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LASIA SPINOSA: AN ETHNOMEDICINAL ANTIDIABETIC PLANT OF ASSAM

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Lasia spinosa:

An Ethnomedicinal Antidiabetic Plant of Assam

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PREFACE

Natural products served humankind as the sources of all drugs, provided most of therapeutic activities from long time. Traditional medicines derived from natural products are widely used because of less side effects than some synthetics. Diabetes mellitus, a chronic metabolic disorder, affects physical, psychological and social health. In this project the physicochemical, phytochemical and in-vivo anti-diabetic activity of the plant *Lasia spinosa* Lour have been studied. The extract of the plant *Lasia spinosa*, belonging to the family Araceae, have the ability to reduce the blood glucose level in disease condition. The lipid profile parameters got corrected by the treatment of the plant extract. The isolated phytoconstituents may emphasize the knowledge of proper investigation of the structural elucidation of bio-marker which will help in future drug development for the treatment of diabetes. Hence development of a drug in future can be effective for the society with further scientific investigation.

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INTRODUCTION:

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products (and their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing *ca*. 25% of the total (Balandrin *et al.,* 1993). The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth, 1988).

TRADITIONAL MEDICINE:

In the past, traditional peoples or ancient civilizations depended greatly on local flora and fauna for their survival. They would experiment with various berries, leaves, roots, animal parts or minerals to find out what effects they had. As a result, many crude drugs were observed by the local healer or shaman to have some medical use. Although some preparations may have been dangerous, or worked by a ceremonial or placebo effect, traditional healing systems usually had a substantial active pharmacopoeia, and in fact most western medicines up until the 1920s were developed this way.

Some systems, like traditional Chinese medicine or Ayurveda were fully as sophisticated and as documented systems as western medicine, although they might use different paradigms. Many of these aqueous, ethanolic, distilled, condensed, or dried extracts actually have a genuine and positive effect, and ethnobotany can help identify which plants should be researched further. Rhubarb root has been used as a purgative for many centuries. In China, it was called "The General" because of its "galloping charge" and was only used for one or two doses unless processed to reduce its purgative qualities. Bulk laxatives would follow or be used on weaker patients according to the complex laxative protocols of the medical system (Dan Bensky *et al.,* 2004).

Cherokee herbalist David Winston, on the other hand, recalls how his uncle, a medicine priest, would frequently misinform visiting ethnobotanists. Acupuncturists who explored Mayan medicine for Wind in the Blood had something to share with the local healers, and so were able to learn things that anthropologists couldn't. (Hernan Garcia *et al.,* 1999).

BRIEF HISTORY:

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. As examples, we have western medicine with origins in Mesopotamia and Egypt, the Unani (Islamic) and Ayurvedic (Hindu) systems centered in western Asia and Indian subcontinent and those of orient (China, Japan, Tibet, etc.)(Evans, 2005).

The contributions of Greek scientists are much to knowledge of natural history. Hippocrates "Father of medicine" (460-370 B.C), Aristotle (384-322 B.C), Dioscorides (40-80 A.D) are the physicians contributed a lot to medical sciences. Theophrastus (370-287 BC) described medicinal plants, some of which like belladonna, ergot, opium, and colchicum are used even today (Shah and Qadary, 2004).

India leads the world in the usage of herbal medicines. It is one of the leading exporters of plant medications and their derivatives, and it also excels in domestic use. According to Indian mythology, when the illness and diseases got rampant on the earth the sages learnt the sciences of healing from lord Indra and recorded them in scripture (Indian Herbal Pharmacopoeia, 2002).

Within 250,000 higher plant species on earth, more than 80,000 plants have medicinal value. India is one of the world's 12 biodiversity centers with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities (Joy *et al.*, 1998).

Many higher plants produce important organic compounds such as oil, resins, tannins, gums dyes, flavours and fragrances, pharmaceuticals and pesticides. However, most species of higher plants have never been described, much less surveyed for chemical or biological active constituent and new source of commercially valuable material remain to be discovered. Advancement in biotechnology, particularly method for culturing plant cell and tissue, provides new means for commercial processing of even rare plants and the chemicals they produce. These new technology will extend and enhance the usefulness of plants as renewable resources of valuable chemicals (Handa and Kapoor, 2003).

Natural goods have long been an important source of medications, with natural products accounting for almost half of all pharmaceuticals now in use. Unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activity of naturally occurring secondary metabolites, the utility of bioactive natural products as bio-chemicals and molecular probes, the development of novel sensitive techniques to detect biologically active natural products, and improved techniques to isolate, purify, and structurally change natural products are all driving interest in natural products. Natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, and pharmacology all have opportunities to use the wide diversity of chemical structures and biological activity of natural products (Farooq, 2005).

HERBAL DRUG: A SCENARIO

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have had a significant impact on global health. Despite the significant improvements in modern medicine over the last few decades, plants continue to play a vital role in health care (Calixto *et al*., 2000). Natural goods have shown to be the most effective medicinal source. Each plant functions as a factory, able to produce an infinite number of very complicated and strange chemical compounds whose structures would otherwise remain a mystery (Kinghorn, 2002). At least 120 different chemical compounds produced from plants are being used as significant medications across the world, while numerous more are simply synthetic adaptations of natural molecules. (Farooqi and Sreeramu, 2001).

WHO has provided some terms related to herbal drugs, according to their definitions. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. Herbal medications in certain places may contain natural organic or inorganic active substances that are not of plant origin, as a matter of tradition (e.g. animal and mineral materials). Herbs are whole, fragmented, or powdered plant parts, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes, or other plant parts. Herbal materials include fresh juices, gums, fixed oils, essential oils, resins, and dry powders of herbs, in addition to plants. These ingredients may be prepared in certain nations using a variety of local methods, such as steaming, roasting, or stir-baking with honey, alcoholic drinks, or other components. Herbal preparations, which can comprise comminuted or powdered herbal ingredients, as well as extracts, tinctures, and fatty oils of herbal materials, form the foundation for completed herbal medicines. Extraction,

fractionation, purification, concentration, and other physical or biological procedures are used to create them. Preparations created by steeping or heating herbal components in alcoholic drinks, honey, or other materials are also included. Herbal preparations created from one or more plants make up finished herbal products. The phrase "mixture herbal product" might be used if more than one herb is utilised. Excipients may be present in finished herbal products and herbal mixtures (WHO guideline, 2000).

INDIAN HERBAL TRADE IN WORLD SCENARIO:

The utilization of herbal drugs is on flow and market is growing step by step (Kamboj, 2000). The annual turnover of Indian medicinal industry is about Rs.2300 crore as against the pharmaceutical industry's turnover of Rs. 14,500 crore with a growth rate of 15 percent (Krishnan, 1998). The global market for herbal medicines currently stands at over $60 billion annually. The sales of herbal medicine are expected to get higher at 6.4% an average annual growth rate (Inamdar *et al*., 2008). Due to contribution of numerous significant factors, the market of herbal medicines has grown at an expressive rate worldwide. Some of them are: preference of consumers for natural therapies: concern regarding undesirable side effects of modern medicines and the belief that herbal drugs are free from side effects (Calixto, 2000). However to achieve the goal in terms of exporting herbal remedies, several steps need to be taken.

- Systematic study of world market demand and short listing of medicinal herbs with good potential.
- Systemic cultivation of medicinal herbs on large scale.
- Encouragement of agro-based phytochemical and pharmaceutical industries to manufacture value added herbal products.
- Strict legislation to control quality and purity.
- Upgradation of cultivation of and collection process.
- Documentation of research work and standardization for quality.

HERBAL DRUG STANDARDIZATION:

Standardization is a system that ensures a predefined amount of quantity, quality & therapeutic effect of ingredients in each dose (Zafar *et al.,* 2005). Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product. Moreover, many dangerous and lethal side effects have recently been reported, including

direct toxic effects, allergic reactions, effects from contaminants, and interactions with herbal drugs (Vaidya and Devasagayam, 2007).

