose lowering effect of Biosynthesized silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* was found to be effective than that of standard drug. Hence it was further chosen for studying the antidiabetic activities in the experimental rats.

This effect was represented in the current study through the elevation of blood glucose and decrease of insulin levels in diabetic control rats. The glucose lowering effect of biosynthesized silver nanoparticles of ethanolic extract of fruits of *Terminalia bellirica* was found to be effective than that of standard drug. Hence it was, further chosen for studying the antidiabetic activities in the experimental rats.The body weight of the experimental rats treated with STZ was significantly decreased ($P<0.05$) when compared to the control rats. The drugs and plant extract treated groups of rats showed an increased percentage of protection towards the disease.

Haematological and biochemical parameters disclosed that the synthesized silver nanoparticles of the ethanolic extracts of fruits of *Terminalia bellirica* were found to contain an effective antidiabetic activity. Histopathological examination was further confirmed the ability of the extracts to reverse the damages caused as a result of induced diabetes in the experimental rats.The simultaneous treatment with the AgNPs EFTB (treateddiabetic rats) had prevented a significant increase in the levels of AST, ALP and ALT.In animal models of diabetes, the insulin level was reduced and restore the levels back to its normal in treated diabetic rats while comparing with the untreated diabetic rats. The streptozotocin induced diabetic rats exhibited significantly increased levels of Triglycerides and Total cholesterol. Histopathological examination ofliver, pancreas and kidney was further confirmed the ability of the extracts to reverse thedamages caused as a result of induced diabetes and obesity in experimental rats.

The body weight of the experimental rats treated with STZ was significantly decreased ($P<0.05$) when compared to the control rats. However significant increase in the body was observed in the treated groups. The animals treated with the standard drugs daily once for 45 days significantly decreased the body mass index, when compared to the diabetic control rats. The drugs and plant extract treated groups of rats showed an increased percentage of protection towards the disease.

Haematological and biochemical parameters disclosed that the synthesized silver nanoparticles of the ethanolic extracts of fruits of *Terminalia bellirica* were found to contain an effective antidiabetic activity. Histopathological examination was further confirmed the ability of the extracts to reverse the damages caused as a result of induced diabetes in the experimental rats.

To conclude the outcome of the present study, the antidiabetic activities of the biosynthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica*, which is used for the animal study, reiterate the fact that the biosynthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica* possess strong antidiabetic activity. And can be suggested that the biosynthesized silver nanoparticles of ethanolic extracts of fruits of *Terminalia bellirica* can be used as a novel candidate in herbal preparations to combat the short comings of the available antidiabetic drug.

BIBLOGRAPHY

- Abad.M.K.M., Haeri.M., Ebrahimi.M., Heidari.R. (2014), Antidiabetic effect of *Capparis spinosa* L. root extract in diabetic rats, Avicenna Journal of Phytomedicine, 5(4): 325-332.
- Abdelghany.T.M., Rajhi.A.M.H., Abboud.M.A., Alawlaqi.M.M., Magdah.A.G., Helmy.E.A.M. and Mabrouk.A. (2018), Recent Advances in Green Synthesis of Silver Nanoparticles and Their Applications: About Future Directions. A Review, Biological Nanosciences, 8:5–16.
- Abell, L.L., Levy, B.B., Brodie, B.B. and Kendall, F.E. (1952), A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity, The Journal of Biological Chemistry, 195: 357.
- Abolfazl,M., Hadi,A., Frhad,M. and Hossein,N. (2014), *In vitro* antibacterial activity and phytochemical analysis of some medicinal plants, Journal of medicinal plant research, 8(3):186-194.
- Adhikari,R. (2016), Applications of upconversion nanoparticles in nanomedicine, Journal of Nanomedicine and Nanotechnology,7(4):2157-2168.
- Ahamad,N. and Sharma,S. (2012), Green synthesis of silver nanoparticles using extracts of *Ananas comosus*, Green and Sustainable Chemistry, 2:141-147.
- Ahmed, D., Kumar, V., Verma, A., Shukla, G.S. and Sharma, M. (2015), Antidiabetic, antioxidant and antihyperlipidemic effect of extract of *Euryale ferox* salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin-induced diabetic rats, *SpringerPlus*, 4(315): 1-17
- Ahmed.S., Ahmad.M., Swami.B.L. and Ikram.S.(2016), A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications:A green expertise, Journal of Advanced Research 7: 17–28.
- Alaqad,K. and Saleh,T.A. (2016), Gold and Silver nanoparticles:Synthesis methods, characterization routes and application towards drugs, Journal of Environmental and Analytical Toxicology, 6(4):1-10.
- American Diabetes Association, Diagnosis and Classification of Diabetes Mellitus, Diabetes care, 37:81-89.

