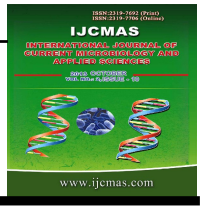
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 537-559**
http://www.ijcmas.com



Original Research Article

AntiHyperglycemic effect of *Spirulina*, Insulin and *Morinda citrifolia* against Streptozotocin induced diabetic rats

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A B S T R A C T

The result of the present study showed that, spirulina and insulin brings back the blood glucose to normal in diabetes-induced rats. After treatment, in the pancreas no **i**nsulitis was observed. From the above results it is shown that the treatment materials used has hypoglycemic activity. Hypoglycemic action of the herbal plant (*Morinda citrifolia)* in diabetic rats may be because of thiobarbituric cid reactive substance (TBARS) and hydroperoxides, which have antioxidant potential, which in turn may be responsible for the hypoglycemic potential. Hypoglycemic action of spirulina is due to the decrease in the activity of hexokinase in liver and increase in glucose 6 phosphatase activity in muscles and the hypoglycemic activity of insulin may be due to the decrease in intestinal glucose absorption, decrease of hepatic glucose production and increase of peripheral glucose disposal. So the experimental evidence obtained from this study indicates that, spirulina and insulin possess hypoglycemic property but has got more hypoglycemic effect than spirulina and insulin, which is standard drug, used for diabetes treatment. The hypoglycemic activity is also confirmed by histopathological examination of pancreas.

Keywords

Morinda citrifolia; thiobarbituric cid reactive substance; hypoglycemic effect

Introduction

Diabetes mellitus is a clinical disorder in which blood sugar levels are abnormally high known as Hyperglycemia. It occurs due to absence or insufficient production of insulin. The term *diabetes* (Greek: *diabetes*) was coined by Aretaeus of Cappadocia. The ancient Indians tested for diabetes by observing whether ants were attracted to a person's urine, and called the ailment "sweet urine disease" (Madhumeha). In 1936 Houssay demonstrated for the first time that, besides insulin, other hormones participate in the pathogenesis of diabetes mellitus namely, the hormone of thyroid gland, ovaries, the anterior pituitary. The

discovery of excess sugar in the blood in diabetes mellitus belongs to Ambrosiani (Kong ,1998).

Epidemiology

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. The Centers for Disease Control has termed the change an epidemic. Diabetes mellitus prevalence increases with age, and the numbers of older persons with diabetes are expected to grow as the elderly population increases in number (Harris *et al.,* 1998).

Types of diabetes mellitus

Type I diabetes mellitus
Type II diabetes mellitus
Gestational diabetes mellitus

Type I diabetes

Type1 diabetes, previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, accounts for approximately 5-10% of all diagnosed cases of diabetes (Cooke DW and Plotnick L ,2008).

Risk Factors for Type I diabetes

Known risk factors for type1 diabetes include:

Family history

Anyone with a parent or sibling with type I diabetes has a slightly increased risk

Genetics

Approximately 20 different genes have been identified which influence risk of developing type I diabetes. The most intensively studied are several genes located within the human leukocyte antigen (HLA) region of chromosome 6.

Weight and growth

Increased height, weight and BMI correlate with childhood onset diabetes.

Lifestyle

Habits related to improved welfare have been correlated with the incidence of childhood diabetes.

Geography

The greatest incidence of type I diabetes is observed in Southeast Asia where 24% of the estimated 480,000 children suffering from type I diabetes are located. European incidence is similar, reporting 23% of global type I pediatric cases.

Type II diabetes

Type II diabetes, previously called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, accounts for approximately 85-95% of all diagnosed cases of diabetes. Once thought to be an adult disease, type II diabetes is increasingly being diagnosed in children and adolescents and is rapidly becoming a global public health issue (Arlan Rosenbloom and Janet H Silverstein , 2003).

Risk Factors for Type II diabetesWeight

Increased weight contributes to the risk of diabetes. In particular, the increasing

prevalence of childhood obesity has led to a rise in the incidence of type II diabetes among children.

Sedentary lifestyle

Inactivity and lack of exercise.

Family history

Although no major susceptibility genes have been identified, around 20 genetic variants are reportedly associated with type- II diabetes.

Race

Blacks, Hispanics, American Indians, and Asian Americans are more likely to develop type II diabetes.

Age

The risk of developing type II diabetes increases with age, especially after 45 years of age, but incidence is increasing dramatically among children, adolescents, and younger adults.

Gestational diabetes

Gestational diabetes mellitus (GDM) is a glucose intolerance, which is first diagnosed during pregnancy. It appears with varying degrees of severity and can affect both the health of the mother and her unborn child(Lawrence J M *et al.*,2005).

Risk Factors for Gestational diabetes

Family history of diabetes among first-degree relatives

Race

African-Americans, American Indians, Asian Americans, Hispanic/Latino, or Pacific Islanders are more likely to develop gestational diabetes.

Other diabetes TypesDiabetes resulting from other etiologies, including specific genetic conditions, surgery, drugs, malnutrition, infections, and other illnesses may account for 1-5% of all diagnosed cases of diabetes (Arlan Rosenbloom and Janet H Silverstein, 2003).

Symptoms of diabetes mellitus

Polyuria, Polydypsia, Polyphagia and Artherosclerosis

Complication of diabetes mellitus

Diabetic angiopathy

Diabetes accelerates hardening of the arteries (atherosclerosis) of the larger blood vessels, leading to coronary heart disease (angina or heart attack), strokes, and pain in the lower extremities because of lack of blood supply (Tomkinson and Jameson,1993).

Diabetic retinopathy

Diseased small blood vessels in the back of the eye cause the leakage of protein and blood in the retina. . Spontaneous bleeding from the new and brittle blood vessels can lead to retinal scarring and retinal detachment, thus impairing vision (Tomkinson AJ and Jameson,1993).

Diabetic nephropathy

Diseased small blood vessels in the kidneys cause the leakage of protein in the urine. Later on, the kidneys lose their ability to cleanse and filter blood (Tomkinson AJ and Jameson,1993).

Diabetic neuropathy

The blood flow to the nerves is limited, leaving the nerves without blood flow, and they get damaged or die as a result. Symptoms of diabetic nerve damage include numbness, burning, and aching of the feet and lower extremities (Tomkinson and Jameson,1993).

Insulin

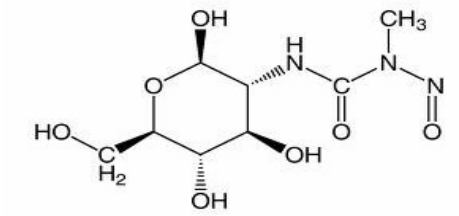
Insulin is secreted by groups of cells within the pancreas called islet cells. Insulin is a rather small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains held together by disulfide bonds. Insulin is a rather small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains held together by disulfide bonds**.**

Most cells of the body have insulin receptors, which bind the insulin, which is in the circulation. When a cell has insulin attached to its surface, the cell activates other receptors designed to absorb glucose (sugar) from the blood stream into the inside of the cell. Those who develop a deficiency of insulin must have it replaced via shots or pumps (type I diabetes). More commonly, people will develop insulin resistance (type II diabetes) rather than a true deficiency of insulin. In this case, the levels of insulin in the blood are similar or even a little higher than in people without diabetes. Many cells of people with type II diabetes respond sluggishly to the insulin they make and therefore their cells cannot absorb the sugar molecules well. This leads to blood sugar levels, which run higher than normal.(Harry *et al.,* 1997).