The phytochemical ingredients of an herbal preparation determine its therapeutic efficacy. Scientists have a tremendous difficulty in developing legitimate analytical techniques that can accurately profile the phytochemical content, including quantitative studies of marker/bioactive chemicals and other important ingredients. In light of the foregoing, standardisation is a key step in establishing a consistent biological activity, chemical profile, or simply a quality assurance programme for herbal medication manufacture and manufacturing. (Patra *et al*., 2010). The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health and to ensure reproducible quality of herbal medicine (Straus, 2002).

CONVENTIONAL METHODS FOR STANDARDIZATION OF HERBAL FORMULATION:

Standardization of herbal raw medications includes passport data for raw plant drugs, botanical authentication, microscopic and molecular investigation, chemical composition identification using various chromatographic methods, and whole plant biological activity. Various workers have reported macroscopic and microscopic assessment as well as chemical profiling of herbal materials for quality control and standardisation (British Herbal Pharmacopoeia, 1996; QC-WHO, 1996; Indian Herbal Pharmacopoeia, 2002).

Sensory assessment factors such as form, size, colour, texture, aroma, and taste are used to determine the macroscopic identification of medicinal plant materials, whereas microscopy entails a comparative microscopic analysis of powdered herbal drugs. Furthermore, developments in microscope technology have improved the precision and capacities of microscopy as a tool for identifying herbal crude materials, thanks to the use of light and scanning electron microscopy (Bhutani, 2003). Furthermore, sophisticated methods for standardisation of herbal pharmaceuticals include chromatographic, spectrophotometric, and combinations of these procedures, electrophoresis, polarography, and the use of molecular biomarkers in fingerprints (Seitz *et al*., 1991; Svicekova, 1993; O'Shea, 1995; Bhutani, 2003; Patel *et al*., 2006; Mosihuzzaman and Choudhary, 2008).

GUIDELINES FOR THE STANDARDIZATION OF HERBAL DRUGS:

The guidelines set by WHO:

a) Botanical characters, sensory evaluation, foreign organic matter, microscopic, histological, histochemical assessment, quantitative measurements

b) Physical and chemical identity, fingerprints chromatography, ash values, extractive values, moisture content, volatile oil and alkaloids tests, quantitative estimation protocols,
c) Estimation of biological activity, the values of bitterness, astringency hemolytic index, a factor swelling, foaming index

d) Detail-toxicity pesticides residues, heavy metals, microbial contamination as viable count total, pathogens such as *E. coli, Salmonalla, P. aeroginosa, S. aureus, Enterobacteriaceae*, etc.

e) Microbial contamination and radioactive contamination are followed (Shrikumar *et al.,* 2006).

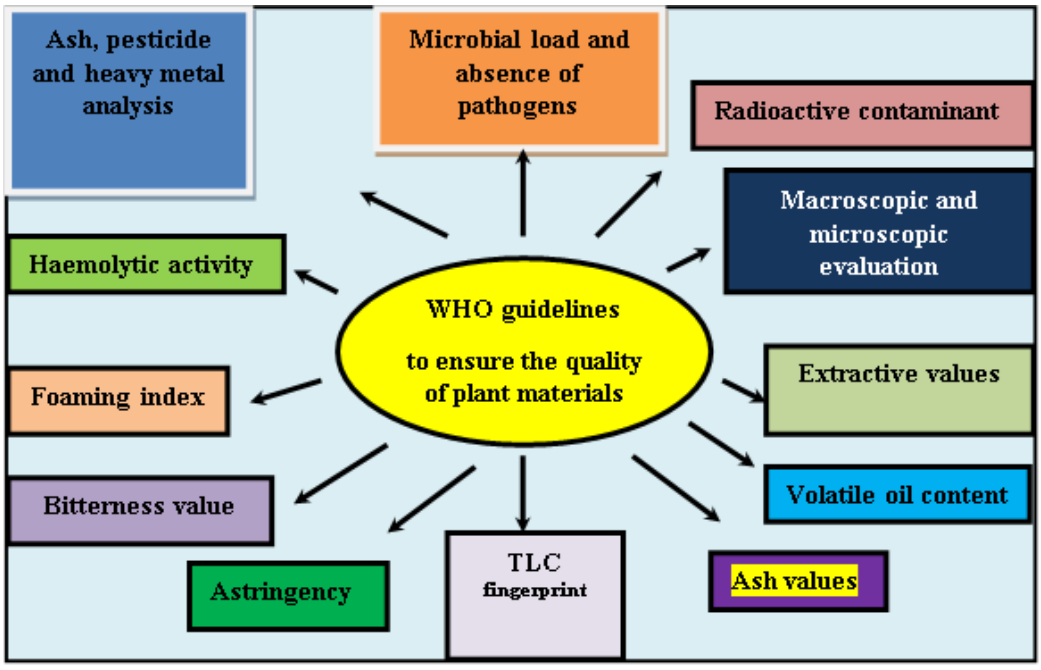

Figure 1: Schematic diagram of WHO monograph for standardization of crude drugs

PREVENTION OF MISUSE OF HERBAL MEDICINE:

The main goal of pharmacognosy is to access the value of raw materials and to ensure that the final product is of required standard. Strict standardization procedure and pharmacognostical studies of medicinal plant would reduce drastically much of the accident in wrong prescription of traditional herbal medicine. WHO has developed several

guidelines for carrying out standardization procedure of raw herbal product, which basically includes pharmacognostical, physicochemical, pharmacological and toxicological methods to standardize the herbal product. Phytochemistry has evolved as major branch of pharmacognosy in developing markers for purpose of identification and standardization.

Most of the cases of accidental herbal medicine misuse start with wrong identification of medicinal plant prescribed. Many of the traditional systems have records where one common vernacular name is supplied in two or more entirely different species. Ginseng, which is a common Indian drug, is sold under13 different names in the market. For example Chinese or Asiatic ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*), Siberian ginseng (*Eleutherococcus senticocus*), Ayurvedic ginseng (*Withania somnifera*) and Russian ginseng (*Acanthopanax senticosus*), to name a few. Such name could create confusion over prescription**.**

The second major reason for accidental herbal medicine misuse is the non-characterization of chemical constituents of the controversial plant. *Aconitum carmichaeli* and *Aconitum kusnezoffii* are both used as anti-inflammatory, analgesic and cardiotonic agent in traditional medicine, but highly toxic C-19 diterpenoid alkaloids of aconitine, mesaconitine and hypaconitine present in them may prove fatal to a subject who ingests them (Sekar and Pokharia, 2007).

REGULATION OF HERBAL MEDICINES:

WHO has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety, and efficacy (Organisation Mondiale De and La Sante, 1992; WHO 1996) In India, traditional medicine is governed by the Drugs and Cosmetics Act, 1940 and the provisions of the Act are implemented by the state governments. The first Indian National Health Policy 1983 claims that India's is the richest source of herbs and the drugs should be standardized (Shrikumar *et al.,* 2006). The department of AYUSH, Government of India, launched a central scheme to develop standard operating procedures for the manufacturing process to develop pharmacopeial standards for Ayurvedic preparations (Kalaiselvan *et al*., 2010). The Regulation for herbal drug products in Europe and United states are more stringent than in India (Singh *et al.,* 2011).

PHARMACOVIGILANCE OF HERBAL MEDICINES:

Pharmacovigilance means the science and activities relating to detect, assess, understand, and to prevent the adverse effects or any other possible drug-related problems, which is not only confined to chemical drugs, but extended to herbal, traditional and complementary medicines, biological, vaccines, blood products and medical devices (Pharmacovigilance of herbal medicines, 2006). There is an increasing recognition of the need to develop safety monitoring systems for herbal medicines (Asiri *et al.,* 2008). The herbal products of ginseng are in great demand as it is considered as a safe herbal drug for human health in spite of few reports on adverse drug reactions. But this is not applicable to every herbal product.