- Amiri,A. Dehkordi,R.A.F., Heidarnejad,M.S., Dehkordi,M.J. (2018), Effect of the Zinc Oxide Nanoparticles and Thiamine for the Management of Diabetes in Alloxan-Induced Mice: a Stereological and Biochemical Study, Biol Trace Elemental Research, 181:258–26.
- Anusooriya, P., Malarvizhi, D., Gopalakrishnan, V.K. and Devaki, K. (2014), Antioxidant and antidiabetic effect of aqueous fruit extract of *Passiflora ligularis* Juss. on streptozotocin induced diabetic rats, *International Scholarly Research Notices*, Article ID 130342: 1-10.
- Arunachalam, K. and Parimelazhagan, T. (2014), Antidiabetic and enzymatic santioxidant properties from methanol extract of *Ficus talboti* bark on diabetic rats induced by streptozotocin, *Asian Pacific Journal of Reproduction*, 3(2): 97-105.
- Ayesha.A. (2017), Bacterial synthesis and application of nanoparticles, Nanoscience and Nanotechnology:An Indian Journal,11(2):119.
- Balamurugan, K., Nishanthini, A. and Mohan, V.R. (2014), Antidiabetic and antihyperlipidaemic activity of ethanol extract of *Melastoma malabathricum* Linn. leaf in alloxan induced diabetic rats, *Asian Pacific Journal of Tropical Biomedicine*, 4(1): S442-S448.
- Barde, K., Loksh, K.R., Kumawat, C., Chaturvedi, M. and Nagar, R. (2016), *In vitro* α- glucosidase and aldolase reductase inhibitory activity of *Pterocarpus marsupium*, *International Journal of Pharmacy, Engineering and Life Sciences*, 1(5): 33-44.
- Brandstrup, N., Kirk, J.E. and Bruni, C. (1957), Determination of hexokinase in tissues, Journal of Gerontology, 12: 166-171.
- Carvalho.H.O., Souza.B.S.F., Santos.I.V.F., Resque.R.L., Keita.H., Fernandes.C.P. (2016) and Carvalho.J.C.T., Hypoglycemic effect of formulation containing hydroethanolic extract of *Calophyllum brasiliense* in diabetic rats induced by streptozotocin, Brazilian Journal of Pharmacognosy, 26 : 634–639.
- Chanda,S. Menpara,D. and Desai, DD. (2013), Antimicrobial activity of *Terminalia bellirica* leaf and stem collected from two different sites, American Journal of Phytomedicine and Clinical Therapeutics,1(9):721-733.
- Chauhan.V., Singh.V. and Tiwari.A. (2017), Applications of Nanotechnology in Forensic Investigation, International Journal of Life Science Scientific Research, 3(3): 1047-1051.

- Choudhari.V.P., Gore.K.P. and Pawar.A.T.(2017), Antidiabetic, antihyperlipidemic activities and herbe-drug interaction of a polyherbal formulation in streptozotocin induced diabetic rats, Journal of Ayurveda and Integrative Medicine, 8: 218-225
- Culling, C.F.A. (1979), Handbook of histopathological and histochemical techniques, 3rd Edition, NewYork, Butterworth and Co. Ltd.,7.
- Das.M.S. and Devi.G. (2015), *In vitro* glucose binding activity of *Terminalia bellirica*, Asian Journal of Pharmaceuticals and Clinical Research,8(2):320-323.
- Deb,A., Barua,S. and Das,B. (2016), Pharmacological activities of Baheda (*Terminalia bellirica*):A review, Journal of Pharmacognosy and Phytochemistry, 5(1):194-197.
- Devi.B.A., Kamalakkannan.N. and Prince.P.S.M. (2003), Supplementation of Fenugreek Leaves to Diabetic Rat's,Effect on Carbohydrate Metabolic Enzymes in Diabetic Liver and Kidney, Phytotherapy Research, 17,1231–1233.
- Drabkin, D.L. and Austin, J.M. (1932), Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood, The Journal of Biological Chemistry, 98: 719-733.
- Elobeid, M.A. (2016), Amelioration of Streptozotocin Induced Diabetes in Rats by Eco-friendly Composite Nano-Cinnamon Extract, Journal of Zoology,48:645-650.
- Ezuruiken.U.F and Prieto .J.M. (2014), The use of plants in the traditional management of diabetes in Nigeria-Pharmacological and toxicological considerations, Journal of Ethanopharmacology,8: 29-39.
- Gancedo, J.M. and Gancedo, C. (1971), Fructose-1,6-bisphosphate, phosphofructokinase and glucose-6- phosphatase dehydrogenase from fermenting and non-fermenting yeasts, *Archives of Microbiology,* 76:132-138.
- Gandhi,H. and Khan,S. (2016), Biological synthesis of silver nanoparticles and its antibacterial activity, Journal of Nanomedicine and Nanotechnology,7(2):1-8.
- Garg.V., Kaur.P., Singh.S.K., Kumar.B., Bawa.P., Gulati.M. and Yadav.A.K. (2017), Solid self nanoemulsifying drug delivery systems for oral delivery of polypeptide-k: Formulation, optimization, *in-vitro* and *in-vivo* antidiabetic evaluation, European Journal of Pharmaceutical Sciences, 109:297–315.
- Gondi,M. and Rao,U.(2015), Ethanol extract of mango (Mangifera indica L.) peel inhibitsα-amylase and α-glucosidase activities, and ameliorates diabetesrelated biochemical parameters in streptozotocin (STZ)-induceddiabetic rats, J Food Sci Technol, , 52(12):7883–7893