Streptozotocin

Streptozotocin is one of the anticancer called alkylation agents. It is available in the U.S. under the brand name Zanosar. It suppresses the immune system (by damaging white blood cells) and interferes with the normal functioning of certain organs and tissues.

Structure



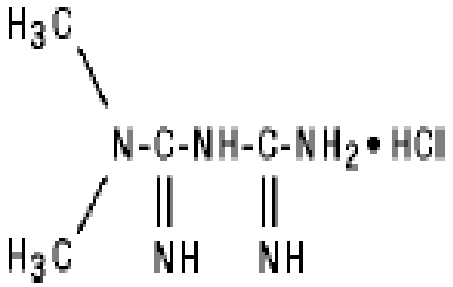
Structure of streptozotocin by Netter (1997)

Mechanism of streptozotocin

Streptozotocin (STZ) experimentally produces diabetes due to beta –cell death by the mechanism of DNA damage in rat's islets. Streptozotocin enters the B cell via glucose transporter (GLUT 2) and causes alkylation's of DNA. DNA damage induces activation of poly ADP – ribosylation, a process that is more important for the diaetogenicity of streptozotocin than DNA damage itself. Poly ADP – ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconites activity and participates in DNA damage. As a result the streptozotocin action, B cells undergo the destruction by necrosis (Netter, 1997).

Insulin

Structure of insulin



Structure of insulin by Webb RG (1999)

Insulin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5$• HCl and a molecular weight of 165.63. Insulin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of insulin is 12.4. The pH of a 1% aqueous solution of insulin hydrochloride is 6.68. Insulin activates AMPK in liver and muscle to improve glucose and lipid metabolism in type 2 diabetes (C.B Freed *et al.*, 2000).

Spirulina

Spirulina is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. Spirulina is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Arthrospira platensis*, and *Arthrospira maxima.* Spirulina occurs naturally in tropical and subtropical lakes with high pH and high concentrations.

It helps in strengthening the immune system, making the body produce more red and white cells as well as the cells, which kills viruses and germs, increasing antioxidant protection, since this super food helps fight free radicals responsible for body aging process. Spirulina promotes body's natural cleansing and detoxification (A. Layam and C. L. K. Reddy, 2000)

Morinda citrifolia

Noni plant belongs to Rubiaceae family.Its botanical name is Noni *Morinda citrifolia*.It is also known as Indian mulberry, Cheese fruit, and beach mulberry. Its natural habitat are volcanic terrains, lava – strewn coast. Noni fruit contains vit C, vit A, niacin, and Vit B_3 ,alanine, glutamic acid, phenylalanine, leucine, isoleucine. It also contains phytochemicals such as lignans, flavonoid**,** iridoids, catechin, terpenoids, alkaloids, arthraquinones are the phytochemicals present (Allen WH and London ,1873).

Free radicals and antioxidants

Antioxidants are substances or nutrients in our foods, which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products), which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, macular degeneration, diabetes, cancer etc are all contributed by oxidative damage. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells (Del Mastero ,1980)

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extra cellular fluids (Clarkson P M ,1995)

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, gluthathione peroxidase and glutaredoxins, as well as reacting directly with oxidants (Clarkson,1995). Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Clarkson P M ,1995).

Aim and objectives

The aim and objective of the present study was to evaluate the hypoglycemic activity of spirulina, noni and insulin extract on STZ induced diabetes mellitus rats, along with antioxidant property of noni and spirulina in STZ induced rats. Both aqueous and methanolic extract of noni, spirulina and aqueous extract of insulin was used *in vivo* test of hypoglycemic and antioxidant property. Histopathological study of pancreatic cells was done.

Materials and Methods

Animals

Adult male albino rat of Wistar strain weighing around 100 to 150gms were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai. Animals are divided into 7 groups, each group containing 6 animals. The animals were kept in polypropylene cages (six in each cage) at an ambient temperature of 25±20°C and 55-65% relative humidity. A 12±1hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

Animal handling

The rats were kept in well-ventilated house conditions (temperature: 28-31 degree C; photoperiod; 12hr natural light and 12hr dark; humidity; 50-55%). The animals were housed in a barbered building and identified by an individual unique tattoo on the head. The 42 animals used for this study were randomly divided into six experimental groups (A, B, C, D, E, F& G) .The body weights of the rat were approximately 100 to 150 gms on the day the experiment began. The rats were kept in individual cages .The cages; cage trays, food hoppers and water bottles were sanitized at regular intervals. All animals were given free access to both food and tap water. There was no indication that any (non-nutrient) substance was present in the diet or in the drinking water that influenced the effects of the test compound.

Chemical

Streptozotocin **(**STZ) was purchased from Sigma Aldrich – Mumbai. About 0.2g of STZ was dissolved in 1ml of water injected to the rats intraperitonially.

Plant material

Morinda citrifolia fruit powder was purchased from Health Indian Laboratory, Chennai.

Spirulina

Spirulina was purchased from Parry's Chennai.

Drug

Insuline was purchased from Apollo medicals, Arcot. About 0.2g of insuline was dissolved in 1ml of water and orally fed to the animal.

Preparation of noni extract

Aqueous extract

0.2g of noni powder was dissolved in 1ml of water and orally fed to the animals.

Methanolic extract

Noni powder was dissolved in methanol and subjected to Soxhelet method and the liquid so got was evaporated to remove alcoholic portion. Finally, the powered material (0.2g) so got was dissolved in 1ml of water and orally fed to the animals.

Preparation of spirulina extract

Aqueous extract

0.2g of Spirulina powder was dissolved in 1ml of distilled water and orally fed to the animals.

Methanolic extract

Spirulina powder was dissolved in methanol and subjected to Soxhlet method and the liquid to got was evaporated to remove alcoholic portion. Finally the powdered material (0.2g) so got was dissolved in 1ml of water and orally fed to the animals.

Parameters

The diagnosis was based on blood sugar, insulin level, antioxidant and histopathological study.

Experimental design

42 rats were classified into seven groups (6 animals/ group) and subjected to treatments as follows:

Group I: Received 1ml distilled water /kg of body weight per day by oral gavages for 45 days and served as a control group.

Group II: Injected intraperitonially with STZ in a dose of 0.2g/kg body weight for 45 days and served as untreated diabetic group (UDG).

Group Ш: Received noni aqueous extract alone, orally in a daily dose 0.2g/kg of body weight for 1 week before induction of diabetes, 3 days during the induction of diabetes and continued for 5 weeks and served as noni-treated group.

Group IV: Received noni methanol extract alone, orally in a daily dose 0.2g/kg of body weight for 1 week before induction of diabetes, 3 days during the induction of diabetes and continued for 5 weeks and served as noni-treated group.

Group V: Received spirulina aqueous extract orally in a dose of 0.2g/kg of body weight for 1week before induction of diabetes, 3days during induction of diabetes and continued for 5 weeks and served as spirullina treated diabetic group.