Therefore, Pharmacovigilance is essential for herbal drug before being considered as a safe for human health (Barnes, 2003). WHO has set specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization. The Medicines and Healthcare Products Regulatory Agencies, UK had launched 'yellow card' scheme for monitoring the safety of herbal medicines. Medicinal herbs as potential source of therapeutics aids have attained a significant role in health care system all over the world for human beings not only in the diseased condition but also as potential material for maintaining proper health (Atmakuri LR and Dath S, 2010). Canadian Health Care department has analyzed various unapproved Ayurvedic medicinal products that contain high levels of lead, mercury, and arsenic in various Indian formulations [Karela capsules (Himalaya Drug, India), Maha Sudarshan Churna (Zandu Pharmaceuticals, India), Safi liquid (Hamdard, India & Pakistan), Shilajit capsules (Dabur, India)] and some herbal products were found to contain 0.1 to 0.3 mg of betamethasone which produced corticosteroid-like side effects. Reports have been received by drug safety monitoring agencies of prolonged prothrombin times, increased coagulation time, subcutaneous hematomas, and intracranial hemorrhage associated with the use of *Ginkgo biloba* (Wal *et al.,* 2011).

QUALITY CONTROL OF HERBAL DRUGS:

Over the years, and with method of analysis continuously improving, the quality control of plant drugs used in the allopathic system of medicine has become very well established and standards covering authenticity, general quality and purity, and the assay of active constituents may be found in all the principal pharmacopoeias. In the past the

situation regarding the quality of the numerous herbal drugs used by manufacturers or sold directly to the public was by no means so well established.

Now, however, as a result of European legislation which has accompanied the enormous demand for such products, there are standards available for a large number of the more commonly used drug. (Evans, 2002). Quality control for efficacy and safety of herbal products is of paramount importance. Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment (Kokate, 1999).

The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage.

In general, quality control is based on three important pharmacopoeial definitions:

- Identity: Is the herb the one it should be?
- Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?
- Content or assay: Is the content of active constituents within the defined limits?

Identity can be achieved by macro and microscopical examinations. Voucher specimens are reliable reference sources.

Purity is connected to medicine safety and includes parameters like ash values, contaminants (for example, foreign materials in the form of other herbs), and heavy metals. Modern purity evaluation now covers microbiological contaminants, aflatoxins, radioactivity, and pesticide residues, thanks to enhanced analytical technologies. Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Several problems not applicable to synthetic drugs influence the quality of herbal drugs:

- Herbal drugs are usually mixtures of many constituents.

- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- Chemo-varieties and chemo cultivars exist.
- The source and quality of the raw material are variable.

The methods of harvesting, drying, storage, transportation, and processing (e.g., mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect (Mukherjee, 2002).

FUTURE INVESTIGATION OF ETHNO MEDICINAL SYSTEM:

In most nations, tribal healers teach local people how to make herbal medicine to cure cut wounds, skin infections, swelling, ageing, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal illnesses, snakebite, and stomach ulcer (Puspangadan and Atal, 1984). They keep no records and the information is mainly passed on verbally from generation to generation (Dhar *et al*., 1968; Sofowora, 1982).

World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants used by tribals from different parts of the world (Kaido *et al*., 1987). Many developing countries have intensified their efforts in documenting the ethno medical data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. People will be better educated about effective pharmacological therapy and improved health status once these native ethno medicinal formulations are professionally examined and disseminated effectively (Manandhar, 1987).

ANTIOXIDANTS: SOME BASIC CONCEPTS:

As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers (Buyukokuroglu *et al*., 2001). Reactive oxygen species (ROS) are potentially reactive derivatives of oxygen that are continually produced inside the human body. The antioxidants in the body detoxify the ROS that are produced. Overproduction of reactive oxygen species (ROS) and/or insufficient antioxidant defences, on the other hand, may quickly impact and induce oxidative damage to a variety of biomolecules, including proteins, lipids, lipoproteins, and DNA (Farber,

1994). This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process.

Phenolic compounds and flavonoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc. (Miller, 1996). Crude extracts of herbs and spices, as well as other plant materials high in phenolics, are gaining popularity in the food industry because they prevent lipid oxidation and thereby increase the quality and nutritional content of food. Flavonoids, on the other hand, are a class of polyphenolic chemicals with well-known capabilities such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory activities (Frankel, 1995).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidant.

ANTHELMINTIC ASPECT:

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. They are a significant hazard to public health in impoverished nations, contributing to malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of worm infections occur in tropical places, they can also affect travellers who have visited those areas, and some of them can even occur in temperate settings (Bundy, 1994).

Helminthiasis is a condition in which worms such as pinworms, roundworms, and tapeworms infest a region of the body. Worms usually dwell in the intestines, but they can also burrow into the liver and other organs, infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation (Idika *et al.,* 2012). Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis (Lukhoba *et al*., 2006). As per WHO only synthetic drugs are frequently used in the treatment of helminthes infestations in

human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

METHODOLOGIES FOR SCIENTIFIC EVALUATION OF ANTHELMINTIC ACTIVITY:

Many of the in vitro investigations on anthelmintic activity of plants, their oils, or extracts have been based on their toxic effects on the earthworm*, Pheritima posthuma* (Gaind and Budhiraja, 1967; Ali and Mehta, 1970; Kokate and Varma, 1971; Dixit and Varma, 1975; Banerjee and Nigam, 1978; Girgune *et al*., 1978; Agarwal *et al*., 1979; Dengre, 1982). Most of the substances which are toxic to earthworms produce a primary irritation or agitation that results in the withdrawal of the worm from the neighborhood of the poison. By virtue of this effect, anthelmintic doubtless often cause expulsion of the parasite when the concentration does not rise sufficiently high to kill the worm (Sollmann, 1918). Some workers have also used hookworms, tapeworms and/or Ascaris lumbricoides for the evaluation of in vitro anthelmintic activity of different plant materials (Dubey and Gupta, 1968; Dixit and Varma, 1975; Kalesaraj, 1975; Girgune *et al*., 1978). In vivo trials have also been conducted for the evaluation of anthelmintic activity of various substances of plant origin. These included expulsion of worms from their hosts (Lawrence, 1990; Philips, 1990; Pradhan *et al*., 1992) or reduction in the number of eggs per gram of feces (EPG) passed by the infected hosts compared with commercial anthelmintic treated animals (Akhtar, 1988).

ANTI –ARTHRITIC PHYTOPRODUCT FROM TRADITIONAL SOURCES:

Arthritis literally means “inflammation of a joint.” It is accompanied by pain, swelling, and changes in joint structure. The distance between the two bones within the joint becomes narrower and the cartilage that forms a smooth lining at the ends of the bones becomes thinner and irregular. There is a restricted range of motion, with cracking and creaking noises caused by the rubbing of the two irregular surfaces. The affected joints usually feel stiff after periods of immobility such as the morning after a night’s sleep.

The two major types of arthritis are Osteoarthritis and Rheumatoid Arthritis. Rheumatoid Arthritis: more severe with development of deformities and loss of function. It is thought to be an autoimmune disease. Osteoarthritis: more common and results from the effects of wear and tear. It tends to affect joints that have been subjected to overuse, trauma, or excessive weight bearing. Unfortunately, there is no cure for arthritis in conventional medicine. The drugs available do not come without side effects, and once

discontinued, the symptoms recur. Conventional medical doctors mainly prescribe anti-inflammatory drugs to treat the symptoms of arthritis (Yu, Vincent Cunhai, 2006).