- Gupta.R. (2017), Diabetes Treatment by Nanotechnology, Journal of Biotechnology and Biomaterials, 7(3), 1-3.
- Haider.A and Kang.I.K. (2015), Preparation of Silver Nanoparticles and Their Industrial and Biomedical Applications: A Comprehensive Review, Advances in Materials Science and Engineering, 1-16.
- Hales, C.N. and Randle, P.J. (1963), Immunoassay of insulin with insulin antibody precipitate, Biochemistry Journal, 88: 137-143.
- Harborne J.B.(1998),Phytochemical methods-A guide to modern techniques of plant analysis,Chapman and Hall ltd,London.
- Hasan.S.(2015), A Review on Nanoparticles: Their Synthesis and Types, Research Journal of Recent Sciences,4:1-3.
- Hassan.S.K., Sammad.N.M., Mousa.A.M., Mohammed.H.M., Farrag.A.R.H., Hashim.A.N.E., Werner.V., Lindequist.U. and Nawwar.M.A.(2015) Hypoglycemic and antioxidant activities of *Caesalpinia ferrea* Martius leaf extract in streptozotocin-induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 2015; 5(6): 462–471.
- Hjelm, M. and de Verdier, C.H. (1963), A methodological study of the enzymatic determination of glucose in blood, Journal of Clinical Laboratory Investigation, 15: 415-428.
- Hoque, M.A., Nyeem, M.A.B, Ullah, M.T., Mannan,A., Nuruzzaman,M.(2017), Evidence based antidiabetic selected indigenous medicinal plants: A review, International Journal of Home Science, 3(2): 305-309.
- http://www.wraltechwire.com/2018/02/05/nanotechnology.
- https://google/images/XQSmEc
- https://www.researchgate.net/figure/Applications-of-different-kinds-nanoparticles.
- Iannaccone,P.M. and Jacob,H.J. (2009) Rats Disease models and mechanisms,2(5-6):206-210.
- Jain,S. and Mehata,M.S.(2017), Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property, Scientific Reports,7:541-547.
- Jamdagni.P., Khatri.P. and Rana.J.S. (2018), Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity, Journal of King Saud University -Science, 30:168–175.

- Jawaad, R.S., Sultan,K.F. and Al-Hamadani,A.H. (2014), Synthesis of silver nanoparticles and its applications, ARPN Journal of engineering and Applied Sciences,9(4):586-592.
- Kakati.M.,Gogoi,L.J. and Sikdar, A.P. (2016), Comparative study of certain phytochemical properties of *Murraya koenigii(L.)*Spreng and *Mentha spicata* L.Two aromatic edible medicinal plants of darrang district, Assam, Journal of Pharmacognosy and Phytochemistry, 5(6):455-458.
- Kannan, R.V., Rajasekar, S.G., Rajesh, P., Balasubramanian, V., Ramesh, V., Solomon, K.E., Nivas, D. and Chandru, S. (2012), Antidiabetic activity on ethanolic extracts of fruits of *Terminalia chebula* Rietz alloxan induced diabetic rats, *American Journal of Drug Discovery and Development*, 2(3): 135-142.
- Kasali.F.M., Fokunang.C.N., Ngoupay.O.J., Fokunang.E.T., Ngameni.B., Njinkio.B., Kechia.F., Kadima.J.N., Marthe.T., Victor.O., Mbacham.W. and Ngadjui.T.B.(2016), Evaluation of the Antidiabetic Properties of Hydro-Alcoholic Extract and Its Fractions from *Physalis peruviana* L. Leaves on Streptozotocin-Induced Diabetic Wistar Rats, Journal of Diseases and Medicinal Plants, 2016; 2(6): 67-73
- Khalil.M.M.H., Ismail.E.H., Baghdady.K.Z and Mohamed.D.(2014), Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity, Arabian Journal of Chemistry, 7:1131–1139.
- Khan, A.K., Rashid,R., Murtaza,G. and Zahra,A. (2014), Gold nanoparticles:synthesis and applications in drug delivery, Tropical Journal of Pharmaceutical Research,13(7):1169-1177.
- Khan.I., Saeed.K and Khan.I. (2017), Nanoparticles: Properties, applications and toxicities, Arabian Journal of Chemistry,1-24.
- King, J. (1965), The hydrolases-acid and alkaline phosphatases, In Practical Clinical Enzymology (Van D. Nostrand Co. Ltd.), London, 191-196.
- Koida, H. and Oda, T. (1959), Pathological occurrence of glucose-6- phosphatase in liver, *Clinica Chemica Acta*, 4: 554-561.
- Kumar.B., Kumari .S., Cumbal.L and Debut.A. (2017), Green synthesis of silver nanoparticles using Andean blackberry fruit extract, Saudi Journal of Biological Sciences, 24: 45–50
- Kumari,S., Krishna,M., Joshi,A,B., Gurav,S., Bhandarkar,A,V., Agarwa,A., Deepak,M., Gururaj,G,M.(2017), A pharmacognostic, phytochemical and

pharmacological review of *Terminalia bellerica*, Journal of Pharmacognosy and Phytochemistry , 6(5): 368-376