Group IV: Received spirulina methanol extract orally in a dose of 0.2g/kg of body weight for 1week before induction of diabetes, 3days during induction of

diabetes and continued for 5 weeks and served as spirullina treated diabetic group.
Group VII: Injected with aqueous extract of insulin, in a dose of 0.2g/kg of body weight for 1 week before induction of diabetes, 3 days during induction of diabetes and continued for 5 weeks and served as insulin treated diabetic group.
Sample collection

The venous blood samples were drawn for all the rats. Blood samples from fasting subjects were collected before and after treatment. The serum was separated and the biochemical parameters where estimated. Even pancreas was excised from the animals and subjected to histopathological and antioxidants analysis.

Test for glucose (Orthotoluidine method)

All the reagents were brought to the room temperature before using the test. Undiluted serum sample was used in this method. 3 sets of test tubes marked as blank (B), Standard (S) and test (T) were taken. 0.1ml of serum sample was taken in the ' T ' then 0.1ml of glucose was added in standard (S) tube, followed by 5.0 ml reagent was added in the test tubes. Then the tubes were mixed well and kept in boiling water bath for 10 minutes. The intensity of the colour developed was read at 620-650nm.

Calculation

Reading of unknown (T) X Concentration of standard X 1OO

Reading of known (S) X Volume of test (T)

Affix the antibody-coated microplate module on the frame. Dispense 95µl sample diluent per well. Pipette 5µl of the sample (or working rat insulin standard******) to the well. Incubate the micro plate for 2 hours at 4°C. Wash the well five times with washing buffer. Dispense 100µl anti-insulin enzyme conjugate per well. Incubate micro plate for 30 minutes at room temperature. Wash each well seven times with washing buffer. Dispense 100µl enzyme substrate solution per well. Incubate micro plate at room temperature while avoiding exposure to light. Stop the reaction by adding 100µl enzyme reaction stop solution per well. Measure the absorbance at A_{450nm} and subtract A_{630nm} values within 30 minutes. Calculate the rat insulin concentration using the standard curve. Each well should be washed with 300µl of wash buffer. Aspirate the wells completely so all-excess solution is removed. Preparation of standards is different for both low and high range assay.

Antioxidant analysis

Experimental Procedure

For antioxidant activity rats were randomly divided into five groups of six animals each. Animals in first group served as control and received vehicle, the second group received Vitamin C at the dose of 3mg/kg body weight and used as standard. Groups III, IV, V and VI received A and B (noni & spirulina) at the doses of 0.5, 1.0, 1.5 and 2.0 ml/100g for 20 days. All the treatments were administered orally.

Determination of lipid peroxidation

Lipid per oxidation was measured by the thiobarbituric acid (TBA) reaction method

of Breton. In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in0.25N hydrochloric acid. The reaction mixtures were placed in boiling water bath for 30 min and centrifuged at 1811g for 15 min. The absorbance of the supernatant was measured at 535 nm. MDA, a measure of lipid peroxidation, was calculated using an extinction coefficient of $1.56x10^5$ / Mcm. The results were expressed as nM/mg protein.

Determination of Catalase activity

Catalase activity was measured according to the method of Aebi. One unit of catalase was defined as a amount of enzymes required to decompose 1mM of H_2O_2.The reaction was initiated by the addition of 1.0 ml of freshly prepared 20nM H_2O_2. The rate of decomposition of H2O2 was measured spectrophotometrically at 240 nm for 1 min. The enzyme activity was expressed as U/mg protein.

Determination of Super oxide dismutase activity

Estimation of SOD was done by autoxidation of hydroxylamine at pH 10.2, which was accompanied by reduction of NBT, and the nitrite produced in the presence of EDTA was detected calorimetrically. One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 10% liver homogenate required to inhibit the reduction of 24mM NBT by 50% and is expressed as units per milligram of protein.

Determination of Glutathione activity

Glutathione was estimated using Ellman's reagent (5, 5¢- dithiobis- (2-nitrobenzoic acid) [DTNB]). The sulphydryl groups present in glutathione forms a colored complex with DTNB, which was measured calorimetrically at 412 nm. The amount of glutathione was determined using its molar extinction coefficient of 13600/m/cm and expressed in terms of µmol/mg of protein.

Statistical analysis

All the data were statistically evaluated and the significance calculated using student's' t ' test. All the results were expressed as mean ±S.D.

Histopathological analysis

After 45 days of treatment, the animals were killed by cervical dislocation. The pancreas was quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and organ weight was measured. Organ slices fixed for 48hrs in 10% formosaline were processed for paraffin embedding following the standard micro technique sections (5 mm) of organs stained with Haemotoxylin and eosin were evaluated for histopathological changes under a light microscope. Histopathological findings were graded for degree of pancreatic cell damage.

Steps

The basic steps of specimen processing include:

1. Fixing
2. Embedding
3. Microtomy
4. Staining
5. Mounting

Specimen

Pancreas of the experimental animals of each set was taken.

Procedure

Fixation

Fixation is a process by which the cells of tissue and its components are fixed in physical and partly in the chemical state. It is the process of killing and hardening the tissue so as to withstand the subsequent processing fluid.

It is carried out by immersion of tissue in the fixative (a chemical substance) to prevent autolysis and bacterial attack.

The fixative used is aldehyde fixative:
10% buffered formalin
10ml commercial formaldehyde,
6.5g disodium hydrogen phosphates

Tissue processing

Tissue when satisfactory fixed, it is to be processed for further, various procedures such as dehydration, clearing, infiltration and embedding.

Dehydration

After fixation the tissues were washed thoroughly over night with tap water and then dehydrated. Dehydration is a stepwise process to remove water from the tissue. In this process-graded dehydration 30%, 50%, 70%, 90% of absolute alcohol was used as grades. These grades are prepared accordingly and stored in large bottles, which constitute the stock. Care should be taken to see that the tissues were carried out for 5-10 minutes.

Cleaning

After absolute alcohol treatment cleaning of the tissue is done using xylene. This is a good clearing agent, which brings about quick removal of alcohol from the tissues and speeds up the infiltration of paraffin into them. Dehydrated tissues were first treated with alcohol,xylene (1:1 ratio) and then with 2 stains of xylene.

Infiltration and embedding

This process involves the impregnation of tissues with a medium that will fill all natural cavities, spaces and interstices of tissues and that will set into firm consistency to allow cutting of suitable thin section. Paraffin wax is the most routinely used for embedding and infiltration.

Infiltration

Cold Infiltration

In this step powdered paraffin is added to xylene containing the processed tissue and each tissue is allowed to dissolve, and made to saturate by mixing it well.

Hot Infiltration

For hot infiltration the paraffin is made to melt in a wax-embedding chamber maintained at 60ºC. three chambers named 1, 2, and 3 are used to give these changes of the tissue in molten wax. Precaution was taken not to char the tissue. Hot infiltration at each charge of components are present which may be distinguished by their ability to retain dyes of contrasting colours when stained in 2 or 3 different types of stains during differential staining.

When specifically desired to study the cells at cytochemical or histochemical levels the common dyes differentiate the nucleus and the cytoplasm.

Processing

Pour paraffin wax in the aluminum tray to about 1-2cm heights. Cssheck the temperature immediately (56ºC - 60ºC). Embed the tissue in the wax down verse with the distance of 0.5cm around each tissue and the specific labels also embedded respectively below the tissue. Kept in room temperature for 10 minutes and immerse in water sink. The paraffin wax becomes hard. Then cut the wax containing tissue slice with its specific label.