STRUCTURE OF A NORMAL JOINT:

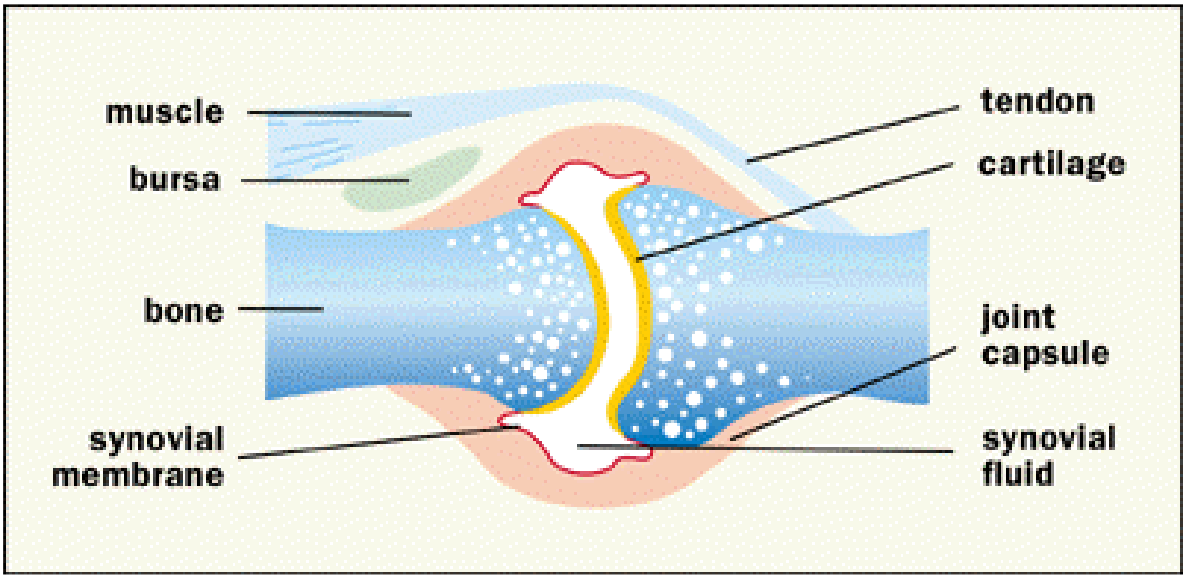

Figure 2: In a normal joint, the muscle and tendon support the bone and aid in movement. The synovial membrane, an inner lining, releases slippery fluid in the joint space between the bones

STRUCTURE OF OSTEOARTHRITIS JOINT:

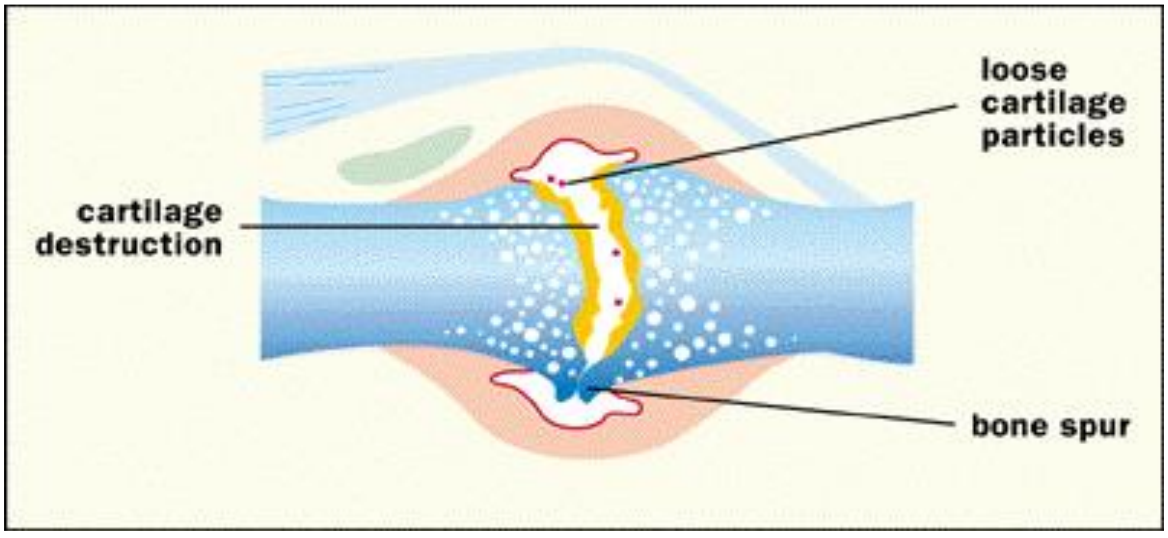

Figure 3: In a joint with osteoarthritis, the cartilage breaks down and the bones rub together

STRUCTURE OF A JOINT WITH RHEUMATOID ARTHRITIS:

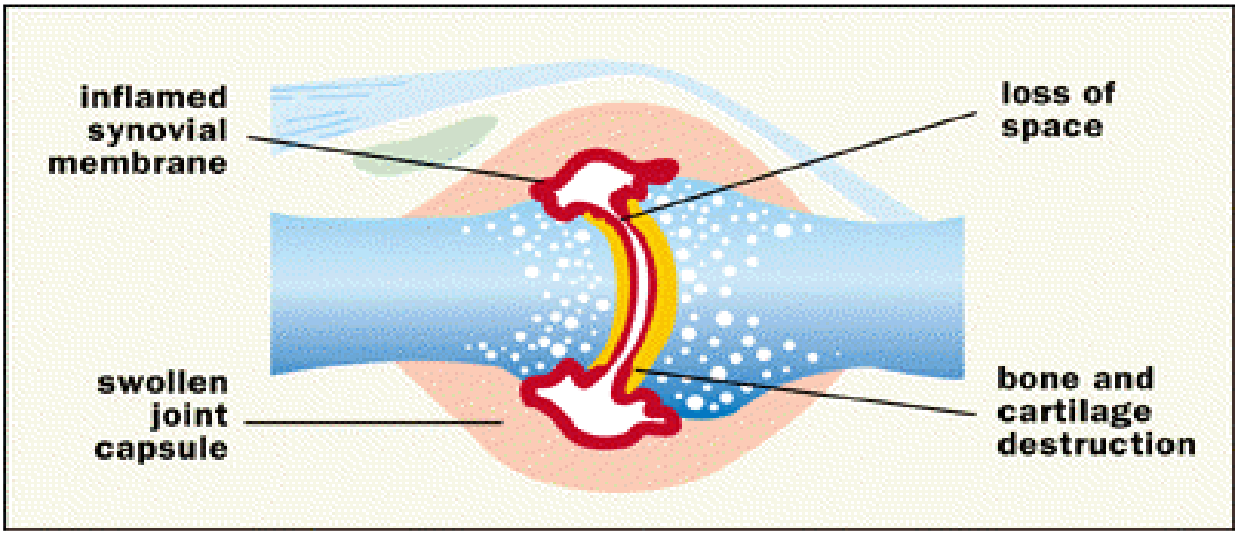

Figure 4: As these all of these various changes occur, the muscles and tendons around the joint also become weak from decreased use and pain, resulting in loss of motion

Rheumatoid arthritis:

Rheumatoid arthritis (RA) is a chronic, relapsing autoimmune disorder that is characterized by pain, synovial membrane inflammation and restricted joint movement due to tissue damages (Salerno *et al*., 2002). In RA, bone deformations and disability of joint function occurred due to progressive erosion of articular cartilage in synovial joint via generation and infiltration of auto antibodies in it, leading to severe pain. Around 1% of the population of the world is suffering from RA (Begum *et al.*, 2004).

Synthetic chemical moieties such as non-steroidal anti-inflammatory drugs (NSAIDs) including ibuprofen, aceclofenac, naproxen, etc. are utilized to minimize the degree of pain. Furthermore in combination with the steroid hormones like cortisone and prednisone are effective for its treatment (Campbell, 1988, Campbell *et al*., 1998). But they fail to produce long-term response treatment for this disease (Vane *et al*., 1994). However long-term treatment with this drug may cause serious side effects, such as gastrointestinal ulcergenicity and renal morbidity (Pincus *et al*., 1992).

There are many treatments like disease modifying antirheumatoid arthritic drugs (DMARDs) including methotrexate, cyclosporin A, leflunomide as well as anti-cytokine therapy such as infliximab, adalimumab, etc. available for RA (Quan *et al.,* 2008) but around 25–30% of patients fail to respond to this treatment (Lawrence and Helmick, 1998).