- Kyu.H., Rahman.M., Kim.G., Sam Na.C., Song.C., Kim.J. and Kang.H., (2015) Antidiabetic Effects of Yam (*Dioscorea batatas*) and Its Active Constituent, Allantoin, in a Rat Model of Streptozotocin-Induced Diabetes, Nutrients, 7, 8532–8544.
- Latha.M. and Pari.L.(2003), Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism, Clinical and Experimental Pharmacology and Physiology 30: 38–43.
- Logeswari.P., Silambarasan.S and Abraham.J. (2015), Synthesis of silver nanoparticles using plants and analysis of their antimicrobial property, Journal of Saudi Chemical Society, 19 : 311–317.
- Mahmoud, A.M. (2013), Hematological alterations in diabetic rats - Role of adipocytokines and effect of Citrus flavonoids, *EXCLI Journal*, 12: 647-657.
- Maiti.R., Misra.D.S. and Ghosh.D. (2014), Hypoglycemic and Hypolipidemic Effect of Seed Hydromethanolic Extract of *Tamarindus indica* L. on Streptozotocin-Induced Diabetes Mellitus in Rat,American Journal of Phytomedicine and Clinical Therapeutics, 2(12)1416-1429.
- Malik,P., Shankar,R., Malik,V., Sharma,N. and Mukherjee,T.K. (2014), Green chemistry based benign routes for nanoparticle synthesis, Materials, 3(2):429-436.
- Mamedov, N. (2015), Medicinal plants used in traditional medicine of the Caucasus and North America, Journal of Medicinally Active Plants, 4(3):42-66.
- Meenakshi.C., Basavaraj.S. and Londonkar.R.L. (2017), Eco-Friendly Approach for Synthesising Silver Nanoparticles (SNPs) from an Exceptional Medicinal Plant *Bombax Ceiba* Bark Extract and its AntiBacterial Activity, International Journal of ChemTech Research, 10(6): 331-336.
- Moosa,A.A., Ridha,A.M. and Kaser, M.A.(2015), Process parameters for green synthesis of silver nanoparticles using leaves extract of *Aloe vera* plant, International Journal of Multidisciplinary and Current Research, 3:345-351.
- Nentwich.M.M. and Ulbig.M.W.(2015), Diabetic retinopathy - ocular complications of Diabetes Mellitus**,** World Journal Diabetes 6(3): 489-499.
- Olufunmilayo, L.A., Johnson, M., Adeleke, A.P. and Modupe, A.S. (2015), Hypoglycemic effects of the methanolic seed extract of *Hunteria umbellate* (Abeere)

and its effect on liver, hematological and oxidative stress parameters in alloxan-induced diabetic male albino rats, *International Journal of Current Research in Biosciences and Plant Biology*, 2(6): 27-34.

- Ortiz.A.V., Miranda.S.L., Covarrubias.M.A.S., Apolinar.A., Leon.R. and Palomares.I.R. (2015), Biocompatible Silver Nanoparticles Synthesized using *Rumex hymenosepalus* extract decreases fasting glucose levels in diabetic rats, Digest Journal of Nanomaterials and Biostructures,10(3): 927 – 933.
- Oyedemi, S.O., Adewusi, E.A., Aiyegoro, O.A. and Akinpelu, D.A. (2011), Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic wistar rats, *Asian Pacific Journal of Tropical Biomedicine*, 1(5): 353-358.
- Pak, Z.H., Abbaspour, H., Karimi,N. and Fattahi,A. (2016), Eco- friendly synthesis and antimicrobial activity of silver nanoparticles using *Dracocephalum moldavica* seed extract, Applied Sciences, 6(69):1-10.
- Pankaj, P.P. and Verma, M.C. (2013), Potential role of *Spirulina platensis* in maintaining blood parameters in alloxan induced diabetic mice, *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(4): 450-456.
- Prabhu.S.,Vinodhini.S.,Elanchezhiyan.C. and Rajeswari.D. (2018), Evaluation of the Antidiabetic Properties of Hydro-Alcoholic Extract and Its Fractions from *Physalis peruviana* L. Leaves on Streptozotocin-Induced Diabetic Wistar Rats, Journal of Diabetes, 10 : 28–42
- Pradeepa, S., Subramanian, S. and Kaviyarasan, V. (2013), Biochemical evaluation of antidiabetic properties of *Pithecellobium dulce* fruits studied in streptozotocin-induced experimental diabetic rats, *International Journal of Herbal Medicine*, 1(4): 21-28.
- Rajaram.K., Aiswarya.D. C and Sureshkumar.P. (2015) Green synthesis of silver nanoparticle using *Tephrosia tinctoria* and its antidiabetic activity,Materials Letters 138:251–254.
- Ramprasad,R. and Madhusudhan,S. (2016). *In vitro* alpha amylase and alpha glucosidase inhibitory activities of ethanolic extract of *Lactuca runcinata* DC, The Pharmaceutical Letter, 8(5):231-236.
- Reddy, J.S. (2015), Silver Nanoparticles – synthesis, applications and toxic effects on humans: A review , International Journal of Bioassays, 4(11): 4563-4573.