Emedding

Tissues completely dehydrated, cleared is to be impregnated with paraffin by immersion in succession of molten wax bath achieved by agitation. Hand processing 3-6 hrs for 3 changes. The term embedding media designates all materials used in histology infiltrate support and encloses specimens, which are subsequently cut into thin sections. Paraffin wax remains popular due to the ease with which large number of tissue blocks may be processed in comparatively short time.

Processing

Pour paraffin wax in the aluminum tray to about 1-2cm heights. Check the temperature immediately (56ºC - 60ºC). Embed the tissue in the wax down verse with the distance of 0.5cm around each tissue and the specific labels also embedded respectively below the tissue. Kept in room temperature for 10 minutes and immerse in water sink. The paraffin wax becomes hard. Then cut the wax containing tissue slice with its specific label.

Microtomy

A microtome is an instrument specially designed to cut very thin section of tissue. The microtome used for section cutting is Rotatory microtome. It is more convenient for cutting serial section. Set the microtome adjustment screws for the desired thickness (normally 4-6m thickness). Block is removed from ice, clamped firmly in the object holder of the microtome. Keep the knife holder and clamp it firmly. Object holder is moved manually until the surface of wax just touches the knife-edge. Avoid too much of cooling. Now rotate the flywheel and start sectioning by moving it gently.

Staining

The staining procedure carried out here is Haematoxylin and Eosin stain.

Solution	**Process**
Xylene I	5 minutes
Xylene II	5 minutes
Absolute alcohol	2 dips
90% alcohol	2 dip
70% alcohol	2 dips
Running tap water	2 dips
Primary stain hematoxylin	5 minutes
Running tap water	5-10 minutes
1% acid alcohol	5minutes2 quick dips
Running tap water	5 minutes
Saturated lithium carbonate	15 seconds

Running tap water	5 minutes
70% alcohol	2 dips
90% alcohol	2 dips
1% alcoholic eosin	15-30 seconds
Absolute alcohol I	2 dips
Absolute alcohol II	2 dips
Xylene I	2 minutes
Xylene II	2 minutes
Xylene III	2minutes

Mounting

Gam dammer or synthetic mounting medium DPX (Distrene dibutyl phthalate xylene) is used. After mounting the slides are viewed under light or electron microscope for the identification of tissue damage due to toxicity.

Results and Discussion

Streptozotocin causes selective destruction of cells of islets of pancreas and brings an increase in blood glucose levels. It is evident from the present investigation that streptozotocin administration at the dose of 200mg/kg albino rats.

Fig 1 shows the normal serum glucose and insulin level due to the normal functioning of pancreas.

Fig 2 shows the increase in blood sugar and insulin level after STZ induction.

Fig3 shows the both aqueous and methanolic extract of noni exhibit antidiabetic and antioxidant property in streptozotocin-induced diabetic rats as evident from changes in serum glucose level and insulin level.

Fig 4 shows both the aqueous and methanolic extract of spirulina exhibits antidiabetic and antioxidant property in streptozotocin-induced diabetic rats as evident from changes in serum glucose and insulin level.

Fig 5 shows that the aqueous extract of insulin exhibit antidiabetic property in streptozotocin-induced rats as evident from changes in serum glucose and insulin level.

Fig 6 shows the effect of noni extract on Superoxide dismutase and lipid peroxidase.

Fig 7 shows the effect of noni on Catalase and Glutathione.

Fig 8 shows the effect of spirulina extract on Superoxide dismutase and lipid peroxidase.

Fig 9 shows the effect of spirulina extract on Catalase and Glutathione.

Thus, it is interesting to note that Noni, Spirulina (algae) and insulin could control the blood glucose and insulin level to normal and also has got antioxidant activity.

Pancreas histology

Histology of the islets of Langerhans of normal control rats showed predominant exocrine pancreatic tissue composed of acini with draining ductules .The endocrine component was found as scattered nodules within the substance of the exocrine pancreas exhibiting no pathological changes. However, in the group II diabetic animals**,** the sections revealed predominant exocrine pancreatic tissue composed of acini with draining ductules. The endocrine component was found as scattered nodules within the substance of the exocrine pancreas relating

Table.1 Glucose and insulin level in control rats

Biochemical Analysis	Control
Glucose (mg%)	138 ±0.23
Insulin (U/L)	38±0.33

Fig.1 Glucose and insulin level in control rats

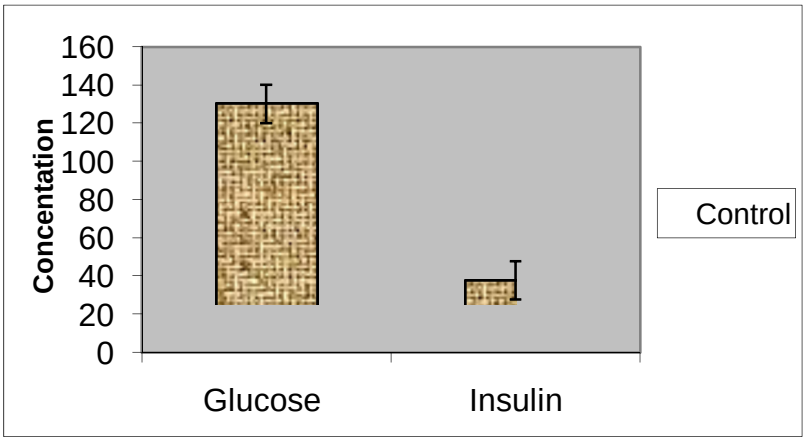


Table.2 Effect of STZ on blood glucose and insulin level

Biochemical Analysis	SZT induction
Glucose (mg%)	321.6 ±0.13
Insulin (U/L)	7.98 ±0.10

Fig. 2 Effect of STZ on blood glucose and insulin level

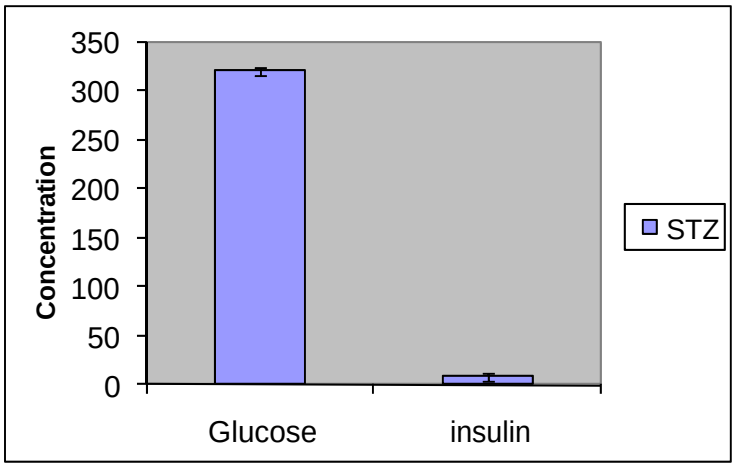


Table.3 Effect of noni extracts on blood glucose and insulin level Treatment

Biochemical Analysis	Noni (aqueous extract) Treatment	Noni (methanol extract) Treatment
Glucose(mg%) Insulin(U/L)	89 27.2	135± 1.80 14.6±0.92