However, besides their high cost, it also associated with severe well-known side effect including gastrointestinal irritation, cardiovascular complication, hematologic toxicity and nephrotoxicity, which limit their utility in treatment of RA (Payne, 2000; Simon, 2005).

Despite considerable progress in the treatment of arthritis by NSAIDs and other drugs, search for newer drugs continues because the existing synthetic drugs have several limitations. The modern medicine has also started admitting that ayurveda and herbal medicine, has a lot of positive influence on the treatment of arthritis. Many medicinal plants have been examined and proven to possess active principles that have anti-arthritis activities. Antiarthritic plants contain phenols, coumarins, essential oils, monoterpenes, catechins, quinones, carotinoids, flavanoids, alkaloids, anthocyanins, and xanthenes, among other chemical elements. (Shah Biren *et al*., 2006).

ANTI –DIABETIC PHYTOPRODUCT FROM TRADITIONAL SOURCES:

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. Diabetes has been a clinical model for general medicine.

Diabetes mellitus has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. Its pharmacotherapy however is over 80 years old. The word diabetes was coined by the Greek physician Aeretaeus in the first century A.D. In the 17th century, Willis observed that the urine of diabetics as wonderfully sweet as if imbued with honey or sugar. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755 (Satoskar *et al*., 1999).

Plant components used as traditional medicine for the treatment of diabetes are thought to be a good source for a novel drug or a lead in the development of a new therapy. Plant extracts or various folk plant medicines are given by traditional practitioners and accepted by consumers in many nations, particularly in third-world countries, for diabetes and other disorders. More than 400 plants are being utilised in various forms for hypoglycemic effects, and all of the claims made by practitioners or consumers are neither unfounded nor absolute. Therefore, a proper scientific evaluation a screening of plant by pharmacological tests followed by chemical investigations is necessary.

Our Vedic literatures like *Charak Samhita* already report the use of plants, herbs and their derivatives for treatment of diabetes mellitus. More than 400 plants have been incorporated in approximately 700 recipes which are used to treat diabetes mellitus in almost two thirds of the world population. A large number of *in vivo* studies have been conducted on animals to test the claimed activity have demonstrated the hypoglycemic property of many plants, already reported in various literatures (Li *et al*., 2004).

AIM AND OBJECTIVE RELATED TO PROJECT WORK:

India being a tropical country is blessed with best natural resources and ancient knowledge for its judicious utilization. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and understand the pharmacological action.

As it is known that oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The oxygen radicals play an important role in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc. Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Now-a-days there is an increasing interest in natural anti-oxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage.

Medicinal plants are of great importance to the health of individual and communities.

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on human body. The most importance of these chemically active (bioactive) constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal are also used for medicinal purposes. The inflammatory diseases like rheumatic diseases are very common through the world. Rheumatoid arthritis is a systemic disease that affects many joints in the body characterized by the inflammation of the membrane lining the joints which causes pain, stiffness, warmth, redness and swelling. Approximately 1-2 % adult population worldwide suffering from rheumatoid arthritis. Women are more prone to rheumatoid arthritis.

Diabetes mellitus is a multi factorial disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion and impaired insulin action. It is a disease of worldwide significance and increasing their prevalence without any plateau. There are lots of chemical components available to control and treat diabetic patients, but total recovery from diabetes may not possible. However, many medicinal plants have been provided a potential source of antidiabetic principles and are widely used for the treatment of DM in various traditional systems of medicine worldwide and many of them are known to be effective against diabetes.

Most of the plants surrounding us contain various medicinal properties, need to screening of their biological activity which may prove beneficial for human life.

I have carried out my project work on a medicinal plant *Lasia spinosa* Lour*.* The plant was explored scientifically under modern pharmacognostic, phytochemical and pharmacological scheme, which may serve as reference standard for future.

The major objectives of present study are:

- To study the physicochemical, preliminary phytochemical screening test parameters for identification of authentic crude drug.
- To evaluate the *in-vitro* anthelmintic activity of crude drug.
- To evaluate the *in-vitro* antioxidant and *in-vivo* anti-diabetic activities of the extracts of *Lasia spinosa* Lour. and hence development of an effective drug from natural product in future.

ANTI-DIABETIC ACTIVITY OF AQUEOUS LEAF EXTRACTS OF *LASIA SPINOSA* LOUR.

INTRODUCTION

Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc (Ayyanar and Ignacimuthu, 2009). Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, they are easily available and cheaper (Iwu *et al*., 1999). Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries (Ayyanar and Ignacimuthu, 2009).

Diabetes mellitus is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins (Patel *et al*., 2011; Warjeet Singh, 2011). It is becoming the third “killer” of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases (Chauhan *et al*., 2010). The principal diagnostic feature of diabetes mellitus is elevated blood glucose level, which leads to increased formation and accumulation of advanced glycation products (AGEs) and sorbitol concentration, which play an important role in diabetic complications, such as retinopathy, neuropathy, and renal dysfunction (Ding *et al*., 2010).

Knowledge about diabetes mellitus existed in ancient Egypt and Greece. The name "diabetes" comes from the Greek word "Diab" (which means "to pass through" and alludes to the cycle of excessive thirst and frequent urination); "mellitus" is the Latin word for "sweetened with honey" (refers to the presence of sugar in the urine). Madhumeha, according to ancient Hindu physicians, is a sickness in which a patient passes sweet urine and displays sweetness throughout the body, including perspiration, mucous, breath, and blood. There are two types of Diabetes mellitus: Type 1, “Juvenile diabetes mellitus” (Insulin dependent diabetes mellitus), which is hereditary and is treated by insulin, and Type 2, “Adult type” (Non-insulin dependent diabetes mellitus), which occurs in elderly people and is treated by controlling the diet and oral hypoglycemic drugs The drugs which

lower the blood sugar level or treat the symptoms of diabetes mellitus are known as hypoglycemic drugs. It can be categorized into insulin and insulin preparation, which are employed only parenterally, and oral hypoglycemic drug which can be administered orally (Warjeet Singh, 2011).

There are various types of phytoconstituent present in the plant material belonging to different chemical classes. Phytoconstituents like alkaloids inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium. Imidazoline compounds stimulate insulin secretion in a glucose-dependent manner. Polysaccharides increase the level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose. Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets. Dietary fibres efficiently adsorbe glucose, slow glucose diffusion, and block alpha-amylase activity, potentially lowering the rate of glucose absorption and postprandial blood glucose levels. Saponin enhances insulin secretion and inhibits glucose synthesis in the circulation, while ferulic acid stimulates insulin secretion. (Bhushan MS *et al*., 2010).

MATERIAL AND METHODS:

Chemicals:

Streptozotocin (SRL laboratory, Mumbai), Carboxy methyl cellulose, total cholesterol diagnostic reagents, triglyceride reagent and high density lipoprotein cholesterol diagnostic reagent.

Collection of plant material:

The whole plants of *Lasia spinosa* were collected in the month of August-September 2012 from Dibrugarh University campus, Assam and nearby area. The plant was identified and authenticated by Dr. N. Odyuo, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (Specimen no. Du/MTJ/2012/07, Reference no. BSI/ERC/2013/Tech/Plant identification/638) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. The leaves was dried under shade for 15 days coarsely powdered and stored under air tight container for further study.

Preparation of Aqueous extract:

Approx 300 gm of powdered crude drug of *Lasia spinosa* (L.) (Araceae) leaves were extracted by continuous hot percolation method with 900 ml of distilled water for 16 hours after pretreatment with petroleum ether. The solvent was recovered after extraction and the extracts were concentrated by rotary evaporator at low temperature (40-45^{0}C) and pressure.