- Reitman, S. and Frankel, A.S. (1957), A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases, American Journal of Clinical Pathology, 28(1): 56-63.
- Sadeghi.B. and Gholamhoseinpoor.F.(2015), A study on the stability and green synthesis of silver nanoparticles using *Ziziphora tenuior* (Zt) extract at room temperature, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134 : 310–315.
- Sanderson, J.H. and Philips, C.E. (1981), An atlas of laboratory, Animal haematology, Clarendon Press, Oxford: 473.
- Sangeetha, M.S., Priyanga, S., Hemmalakshmi, S. and Devaki, K. (2015), *In vivo* antidiabetic potential of *Cyclea peltata* in streptozotocin induced diabetic rats, *Asian Journal of Pharmaceutical and Clinical Research*, 8(1): 103-108.
- Sayin.N., Kara.N. and Pekel.G.(2015), Ocular complications of diabetes mellitus, World Journal of Diabetes, 6(1): 92-108.
- Shah, M., Fawcett,D., Sharma,S., Tripathy,K.S. and Poinern.G.E.J. (2015), Green synthesis of metallic nanoparticles via biological entities, Materials, 8:7278-7308.
- Shanker,K and Mohan,G,K.(2017), *In vivo* Therapeutic Potential of Biologically Synthesized Silver Nanoparticles, International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering,11(3):1-4
- Sia.P.D.(2017) Nanotechnology among innovation, health and risks, Procedia - Social and Behavioral Sciences 237 : 1076 – 1080.
- Singh.A., Srivastav.R. and Pandey.A.K.(2018), Effect of the seeds of *Terminalia chebula* on blood serum, lipid profile and urine parameters in STZ induced Diabetic rats , Journal of Pharmacognosy and Phytochemistry, 7(2): 1-5.
- Singh.P., Kim.Y., Zhang.D and Yang.D.C. (2016), Biological Synthesis of Nanoparticles from Plants and Microorganisms, Trends in Biotechnology,34:588-598.
- Srikar,S.K., Giri, D.D., Pal.D.B., Mishra, P.K. and Upadhayay.S.N. (2016), Light induced green synthesis of silver nanoparticles using aqueous extract of *Prunus amygdalus*, Green and Sustainable chemistry,6:26-33.
- Sundarajan,B. and Kumara,R.B.D(2014),Biosynthesis of silver nanoparticles in *Langersteroemia speciosa(L)* per and their antimicrobial activities, International Journal of Pharmacy and Pharmaceutical Sciences, 6(3):30-34.
- Temps.L.V. (2016), Nanotechnology in medicine, Indian Heart Journal, 68: 437–439.

- Tran,Q.H., Nguyen,V.Q. and Le, A.T. (2013), Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives, Advances in Natural Sciences: Nanoscience and Nanotechnology,4:2043-6262.
- Ullah.A., Khan.A. and Khan.A. (2016), Diabetes mellitus and oxidative stress–A concise Review, Saudi Pharmaceutical Journal, 24, 547–553.
- Van Handel, E. and Zilversmit, D.B. (1957), Micromethod for the direct determination of serum triglycerides, Journal of Laboratory and Clinical Medicine, 50: 152-157.
- Vasundhara.C.S.S and Devi.G. (2018), Effect of ethanolic extract of leaves of *Boerhavia diffusa* on carbohydrate metabolising enzymes, renal and hepatic markers in streptozotocin induced diabetic rats, International Journal of Pharmaceutical and Clinical Research , 10(2): 43-47.
- Vasundhara.C.S.S. and Devi.G. (2018), Antihyperlipidemic property of *Boerhavia diffusa* leaf extract in streptozotocin induced diabetic rats,11(4):173-176.
- Verma,S. (2016), Phytochemical and pharmacological study on *Abrus precatorius*, Asian Journal of Plant Science and Research, 6(2): 24-26.
- Zhou.J., Yan.J., Bai.Z. and Li.K., Huang.K. (2015), Hypoglycemic activity and potential mechanism of a polysaccharide from the loach in streptozotocin-induced diabetic mice, Carbohydrate Polymers 121 : 199–206.

APPENDICES

APPENDIX I

ESTIMATION OF HAEMOGLOBIN

(Drabkin and Austin, 1932)

Principle

Haemoglobin is converted into acid haematin by the action of HCl. The acid haematin solution is further diluted with distilled water until its colour matches with exactly that of permanent standard of comparator block. The Hb concentration is read directly from the calibration tube.

Procedure

0.1N HCl was added in the haemoglobin meter up to the lowest marking. 20µl of blood was drawn up to 20µl in the Sahli's pipette. Adjusted the blood column carefully without bubbles. Wiped the excess of blood on the sides of the pipette by using a dry piece of cotton. Blown the blood into the acid solution in the graduated tube, rinsed the pipette well. Mixed the reaction and allowed the mixture to stand at room temperature for 10minutes. Diluted the]solution with distilled water by adding a few drops of water carefully and by mixing the reaction mixture until the colour matches the colour in the comparator. The lower miniscus of the fluid was noted and reading was noted in g/100ml.

APPENDIX II

ESTIMATION OF RED BLOOD CORPUSCLES

(Sanderson and Phillips, 1981)

The total erythrocyte count was determined accurately by diluting a measured quantity of red blood corpuscles with a fluid isotonic solution by the method of Huxtable.

Reagents

Red blood cell diluting fluid (Hayem's fluid) - 5g of sodium sulphate, 1g of sodium chloride, 0.5g of mercuric chloride were dissolved in 200ml of distilled water.

Procedure

Blood was sucked exactly up to the 20µl mark in the RBC pipette and the diluting fluid was drawn immediately up to the mark and the blood mixed thoroughly with the diluting fluid. It was left for 2-3 minutes for proper mixing. The Neubauer counting chamber was placed along with the cover glass in position. The capillary stem of the pipette was emptied which contains only the diluting fluid. This was done by discarding first 3-5 drops.