Fig .3 Effect of noni extracts on blood glucose and insulin level Treatment

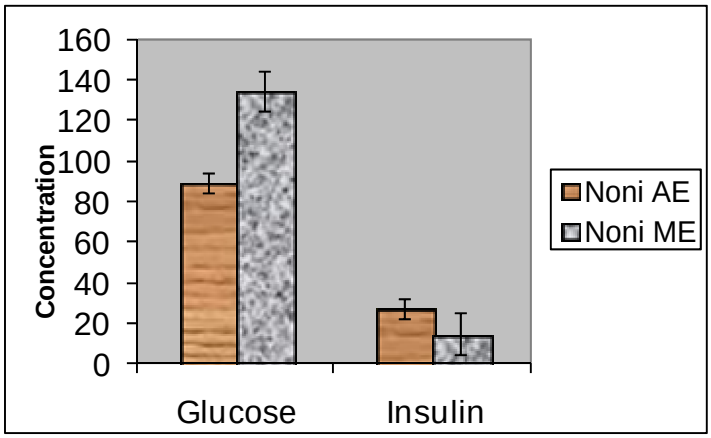


Table.4 Effect of spirulina extracts on blood glucose and insulin level

Biochemical Analysis	Spirullina(aqueous extract) Treatment	Spirullina (methanol extract) Treatment
Glucose(mg%) Insulin(U/L)	98.6 18.4	14.3 ±0.96 16.8 ±1.02

Fig.4 Effect of spirulina extracts on blood glucose and insulin level

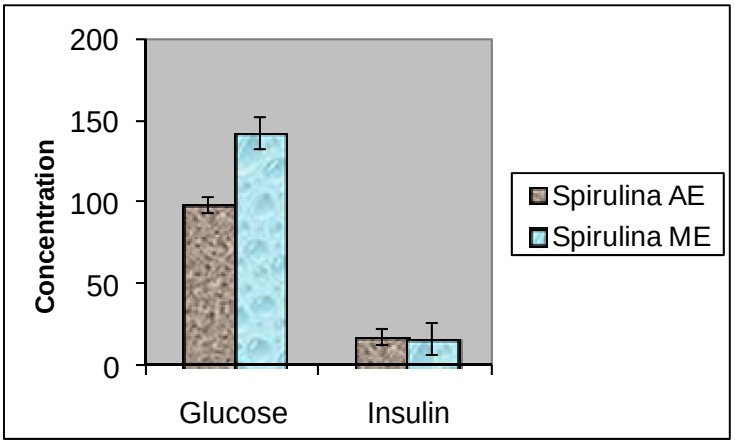


Table. 5 Effect of insulin on blood glucose and insulin level

Biochemical Analysis	**Insulin**
Glucose (mg%) Insulin (U/L)	93±0.95 23.8±0.18

Fig.5 Effect of insulin on blood glucose and insulin level

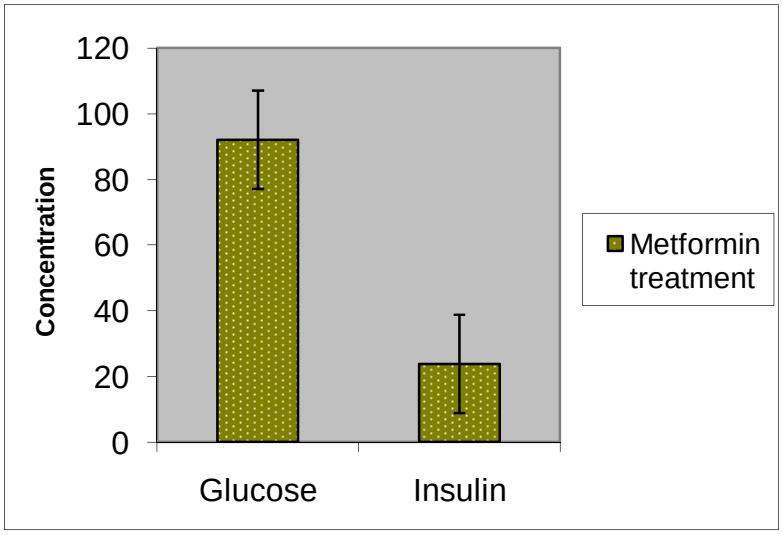


Table.6 Effect of noni extract on Superoxide dismutase and lipid peroxidase

S.No	**Treatment**	**LP Nm/Mg protein**	**SOD unit /min/mg**
1.	Control saline	2.87±0.10	11.38±0.26
2.	Control Vit C	1.13±0.70	25.17±0.42
3.	Test 1	2.79±0.74	13.36±0.17
4.	Test 2	2.58±0.65	17.21±0.15
5.	Test 3	2.29±0.76	20.79±1.24
6.	Test 4	2.03±0.71	21.41±1.19

Fig.6 Effect of noni on Superoxide dismutase and lipid peroxidase

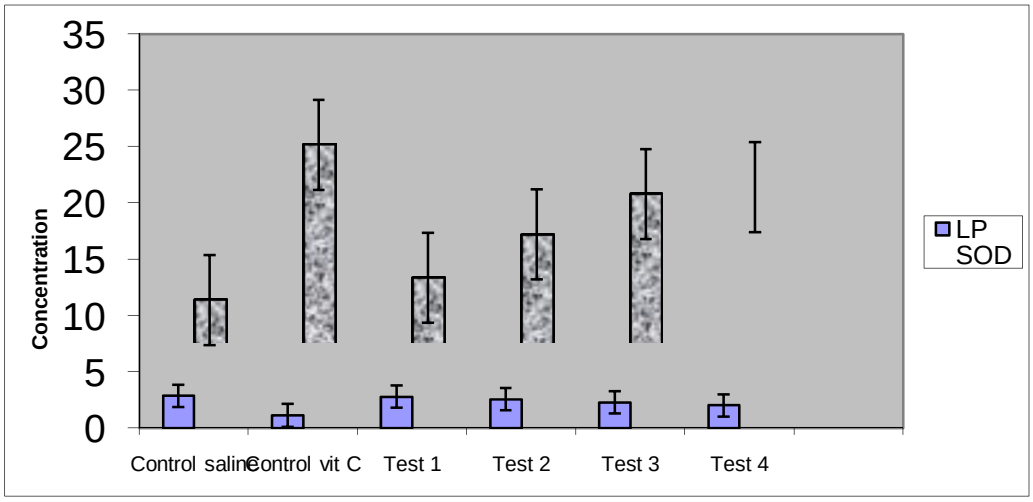


Table.7 Effect of noni on Catalase and Glutathione

S.No	Treatment	Catalase nMH202/min/mgprotein	Glutathione
1.	Control saline	78.56±1.5	6.79±0.9
2.	Control Vit C	176.28±0.97	16.23±1.86
3.	Test 1	91.32±0.87	8.21±0.97
4.	Test 2	109.76±0.90	9.73±0.09
5.	Test 3	123.36±0.91	10.03±0.10
6.	Test 4	129.59±0.94	11.69±1.29