Animals:

Protocol of the study was passed by Institutional Ethics Committee of Department of Pharmaceutical Sciences, Dibrugarh University (Regd. No. 1576/GO/a/11/CPCSEA Dated: 17/2/2012). The study was carried out with adult male Wistar rats weighing 90-120 g. Animals were acclimatized to experimental conditions in cages and kept under standard environmental conditions (22 ± 3◦C; 12/12 h light/dark cycle). Rats were allowed to feed and water *ad libitum*.

Oral hypoglycemic effect of extracts in normal rats:

The oral glucose tolerance test performed on overnight fasted normal animals. Rats divided into four groups, were administered 0.5% CMC solution, metformin hydrochloride 5mg/kg *L.spinosa* leaf extract (250mg/kg & 500 mg/kg) dissolved in vehicle respectively. Glucose (3g/kg) was feed 30 min. after the administration of samples. Blood was

withdrawn from puncture of tail vein at 0, 30, 60, 90 and 120 mins of sample administration. Blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Chattopadhyay, 1996)

Experimental design of anti-diabetic activity:

Overnight fasted normal animals were randomly divided into five groups of five rats in each group. The group I served as control, which received vehicle i.e. 0.3% CMC solution. Group II diabetic control which received streptozotocin (STZ) (40 mg/kg body weight) where as group III received metformin hydrochloride 5 mg/kg- body weight orally as standard drug. Group IV & V were treated orally with aqueous leaf extract (AELS) of *L.spinosa* 250 mg/kg and 500 mg/kg body weight respectively (Babu *et al.,* 2003).

GROUP-I	Normal control and received vehicle i.e. 0.3% CMC solution orally
GROUP-II	Diabetic control and received 40mg/kg body weight streptozotocin (STZ) intra-peritoneally (ip)
GROUP-III	Received metformin hydrochloride 5mg/kg body weight, orally on 3rd day after induce STZ (i.e. 1st day of treatment)
GROUP-IV	Treated orally with AELS, 250mg/kg body weight
GROUP-V	Treated orally with AELS 500mg/kg/ body weight

Induction of experimental diabetes:

Overnight fasted albino rats were made diabetic by injecting streptozotocin intraperitonially at a dose of 40 mg/kg body weight. Diabetes was confirmed in STZ injected rats by measuring the fasting blood glucose concentration, after 72 hours. Rats with blood glucose level above 180 mg/dl were considered to be diabetic and were used in this study. Blood samples were collected for measurement of blood glucose level on 0, 5, 10 &15 days. The blood glucose levels were measured by one touch glucometer. The rats were fasted overnight and blood was withdrawn by rupturing the tail vein.

In single-dose, short term study:

Fasting Blood Glucose was estimated from the tail vein prior and 1, 3 and 6 hr after administration of test drugs and vehicle.

In multi dose long term study:

The same animals were continued with the same dose of vehicle, AELS and metformin hydrochloride once daily for 15 days.

Fasting Blood Glucose and measured 24 hr after the previous dose.

Biochemical Estimation:

Serum Glutamate Oxaloacetate Transaminase (SGOT) (Alberti, 1982):

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidney. Injury to this tissues result in the release of the enzyme in blood. Elevated levels are found in myocardial infarction, cardiac operations, hepatitis etc. decreased levels may found in diabetic ketoacidosis.

Principle:

SGOT catalyses the transfer of amino group between L aspartate and alpha keto glutarate to form oxaloacetate and glutamate. The formed oxaloacetate reacts with NADH in the presence of malate dehydrogenease to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT activity in the sample.

Reaction:

$$\text{L- aspartate + } \alpha\text{ keto glutarate} \xrightarrow{\text{aminotransferase}} \text{oxaloacetate + L- Glutarate}$$

$$\text{Oxaloacetate + NADH + } H^{+} \xrightarrow{\text{Malate dehydrogenase}} \text{L- malate + } NAD^{+}$$

Requirements:

SGOT Enzyme Reagent

SGOT substrate Reagent

Procedure:

The required reagent and samples were taken in test tubes as shown in the following table:

Reagents	Test
Working Reagent	1000 µl
Sample	1000µl

The mixture were shaken well and read the initial absorbance (A) after 1 min and repeat the absorbance reading after every 1, & 2 minutes. Calculate the mean absorbance change per minute (δA/min)

Calculation:

SGOT activity (U/L) = δA/min × 1746.

9.2.8.2 Serum Glutamate Pyruvate Transaminase (SGPT) (Cox and Nelson, 2004)

SGPT is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

Principle

SGPT catalyses the transfer of amino group between L-alanine and alpha keto glutarate to form pyruvate and glutamate. The formed pyruvate reacts with NADH in the presence of lactate dehydrogenease to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT activity in the sample.

Reaction

$$\text{L- alanine} + \alpha\text{ keto glutarate} \xrightarrow{\text{aminotransferase}} \text{pyruvate} + \text{L- Glutarmate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{Lactate dehydrogenase}} \text{L- lactate} + NAD^{+}$$

Requirements:

SGPT Enzyme Reagent

SGPT substrate Reagent

Procedure:

The required reagent and samples were taken in test tubes as shown in the following table-

Reagents	Test
Working Reagent	1000 µl
Sample	1000µl

The mixture was shaken well and read the initial absorbance (A) after 1 min and repeat the absorbance reading after every 1, & 2 minutes. Calculate the mean absorbance change per minute (δA/min)

Calculation:

SGPT activity (U/L) = δA/min × 1746.

Alkaline Phosphatase (Reitman and Frankel, 1957; Bergmeyer and Brent, 1974)

ALP is an enzyme of the hydrolase class of enzymes and act in an alkaline medium. It is found in high concentrations in the liver, bilary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease. Moderate hydrolases are seen in Hodgkin's disease and congestive heart failure.

Principle

ALP in serum catalyse the hydrolysis of para nitrophenyl phosphate to para nitrophenol is measured as an increase in the absorbance which is proportional to the ALP activity in the sample.

Reaction

$$\text{P-nitrophenylphosphatase} \xrightarrow{\text{ALP}} \text{p-nitrophenol + phosphate}$$

Requirements

ALP Enzyme Reagent & ALP substrate Reagent.

Procedure:

The required reagent and samples were taken in test tubes as shown in the following table:

Reagents	Test
Working Reagent	1000 µl
Sample	20µl

The mixture was shaken well and read the initial absorbance A after 1 min and repeat the absorbance reading after every 1, & 2 minutes. Calculate the mean absorbance change per minute (δA/min)

Calculation:

ALP activity (U/L) = δA/min × 2764.

Total cholesterol estimation (Allain *et al.,* 1974; JAMA, 2001):

The serum cholesterol level was estimated by wybenga and pileggi method using cholesterol diagnostic reagent kit (Span Diagnostics, Surat, India)

Principle:

Cholesterol is determination of the colored complex which is measured at 505 nm. According to the following reaction.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

(4-AAP= 4-Aminoantipyrine)

Reagents:

Reagent-1: Cholesterol reagent

Reagent-2: Working cholesterol standard, 200 mg%

Procedure:

	Blank	**Standard**	**Test**
Cholesterol reagent	1000 µl	1000 µl	1000 µl
Working cholesterol standard 200 mg%	-	10 µl	-
Serum	-	-	10 µl

Reagents were mixed and incubate for 5 min. at 37^0.C. Mixer was allowed to cool to room temperature under running tap water. Absorbance measured at 505 nm.

Calculation:

The Total cholesterol (mg/dl) was calculated using the following formula-

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

Triglycerides estimation (Fossati and Prencipe, 1982; Vassault *et al.,* 1986)

The triglycerides level estimated by Glycerol phosphate oxidase (GPO) method.

Principle

Triglycerides in samples were hydrolysed by microbial lipases to glycerol and free fatty acids (FFA). Glycerol is phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G-3-P) in reaction catalyzed by glycerol kinase (GK).G-3-P is oxidized to dihydroxyacetone phosphate (DAP) in reaction catalysed by the enzyme glycerol phosphate oxidase (GPO). In this reaction hydrogen peroxide (H_2O_2) is produced in equimolar conc. to the level of triglyceride conc. in the sample. H_2O_2 reacts with 4-aminoantipyrine (4- AAP) and 3,5 di-choloro 2 hydroxy benzo sulfonic acid (DHBS) in a

reaction catalysed by peroxidase (HPOD). The result of this oxidative coupling is a quinoeimine red colour dye.