Charging of the counting chamber

One drop of diluted blood was released into the groove of the Neubauer counting Chamber. It was left for cells to settle for 2-3 minutes, the counting chamber was put under the microscope and the ruled area was located. Erythrocytes were counted in the 5 squares of the counting area of 1mm square. The number of cells in the 4-corner square was counted.

Calculation

The total number of cells found in 5 groups of 16 squares is multiplied by 10,000 to give the number of cells in millions/mm of blood.

APPENDIX III

ESTIMATION OF WHITE BLOOD CORPUSCLES

(Sanderson and Phillips, 1981)

WBC diluting fluid or Turk's fluid was used as the diluents which can destroy RBC'S.

Reagents

White blood cells diluting fluid - Glacial acetic acid, Gentian violet 1%, Water 95ml

Procedure

The method of counting is similar to RBC counting except that the count is made in 4 large (1mm) cover squares of the Neubauer counting chamber.

Calculation

The total number of cells in 4 squares is multiplied by a factor of 2500 to give the count/mm of blood.

APPENDIX IV

ESTIMATION OF BLOOD GLUCOSE

(Hjelm and de Verdier, 1963)

Glucose level in serum was estimated by glucose oxidase/peroxidase method using a commercial kit from Med source Ozone Biomedicals Pvt Ltd.

Reagents

1. Enzyme reagent
2. Buffer solution
3. Glucose standard (100mg/dl)

Procedure

10µl of serum was added to 1.0ml of working enzyme reagent, mixed well and incubated at 37°C for 15 minutes. The colour developed was read at 505nm against blank containing distilled water instead of the sample. A standard was also processed similarly. The level of glucose is expressed as mg/dl.

APPENDIX-V

ESTIMATION OF PLASMA INSULIN

(Hales and Randle, 1963)

Plasma insulin was assayed by the solid phase enzyme linked immunosorbent assay (ELISA) using kit obtained from Monobind microwells Inc., CA., USA. Standards or samples containing insulin are allowed to react with capture antibodies coated on plastic wells and with monoclonal antibodies labeled with horseradish peroxidase. The plates were washed and then treated with revelation solution. The fluorescent product formed was measured at 450nm.

Reagents

1. Microtitre plate with anti-insulin coated wells
2. Anti-insulin horseradish peroxidases (HRP) conjugate in Tris-HCI buffer with bovine serum albumin and preservatives.
3. Standards containing insulin (5-500µU/ml) in sodium merthiolate
4. Washing solution - 20% tween 20. This was diluted with distilled water prior to use.

5. Revelation solution - 0.2ml of the chromogen tertramethyl benzidine (TMB) was mixed with 1 vial (21ml) of H_2O_2 in acetate/citrate buffer.

6. Arresting agent - 1N sulphuric acid, H2SO4

Procedure

Accurately 50µl each standard, control or sample was dispensed into the appropriate wells. Time between distribution of first standard and last sample was kept minimum. 50µl of anti-insulin HRP conjugate was dispensed into all wells and incubated for 30 min at room temperature on a horizontal shaker set at 700RPM. The plates were washed after aspirating the liquid from the well. Then, 0.4ml of washing solution was dispensed into each well and the contents were aspirated. Washing was repeated twice. Then, 200µl of the freshly prepared revelation solution was added into each well within 15 minutes after washing. Then the plate was incubated for 15 minutes on a horizontal shaker set at 700RPM at room temperature, avoiding direct sunlight. 50µl of arresting agent was added into each well. The absorbance was read within one hour at 450nm. Insulin concentrations were expressed in µU/ml **.**

APPENDIX VI

ESTIMATION OF TOTAL CHOLESTEROL

(Abell *et al*., 1952)

Reagents

1. Ferric chloride-acetic acid reagent: 0.05% ferric chloride in acetic acid.
2. Concentrated sulphuric acid.
3. Cholesterol standard

Procedure

0.1ml of extract was evaporated to dryness and 5ml ferric chloride- acetic acid reagent was added, mixed and centrifuged. To the supernatant 3ml of concentrated sulphuric acid was added and the absorbance was read after 20 minutes at 560nm against a reagent blank. A standard in the concentration range of 40-200µg was treated similarly. Values were expressed as mg/dL serum.

APPENDIX VII

ESTIMATION OF TRIGLYCERIDES

(Van Handel and Zilversmit, 1957)

Triglycerides were determined by the following method. Triglycerides were extracted by isopropanol, which upon saponification with potassium hydroxide yield glycerol and soap. The glycerol liberated is treated with meta periodate, which releases formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow colored compound, the intensity of which is measured at 420nm.

Reagents

1. Isopropanol
2. Activated aluminium oxide (Neutral)
3. Saponification reagent - 5g of potassium hydroxide was dissolved in 60ml of distilled water and 40ml of isopropanol was added to it
4. Sodium meta- per iodate reagent - 77g of anhydrous ammonium acetate was dissolved in about 700ml of distilled water, 60ml glacial acetic acid was added to it followed by 650mg of sodiummeta- periodate. The mixture was diluted to 1liter with distilled water
5. Acetyl acetone reagent - 0.75ml of acetyl acetone was dissolved in 60ml of distilled water and40ml of isopropanol was added to it
6. Standard triolein solution - 1g of triolein was dissolved in 100ml isopropanol. 1ml of stock standard was diluted to 100ml to prepare a working standard 100µg of triolein/ml.