Fig.7 Effect of noni on Catalase and Glutathione

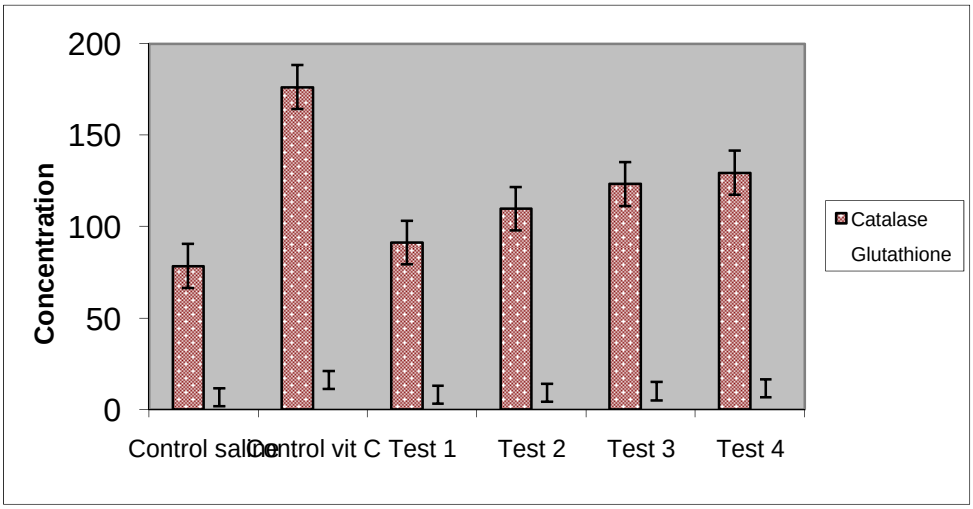


Table.8 Effect of spirulina extract on Superoxide dismutase and lipid peroxidase

S.No	Treatment	LP Nm/Mg protein	SOD unit /min/mg
1.	Control saline	3.17±0.33	11.38±1.80
2.	Control Vit C	1.44±0.33	21.07±0.78
3.	Test 1	2.98±0.33	12.36±0.84
4.	Test 2	2.89±0.33	14.21±0.81
5.	Test 3	2.68±0.33	18.79±0.83
6.	Test 4	2.37±0.32	19.41±0.76

Fig.8 Effect of spirulina extract on Superoxide dismutase and lipid peroxidase

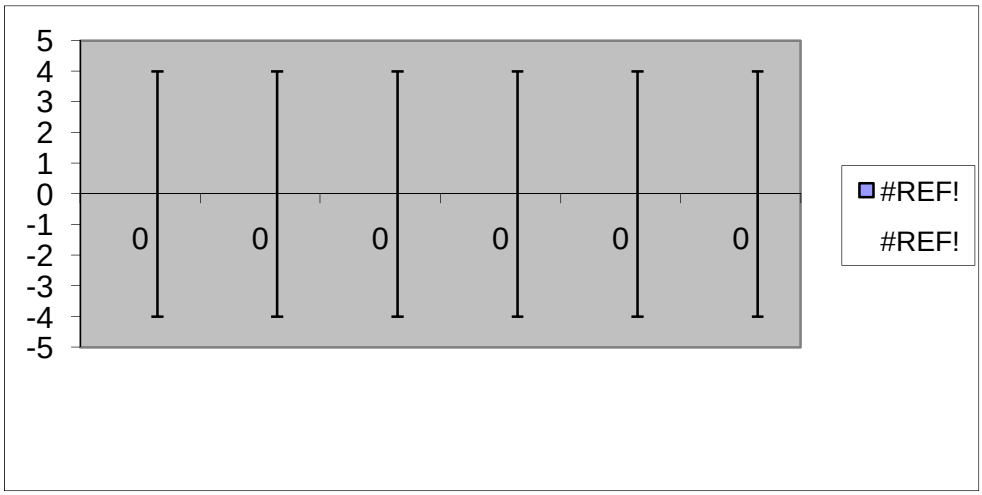
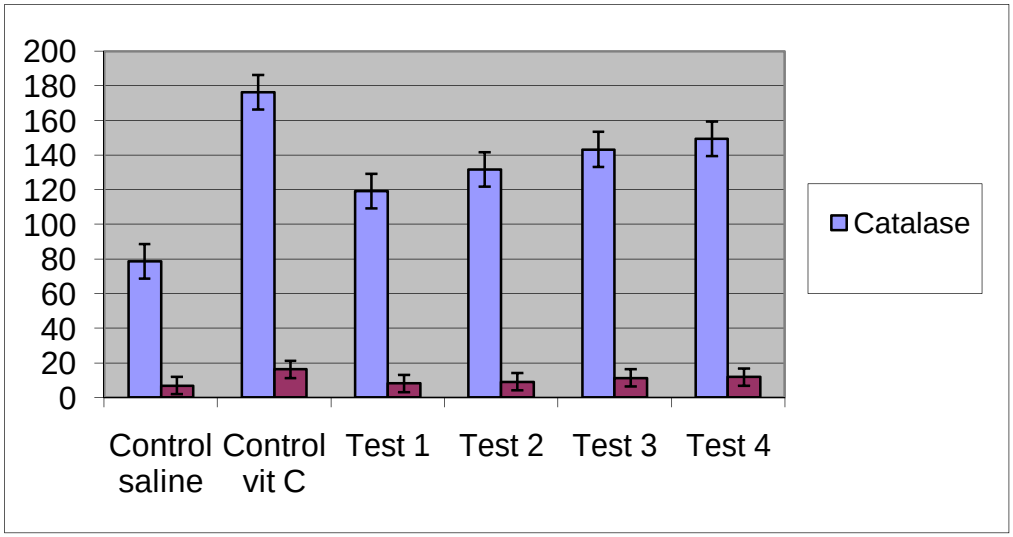


Table. 9 Effect of spirulina extract on Catalase and Glutathione

S.No	Treatment	Catalase nMH202/min/mg protein	Glutathione
1.	Control saline	78.53±0.5	6.79±0.02
2.	Control Vit C	176.27±0.54	16.23±1.11
3.	Test 1	119.32±0.33	8.11±0.28
4.	Test 2	131.73±90.41	8.99±0.21
5.	Test 3	143.32±0.59	11.21±1.32
6.	Test 4	149.51±0.38	11.69±1.42

Fig.9 Effect of spirulina extract on Catalase and Glutathione



to focal mild infiltration by mononuclear cell. However, in group III, IV, V ,VI ,VII animals the endocrine component was found as scattered nodules within the substance of the exocrine pancreas showing no signs of insulitis as observed in group II.

Diabetes mellitus is characterized by hyperglycemia, which usually produces many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, obesity, atherosclerosis, and even cardiovascular disease (Defronzo *et al.*, 1992; Alberti et al., 1997).

Oxidative stress is produced under diabetic conditions and it is likely involved in progression of pancreatic β-cell dysfunction (Kajimoto and Kaneto, 2004). Also, because of the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic β- cells may be vulnerable to ROS attack when the system is under oxidative stress situation (Lenzen *et al.*, 1996; Tiedge *et al.*, 1997). Similarly, elevated levels of free radicals, due to insufficiency of the antioxidant defense system, may lead to disruption of cellular function, oxidative damages to membranes and enhance their susceptibility to lipid peroxidation (Baynes, 1991).

In recent years, it has been shown that dietary supplementation with natural antioxidants such as, vitamins C and E, melatonin and flavonoids attenuated the oxidative stress and diabetic state induced by STZ (Montilla *et al*., 1998

The levels of blood glucose, plasma insulin and serum C-peptide were estimated and studied in streptozotocin diabetic rats. The findings were compared between normal, diabetic and spirulina supplemented diabetic rats. The findings indicated that the administration of spirulina tended to bring the parameters significantly towards the normal. The effect of spirulina at a dose of 15mg/kg body weight yield a higher level of significance than the doses of 5 and 10mg/kg body weight, therefore the former was used in further biochemical; and clinical studies. (Anitha and Chandra Lekha Kasi Reddy, 2006).