Requirements:

Enzyme Reagent	3 ml
Buffer	30 ml
Triglyceride Standard	3 ml

Procedure

The required reagent and samples were taken in test tubes as shown in the following table

Reagents	**Test**	**Standard**	**Blank**
Working Reagent	1000 µl	1000 µl	1000 µl
Sample	10µl	----	----
Standard	----	10µl	----

The mixture were shaken and Incubated at 37 ^{0}C for 10 min. The absorbance was read against reagent blank on a spectrophotometer at 505 nm against reagent blank. The final colour stable for 30 min.

Calculation:

The Triglyceride conc. (mg / dl) was determined using the following formula-

$$\text{Triglyceride conc. (mg / dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of Std.}} \times 200$$

We can also measure the change of optical density directly from Bio Chemical analyzer at 505 nm.

HDL_C (High-density lipoprotein cholesterol (Young, 1995, 2001)

Principle

HDL_C levels are determine directly serum without the need for any pre-treatment of centrifugation of the sample. The assay takes place in two steps.

1. Elimination of lipoprotein no-HDL

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ 4-Cholestenone +H_2O_2

$2H_2O_2$ $\xrightarrow{Catalase}$ $4H_2O + O_2$

2. Measurement of HDL_C

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol + Fattay acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ 4-Cholestenone +H_2O_2

$2H_2O_2$+ HDAOS+ 4-AA $\xrightarrow{POD}$ Quinoneimine +4 H_2O

Reagents:

Reagent-1: HDL_C reagent

Reagent-2: HDL_C reagent

Reagent-3: Working HDL_C standard

Procedure:

The required reagent and samples were taken in test tubes as shown in the following table:

	Blank	**Standard**	**Sample**
Reagents-1	1000 µl	1000 µl	1000 µl
Reagents-2	300 µl	300 µl	300µl
HDL_C standard	--	10µl	--
Sample	--	--	10µl

The mixture was shaken and incubated for 5min at 37 ^{0}C. The absorbance (A_1) was read of the sample and calibratior, against the blank. Add Reagents-2

The mixture was shaken and incubated for 5min.at 37 ^{0}C.The absorbance (A_2) was read of the sample and calibrator against the blank. Calculate the increase of the absorbance.

Calculation:

The HDL_C Cholesterol (mg / dl) was determined using the following formula

$$HDL_C \text{ Cholesterol (mg / dl)} = \frac{\text{Sample (A)}}{\text{Standard (A)}} \times \text{Calibrator conc.}$$

Conversion factor mg / dl × 0.0259 = mmol/L.

Low density Lipoprotein & Very low density Lipoprotein (American Diabetes Association, 1997; Satyanaraya and Chakrapani, 2008; Patel *et al*., 2009):

Lipoproteins are molecular complexes that consist of lipids and proteins. They functions as transporter vehicles for lipids in blood plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization. Low density lipoproteins are formed from VLDL in the blood circulation. They transport cholesterol from liver to other tissues. VLDL is produced in liver and intestine and are responsible for the transport of endogenously synthesized triacyglycerols.

Serum samples were analyzed in spectrophotometrically for total cholesterol & HDL cholesterol. Low density lipoprotein (LDL) and very low density lipoproteins (VLDL) were calculated as per Friedvald's equation.

LDL = Total Cholesterol – (Total Triglyceride ÷ 5) - HDL

VLDL = (Total serum Triglyceride ÷ 5)

RESULT AND DISCUSSION:

Oral Glucose Tolerance test (OGTT):

The results of oral glucose tolerance test is shown in table 9.1

Table 1: Results of Oral Glucose Tolerance test (OGTT)

Animal Group	0 min	30 min	60 min	90 min	120 min
Normal Control	88.16±1.22	140.50±4.32	152.16±2.12	167.00±1.62	186.51±1.2
AELS (250 mg/kg)	77.32±3.14	127.10±2.38	144.32±2.33	116.12±3.84	98.22±2.74
AELS (500 mg/kg)	79.12±2.43	115.24±3.38	139.50±5.83	112.23±2.95	84.00±1.22
Standard drug (Metformin hydrochloride)	80.15±1.51	128.15±3.21	118.20±1.86	101.32±1.62	86.21±1.53

The Blood glucose levels of control group reach a peak decreased at 30 min. and continuously decrease to attain basal glucose level. Both extracts of plant (250mg /Kg & 500 mg/Kg) decreased blood glucose level gradually at 90 and 120 min. The glucose level tends to decreased after 90 minutes both extract fractions and standard drug dose. Administration of metformin hydrochloride cause fall of glucose level continuously except at starting dose.

Blood glucose estimation:

The results of oral glucose tolerance test is shown in table 2 and figure 5

Table 2: Results of Blood glucose level of the experimental animal groups

Treatment	0 day	5th day	10th day	15th day
G-I- Normal control	92.52 ± 2.89	103.36±3.32	101.12± 4.23	98.15± 2.25
G-II Diabetic control	245.23 ±4.45	262.00±4.35	265.13± 8.16	271.23± 4.61
GIII Standard Drug	240.54 ±4.12	160.45± 3.38	135.13± 5.28	116.12± 6.77
G-IVAELS (250mg/kg)	236.04 ±3.52	174.14± 6.62	167.32± 3.25	124.33±2.56
G-V AELS (500mg/kg)	235.12± 4.50	153.20 ± 6.74	124.25 ± 2.61	108.26 ± 2.14

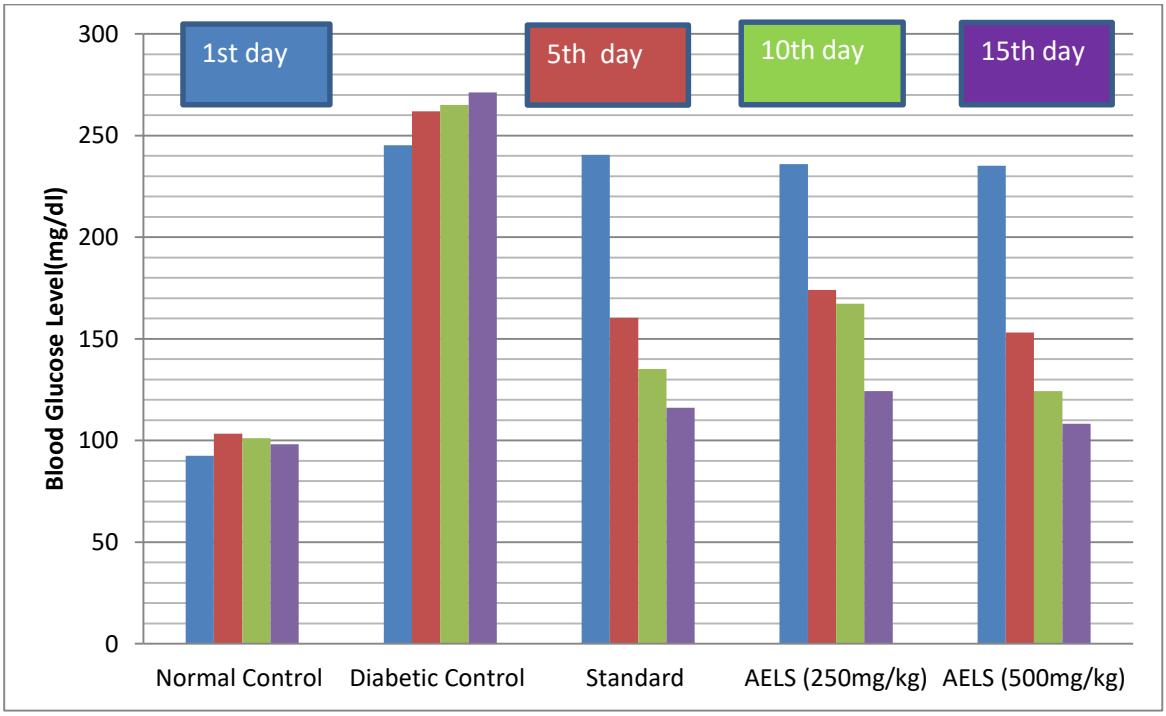

Figure 5: Graphical presentation of blood glucose level of the experimental rats

STZ-induced diabetes due to the selective destroying of pancreatic β-cells. However the animal survived without insulin treatment and shows improvement by metformin hydrochloride drug which acts stimulating β-cells of pancreas, indicates incomplete destruction of pancreatic β-cells of diabetic animals. The drugs also improved the B cells for the production of insulin. In present study increased glucose level and rats' body weight loss confirmed the induction of hyperglycemia by streptozotocin (STZ). Oral supplements

of aqueous extracts of both fractions of the plants under study caused a rapid decrease in hyperglycemic peak after glucose loading in rats.