Procedure

An aliquot of serum/lipid extract was evaporated to dryness. 0.1ml of methanol was added, followed by 4ml of isopropanol. 0.4g of alumina was added to all the tubes and shaken well for 15 minutes. Centrifuged and then 2ml of the supernatant was transferred to labeled tubes.The tubes were placed in a water bath at 65°C for 15 minutes for saponification after adding 0.6ml of the saponification reagent followed by 0.5ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40µg triolein

were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm. The triglyceride content was expressed as mg/dl – serum.

APPENDIX - VIII

ESTIMATION OF ASPARTATE TRANSAMINASE (AST)

(Reitman and Frankel, 1957)

Principle

Serum glutamine oxaloacetate transaminase (SGOT) catalyses the reversible transfer of an amino group from aspartate to α-keto glutarate forming glutamate and oxaloacetate. SGOT catalyses the following reaction:

SGOT (pH 7.4)

L – Aspartate + α – Keto glutarate Oxaloacetate + L – Glutamate

Alkaline medium

Oxaloacetate + 2,4 DNPH 2,4 dinitrophenyl hydrazine (brown colored)

Reagents

1. Tris buffer, pH 7.5 - 100mmol/l
2. L-aspartate - 500mmol/l
3. 2-oxoglutarate - 15mmol/l
4. 2, 4 dinitrophenyl hydrazine reagent
5. Working sodium hydroxide (4N

Procedure

Five hundred microlitre of buffered substrate was incubated at 37°C for 3 minutes and 0.1ml of serum was added, mixed well and incubated at 37°C for 30 minutes. Then 0.5ml of 2, 4 - dinitrophenyl hydrazine (DNPH) reagent was added, mixed well and kept at room temperature for 20 minutes and 0.5ml of 4N working sodium hydroxide was added and kept at room temperature for 10 minutes. Blank and standards were also processed in a similar way and the absorbance was measured spectrophotometrically at 505nm. Activity of SGOT was expressed as U/L**.**

APPENDIX – IX

ESTIMATION OF ALANINE TRANSAMINASE (ALT)

(King, 1965)

Principle

SGPT catalyses the reversible transfer of amino group from L-alanine to alpha ketoglutarate with the formation of pyruvate and glutamate. The pyruvate so formed is allowed to react with 2-4 dinitrophenylhydrazine (DNPH) to produce 2, 4- dinitrophenyl hydrazone derivative, which is measured spectrophotometrically.

SGPT (pH 7.4)

α – Keto glutarate + L Alanine L - Glutamate + Pyruvate

Alkaline Medium

Pyruvate + 2, 4 DNPH 2,4 dinitrophenyl hydrazine (Brown Colored)

Reagents

1. Tris buffer, pH 7.5 - 100mmol/l
2. L-alanine - 500mmol/l
3. 2-oxoglutarate - 15mmol/l
4. 2, 4 dinitrophenyl hydrazine reagent
5. Working sodium hydroxide (4N)

Procedure

Five hundred microlitre of buffered substrate was incubated at 37°C for 3 minutes and 0.1ml of serum was added, mixed well and incubated at 37°C for 60 minutes. Then 0.5ml of DNPH reagent was added, mixed well and kept at room temperature for 20 minutes and 0.5ml of 4N working sodium hydroxide was added and kept at room temperature for 10 minutes. Blank and standards were also processed in a similar way and the absorbance was measured spectrophotometrically at 505nm. Activity of SGPT was expressed as U/L.

APPENDIX – X

ESTIMATION OF ALKALINE PHOSPHATASE (ALP)

(Reitmen and Frankel, 1957)

Principle

Alkaline phosphatase (ALP) is an enzyme which catalyses the splitting of phosphoric acid from certain monophosphoric esters. In this method disodium phenyl phosphate was

hydrolyzed with the liberation of phenol and formation of sodium phosphate. The amount of phenol formed was estimated in a spectrophotometer at 650nm.

Reagents

1. Disodium phenyl phosphate (0.01M) – 1.09g of disodium phenyl phosphate was dissolved inwater and made up to 500ml. It was then boiled, cooled and little chloroform was added andkept in refrigerator (Solution A).

2. Sodium carbonate-sodium bicarbonate buffer (0.1M) - 3.18g of anhydrous sodium carbonate and 1.68g of sodium bicarbonate was dissolved in water and made up to 500ml (Solution B).

3. Buffered substrate for use - Equal volume of solution A and solution B was mixed which has pH of 10.

4. Trichloro acetic acid (20%) – Acid molybdate reagent - 5g of ammonium molybdate dissolved in 5N sulphuric acid.

5. 1, 2, 4 – ANSA : 0.25% of 1,2,4 – ANSA was prepared by adding 0.5g of dry powder ANSA to 190 ml of 15% sodium bisulphate and 5ml of 20% sodium sulphite, stoppered the bottle and shaken until it dissolved.

6. Stock phosphate solution – 2.194g of pure potassium dihydrogen phosphate was dissolved in water and made up to 500ml. A few drops of chloroform was added to it (1mg/1ml of phosphate).