Streptozotocin (STZ) is an antibiotic that can cause pancreatic *β*-cell destruction, so it is widely used experimentally as an agent capable of inducing insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes mellitus (T1DM). The *Morinda citrifolia* is particularly adapted to treat Type II diabetes. The *Morinda citrifolia* product is preferably a leaf extract, but may also be in the form of a juice, a puree juice, a dietary fiber, or other similar forms and is incorporated into various carriers or naturaceutical compositions suitable for in The naturaceutical may also combine other food products into the naturaceutical, such as fruit juices, dietary supplements, vitamins and minerals, and others vivo treatment of a patient. . (Jensen *et al.*, 2007).

Insulin is a biguanide derivate used as an oral hypoglycaemic drug in diabetics. The animals were rendered diabetic by intraperitoneal injection of 65 mg/kg STZ. Fourteen days later, insulin was given at 25 mg/kg by gavage, daily for 28 days, to STZ diabetic rats and a control group. In the STZ-diabetic group, some degenerative changes were observed by light microscopic examination. But the degenerative changes were decreased in the STZ-diabetic group given insulin. In the STZ-diabetic group, blood glucose levels decreased. In the STZ-diabetic group given insulin, blood glucose level and liver GSH increased. So it was concluded that insulin has a protective effect on hepatotoxicity produced by STZ. (Yanardag *et al.*, 1999).

Aqueous and alcoholic extracts of spirulina was prepared for antioxidant vitamin content (vitamin C and E), total phenolic coumpounds. Antioxidant status, reducing power and the effect on glutathione S-transferase (GST) activity were evaluated in vitro. Total antioxidant activity of aqueous extract of spirulina at1mg/ml concentration was 1.33mmol/l. At similar concentration the total antioxidant activity of alcoholic extract of spirulina was1.73mmol/l respectively. Both aqueous and alcoholic extracts of spirulina showed protection against t-BOOH induced cytotoxicity and production of ROS in cultured C6 glial cells. (Vasudha shukla *et al*., 2009).

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The elevated levels of blood glucose, glycosylated hemoglobin, blood urea, and serum creatinine in the diabetic rats reverted back to near normal after treatment with the noni fruit extract. Similarly significant decrease in the levels of plasma insulin and haemoglobin were elevated to near normal after treatment with fruit extract, suggesting antihyperglycemic effect of *Morinda citrifolia* fruit. Determination of thiobarbituric acid reactive substance (TBARS), hydroperoxidase and both enzymatic and non-enzymatic antioxidants evidenced the antioxidant potential of the

extract of noni fruit, which in turn may be responsible for its hypoglycemic potential. (U.S Mahadeva Rao and S. Subramanian, 2008)

References

Acworth, I.N., and B. Bailey 1997. Reactive Oxygen Species. In: The handbook of oxidative metabolism. Massachusetts: ESA Inc. p. 1-1 to 4-4.

Alessio, H.M., and E.R. Blasi2000. Physical activity as a natural antioxidant booster and its effect on a healthy lifestyle. Res. Q. Exerc. Sport. 68 4: 292-302.

Allen WH, London C 1873 .Some information on the ethnobotanical properties of Noni*Morinda citrifolia*. In: The useful plants of India.

American Diabetes Association 2005. "Total Prevalence of Diabetes & Pre-diabetes". http://www.diabetes.org/diabetes-statistics/prevalence.jsp. Retrieved 2006-03-17.

Anitha, L., and K. Chandralekha2006.Effect of Supplementation of Spirulina on Blood Glucose,Glycosylated Hemoglobin and Lipid Profile of Male Non insulin Dependent Diabetics.

Anuradha V, Vidhya D. Impact of administration of spirulina on the blood glucose levels of selected diabetic patients. Indian J Nutr Dietet 2001;38:40-44. 27. Babu DY. Hypoglycaemic effects of algae spirulina in non-insulin dependent diabetes mellitus NIDDM patients. MS thesis. Bharathiar University, Coimbatore, 1989:1-70. 28. Iyer U, Pariskh S. Glycemic index of spirulina supplemented recipes in NIDDM subjects. MS thesis.

Arlan Rosenbloom and Janet H Silverstein 2003. Type 2 Diabetes in Children and Adolescents: A Clinician's Guide to Diagnosis, Epidemiology, Pathogenesis, Prevention, and Treatment. *American Diabetes Association,U.S.*. pp. 1. ISBN 978-1580401555.

Balakrishna S, Seshadri TR, Venkataramani B.1961Special chemical component of commercial woods and related plant materials: Part X-Heartwood of *Morinda citrifolia* Linn. J Sci Industrial Res; 20B: 331-3.

Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA November 1991. "Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. 1922". *CMAJ* 145 10: 1281–6. PMID 1933711.

Baynes KCR, Boucher BJ, Feskens EJM, Kromhout D1997.Vitamin D, glucose tolerance anal insulinaemia in elderly men. *Diabetologia.;*403:344–347.

Belay, A.; Ota, Y.; Miyakawa, K.; Shimamatsu, H. 1993, Current knowledge on potential health benefits of *Spirulina*. *Journal of Applied Phycology*, 5, 235-241.

Brancati FL, Kao WHL, Folsom AR, Watson RL, Szklo M2000 . Incident type 2 diabetes mellitus in African American and white adults: the Atherosclerosis Risk in Communities Study. Journal of the American Med Association.;28317:2253–2259.

Brian J. Welch, MD and Ivana Zib, MD 2004 Case Study: Diabetic Ketoacidosis in Type 2 Diabetes: "Look Under the Sheets", *Clinical Diabetes*, , 22 4, 198-200.

Bruggnecate JT1992. Native plants can heal your wounds. Honolulu Star-Bulletin Local News Feb 2.

Clarkson P. M1995. Antioxidants and its type. Crit.Rev. Food Sci. Nutr. 35: 131-141..

Clarkson P. M1998. Antioxidants and diabetes mellitus. Crit.Rev. Food Sci. Nutr. 35: 131-141.

Cooke DW, Plotnick L November 2008. "Type 1 diabetes mellitus in pediatrics". *Pediatr Rev* 29 11: 374–84; quiz 385. doi:10.1542/pir.29-11-374. PMID 18977856.

Costa, J.A.V.; Linde, G.A.; Atala, D.I.P.; Mibielli, G.M.; Kruger, R.T 2000, Modelling of growth conditions for cyanobacterium *Spirulina platensis* in microcosms.

Dekkers, J. C., L. J. P. van Doornen, and G.Kemper1996. The Role of Antioxidant Vitamins and Enzymes in diabetes mellitus. Clinical sMed 21: 213-238.

Del Mastero, R.F1980. An approach to free radicals in medicine an biology. Acta. Phyiol. Scand. 492: 153-168.

Del Mastero, R.F1980. An approach to free radicals in medicine an biology. Acta. Phyiol. Scand. 492: 153-168.

Dillard, C.J., R.E. Litov, W.M. Savin, E.E. Dumelin, and A.L. Tappel 1978 Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J. Appl. Physiol. 45: 927.