Animals of Group II, blood glucose level increased gradually throughout the 15 days from 245.23 to 271.23 mg/dl. The administration of AELS 250mg/Kg failed blood glucose level 236.04 to 124.33 mg/dl, but the long dose (500 mg/Kg) decreased it 235.12 to 108.26 during this time. Whereas the animals from standard drug treated (Group III), shows the dramatic fall of blood sugar level, which was 240.54 on 0 day & reached to 116.12 mg/dl on 15th day.

The extract fractions have the activity to reduce the elevated blood sugar and the high dose has the better activity with compared to low dose of AELS.

Determination of SGOT, SGPT and ALP (Blood parameters)

The results of SGOT, SGPT and ALP are shown in table 3 and figure 6

Table 3: Effects of AELS (Aqueous extract of *Lasia spinosa*) on control and experimental rats

Groups	SGOT	SGPT	ALP
G-I- Normal control	75.46±5.9	43.21±4.4	105±2.4
G-II Diabetic control	120.79±6.2	84.23±3.3	175±3.6
GIII Standard Drug	82.38±4.6	49.33±2.8	122±2.8
G-IV HLE (250mg/kg)	98.20±3.8	60.13±5.1	138±4.2
G-V HLE (500mg/kg)	82.42±4.3	54.32±3.4	127±5.1

Liver enzymes are liberated into blood whenever liver cells are damaged and enzyme activity in the plasma is increased. The leaf extracts of *L. spinosa* at doses of 250 and 500 mg/kg body weight showed varied effect on biochemical parameters.

Increased serum concentration of qualitative diagnostic enzymes such as SGPT, SGOT and ALP were observed in diabetic rats indicating an altered liver function and/ or liver mitochondrial injury in comparison to normal control rats. Insulin deficiency contributes to increased serum level of transaminase enzymes due to easily availability of

amino acids which leads to enhanced occurrence of gluconeogensis and ketogenesis processes during diabetes.

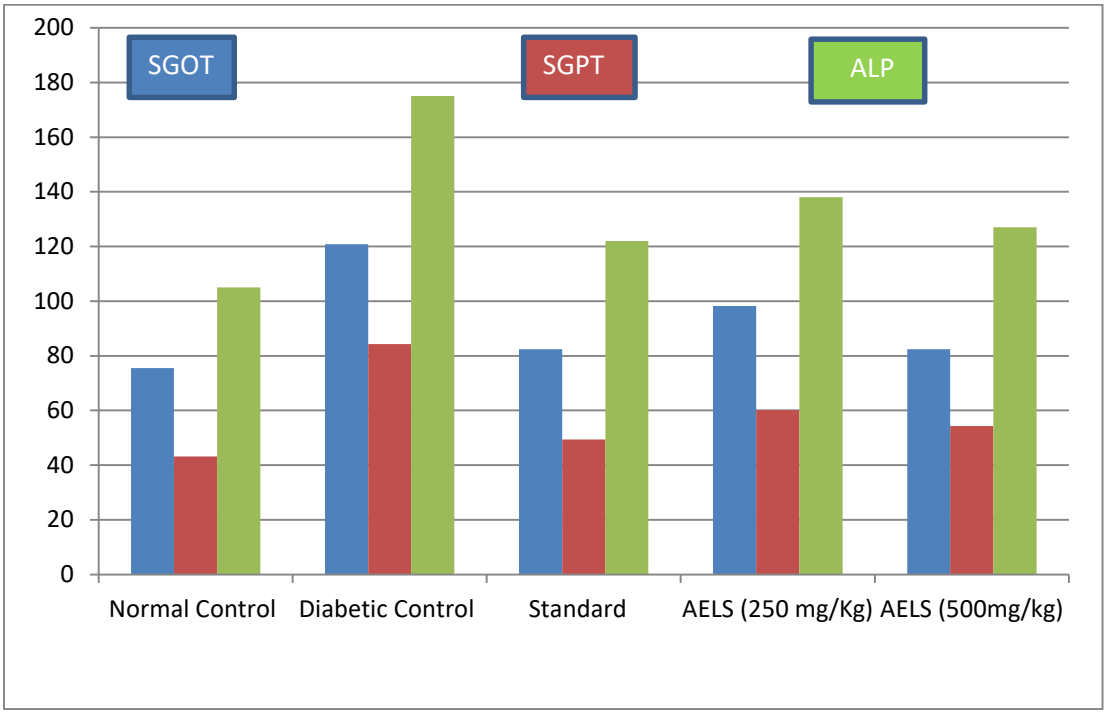

Figure 6: Graphical presentation of Effects of AELS on blood parameters of control and experimental rats

On treatment with AELS significantly reversed the elevated marker enzymes i.e. SGOT, SGPT, ALP and restored to normal values indicates a revival of insulin secretion into circulations and its hepatoprotective effect.

Lipid profile determination:

The results are summarized in table 4

The lipid profile of STZ-induced rats deals with significant disturbance of metabolic pathways. The higher concentration of serum triglycerides may be attributed to inhibition of cholesterol catabolism or may be insulin deficiency and mobilizations of fatty acids from adipose tissues by lipolysis and increase in serum triglyceride level due to increase biosynthesis or dismissed clearance from blood, elevation of serum triglyceride are also associated with an increased risk of pancreatitis.

On treatment with AELS decreased triglyceride & cholesterol level in respect to diabetic control group. While the HDL-cholesterol increased slightly in case of low dose

fraction but high dose effect shows reverse. LDL cholesterol decreased for AELS 250 mg/Kg dose treated.

Table 4: Lipid profile of the experimental animal groups

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
G-I- Normal control	172.22 ±6.80	194.32 ± 7.80	52 ± 2.30	81.35 ± 3.20
G-II Diabetic control	240.15 ±6.23	305.12 ± 2.29	35 ± 2.12	105.26 ± 6.53
GIII Standard Drug	152.28 ± 5.26	220.02 ± 3.83	46 ± 1.43	62.27 ± 2.78
G-IV AELS (250mg/kg)	198.34 ± 4.38	255.33 ± 4.23	38 ± 1.50	109.27 ± 4.28
G-V AELS (500mg/kg)	174.25 ± 2.29	232.23 ± 9.25	32 ± 1.72	95.78 ± 5.32

CONCLUSION:

The leaves extracts (aqueous extracts) of *Lasia spinosa.* Lour have the activity to reduce the blood glucose level in disease condition. The parameters of lipid profile (Total cholesterol, triglyceride. High density & low density lipoprotein cholesterol) also corrected by the treatment of aqueous extracts. The bio-activity of the isolated compound evaluated with *in-vitro* and *in-vivo* model may be emphasized the knowledge of proper investigation of the plant extracts and structural elucidation of the bio-marker may signify the new drug development of this natural biodiversity fields for treatment of diabetes.

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