7. Working standard: Two ml of stock standard was diluted to 500ml.

Procedure

Six ml of buffered substrate was pipette out in a test tube and placed in water bath at 37^0C for few minutes. Then, 0.3ml of serum was added, mixed well and incubated for 15 minutes. At the same time control and blank were also kept. For blank, 0.3ml of water was added to 6ml buffered substrate. For control, 0.3ml of serum was added to 6ml of distilled water. Later, 1.2ml of 20% TCA was added and shaken well. Five ml of the filtrate was taken in separate test tubes. To the blank and control, 0.8ml of acid molybdate was added followed by 0.2ml of ANSA. It was then mixed well and allowed to stand for 10 minutes at 37^0C and the colour developed was read at 650nm. Pipetted out 1.0 to 4.0 ml of standard solution and made up to 5ml with distilled water. Acid molybdate (0.8ml) was added followed by 0.2ml of ANSA. Standards were also read at 650nm. Alkaline phosphatase activity in serum was expressed as U/L. The activity in tissue homogenate was expressed as mole of phenol liberated/min/mg protein.

APPENDIX - XI

ESTIMATION OF GLUCOKINASE

(ATP: D-hexose-6-β-phosphotransferase)

(Branstrup *et al.*, 1957)

Reagents

1. 0.005M glucose
2. 0.72M ATP
3. 0.05M MgCl2
4. 0.0125M KH2PO4
5. 0.1MKCl
6. 0.5M sodium fluoride
7. 0.01M tris-HCl buffer, pH 8.0
8. 10% TCA

Procedure

The incubation mixture containing 2.5ml buffer, 1ml of substrate, 0.5ml ATP, 0.1ml each of MgCl2 and sodium fluoride and 0.5ml each of KH2PO4 and KCl was preincubated at 37oC for 5minutes room temperature. The reaction was initiated by the addition of 2ml of enzyme extracts. 1ml of aliquot of the reaction mixture was removed immediately (zero time) and added to tubes containing 1ml of 10% TCA. After 30 minutes incubation, 1ml of aliquot of the above reaction mixture was added to a separate set of tubes and the reaction was stopped by the addition of 1ml of TCA. After the samples precipitated and centrifuged, the supernatants were used for the estimation of glucose by ortho toluidine method.

APPENDIX - XII

DETERMINATION OF GLUCOSE-6-PHOSPHATASE

(Glucose-6-phosphate phosphohydrolase)

(Koda and Oda, 1959)

The enzyme catalyses the reaction

Glucose-6-phosphate +H2O glucose + Pi

Reagents

1. 0.1M citrate buffer, pH 6.5
2. Substrate Glucose-6-phosphate, 0.01M in distilled water
3. 2.5% ammonium molybdate solution
4. ANSA

5. 10% TCA

Procedure

The incubation mixture in a total volume of 0.1ml contained 0.3ml of buffer, 0.5ml of substrate, and 0.2ml of enzyme solution. Incubation was carried out at 37oC for 60 minutes. The reaction was terminated by the addition of 1ml of 10% TCA solution. The suspension wascentrifuged and the phosphorus content in the supernatant was estimated by the method of Fiske and Subbarow (1925). The enzyme activity is expressed as nmoles of Pi liberated/min/mg protein.

APPENDIX - XIII

ESTIMATION OF FRUCTOSE - 1, 6-BISPHOSPHATASE

(Gancedo and Gancedo, 1971)

This enzyme catalyses the reaction

Fructose-1,6-diphosphate + H2O fructose-6-phosphate + Pi.

Reagents

1. 0.1M tris-HCl buffer, pH 7.0
2. Substrate 0.05M Fructose-1,6-diphosphate solution
3. 0.1M MgCl2
4. 0.1M KCl
5. 0.001M EDTA
6. 10% TCA
7. 2.5% ammonium molybdate solution
8. ANSA

Procedure

The assay medium in a final volume of 2ml contained 1.2ml of buffer, 0.1ml of substrate solution, 0.25ml of MgCl2, 0.1ml of KCl solution, 0.25ml of EDTA solution and 0.1ml of enzyme. The incubation was carried out at 35^{o}C for 15 minutes. The reaction was terminated by the addition of 1ml of TCA. The suspension was centrifuged, and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow (1925). Protein was determined by the method of Lowry *et al.* (1951).The enzyme activity is expressed as nmoles of Pi liberated/min/mg protein.

APPENDIX - XVII

HISTOPATHOLOGICAL EXAMINATION

(Culling, 1979)

The rats were sacrificed by cervical dislocation and an autopsy was carried out to obtain liver, heart and tail of the rats. Tissue samples were taken and preserved in 10% formalin solution for a minimum of one hour. Formalin was removed from the tissue samples with running water. Dehydration of the fixed tissue was done by giving three changes of acetone (each 100ml). Cleaning of tissue from acetone was followed by three changes of xylene (each 500ml) in a total duration of three hours. Incubation of processed tissue in melted paraffin was done by two changes for 3-4 hours in an incubator maintained at 58-60˚C. Embedding of the tissue in paraffin wax was then done by immersing the tissue in molten paraffin and then cooling it to harden the paraffin. Sections of the paraffin embedded tissue were done using a microtome adjusted to 1-3µ thickness. The paraffin sections were carefully taken on glass slides. The sections were then cleaned by immersing in xylene. The sections were stained with hematoxylin and eosin stain and screened to evaluate the morphology and cellular composition.