Dillard, C.J., R.E. Litov, W.M. Savin, E.E. Dumelin, and A.L. Tappel 1999. Effects of antioxidants on STZ induced rats.

Dobson, M. 1776. "Nature of the urine in diabetes". *Medical Observations and Inquiries* 5: 298–310.

Duke JA1992. Handbook of phytochemicals. Boca Raton, FL: CRC Publishing.Heinicke R. The Xeronine sys tem: a new cellular mechanism that explains the health promoting action of NONI and Bromelian. Direct Source Publishing; 2001.

Estrada, J.E.; Bescós, P.; Villar Del Fresno, A.M. 2001, Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *Il Farmaco*, 56, 497-500.

Farine JP, Legal L, Moreteau B, Le Quere JL1996. Volatile components of ripe fruits ofMor inda citr ifolia and their effects on Drosophila. Phytochemistry; 41:433-8.

Freed, C.B., Zaul , Rayman ,James 2000.Activation of AMPK pathway by insulin in STZ diabetic rats.

Geethan PK, and Prince PS 2008. Antihyperlipidemic effect of D-pinitol on streptozotocin-induced diabetic Wistar rats. *J Biochem Mol Toxicol*.;224:220-4.

Goldfarb, A 1999. H. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Can. J. Appl. Physiol. 24: 249-266.

Halliwell, B., and J.M.C. Gutteridge1985. The chemistry of oxygen radicals and other oxygen-derived species. In: Free Radicals in Biology and Medicine. New York: Oxford University Press, p. 20-64.]

Harris MI, Flegal KM, Cowie CC, *et al.* April 1998. "Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988–1994". *Diabetes Care* 21 4: 518–24. doi:10.2337/diacare.21.4.518. PMID 9571335..

Heinicke R1985. The pharmacologically active ingredient of Noni. Bulletin of theNational Tropical Botanical Garden, Wang M, Kikuzaki H, Csiszar K, Boyd CD, Maunakea A, Fong SF, et al. Novel trisaccharide fatty acid ester identified from the fruits of *Morinda citrifolia* Noni.

Himsworth 1936. "Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types". Lancet

i: 127–30. doi:10.1016/S0140-67360136134-2.

Inzucchi SE, Sherwin RS2008 .The Prevention of Type 2 Diabetes Mellitus. *Endocrinol Metab Clin* N Am 34 2205 199-219. .

Jack L, Boseman L, Vinicor F2004. "Aging Americans and diabetes. A public health and clinical response". *Geriatrics* 59 4: 14–7.

Jensen, R.S Roddy ,C.K Ellie 2007Clinical Research on *Morinda citrifolia* L. – Noni

Jeppesen PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A, Xiao J, Kruhøffer M, Orntoft T, Hermansen K. 2003. *Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism.* 523:372-8.

Kapoor R, Mehta U. Effect of supplementation of blue green algae on outcome of pregnancy of rats..

Kong 1998.Definition, classification Type I ,Type Iiand Gestational diabetes , its symptoms and treatment.

Krauss B1993. Plants in Hawaiian culture. Honolulu: University of Hawaii Press.

Lawrence JM, Contreras R, Chen W, Sacks DA May 2008. "Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005". *Diabetes Care* 31 5: 899–904. doi:10.2337/dc07-2345. PMID 18223030.

Layam, A., C. L. K. Reddy 2000.Antidiabetic property of spirullina.

Liu G, Bode A, Ma WY, Sang S, Ho CT, Dong Z2001 Two novel glycos ides from the fruitsof *Morinda citrifolia* noni inhibit AP-1 transactivation and cell trans formationin the mouse epidermal JB6 cell line. Cancer Res ; 61: 5749-56.

Mahadeva Rao,U.S., and S. Subramanian 2008.Hypoglycemic and antioxidant effect of *Morinda citrifolia* on streptozotocin induced rats.

Maiden JH1889.. Useful native plants of Australia including Tasmania. Sydney: Tunerand Henderson Publisher; p 45.

McCarty MF. 2000.Toward practical prevention of type 2 diabetes. *Med.* May;545:786-93.

Merrill ED1992. Noni *Morinda citrifolia* as an edible plant. In: Technical manual:emergency food plants and poisonous plants of the islands of the pacific.

Miranda, M.S, R.G Cintra AND SBM Barrow1998. Antioxidant activity of the microalga *Spirulina maxima*.

Miranda, M.S.; Cintra, R.G.; Barros, S.B.M.; Filho, J.M. 1998, Antioxidant activity of the microalga Spirulina maxima. *Brazilian Journal of Medical and Biological Research*, 31, 1075-1079

Mohammad Mohiuddin, Md. Shah Amran and Md. Amjad Hossain2009.The *In vivo* Effects of Caffeine on the Hypoglycemic Activity of Gliclazide and Insulin in Healthy Rats

Montilla ,PoterW,Wang,Timms1998.Natural antioxidants in scavenging oxidative stress and streptozotocin induced diabetes

Morinda citrifolia, Linn. Antiseptic 1970; 67: 167-71.

Morton JF1992. The ocean-going Noni, or Indian mulberry *Morinda citrifolia*,Rubiaceae and some of its 'colorful' relatives. Economic Botany ; 46: 241-56.

Netter1997 Structure and mechanism of streptozotocin

Parikh P,Mani,A.N Sharma 2000. Study of hypoglycemic activity of spirulina.

Rother KI 2007. "Diabetes treatment-bridging the divide". The New England *Journal of Medicine* 356 15: 1499–501

Sang S, Cheng X, Zhu N, Stark RE, Badmaev V, Ghai G, et al2001. Flavonolglycosidesand novel iridoid glycoside from the leaves of *Morinda citrifolia*. J Agric FoodChem; 49: 4478-81.

Sang S, Cheng X, Zhu N, Wang M, Jhoo JW, Stark RE, *et al* 2001 Iridoid glycosides from the leaves of *Morinda citrifolia*. J Nat Prod ; 64: 799-800.

Saydah SH, Miret M, Sung J, Varas C, Gause D, Brancati FL August 2001. "Postchallenge hyperglycemia and mortality in a national sample of U.S. adults". *Diabetes Care* 24 8: 1397–402.

Saydah SH, Miret M, Sung J, Varas C, Gause D, Brancati FL August 2001. "Postchallenge hyperglycemia and mortality in a national sample of U.S. adults". *Diabetes Care* 24 8: 1397–402. doi:10.2337/diacare.24.8.1397. PMID 11473076.

Sena CM, Nunes E, Louro T, Proença T, Seiça RM. 2007.Endothelial dysfunction in type 2 diabetes: effect of antioxidants. *Rev Port Cardiol.* 266:609-19.

Simonsen JL1920.Note on the constituents of *Morinda citrifolia*. J Chem Soc;117: 561-4.

Singh J, Tiwari RD1976. Flavone glycosides from the flowers of *Morinda citrifolia*. J Indian Chem Soc 1976; 53: 424.

Solomon N1999. The tropical fruit with 101 medicinal uses, NONI juice. 2nd ed.Woodl and Publishing.

Tabrah FL, Eveleth BM1966. Evaluation of the effectiveness of ancient Hawaiian medicine.Hawaii Med J ; 25: 223-30.

Tomkinson AJ and Jameson1993.The Diabetes Control And Complications. "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group". *The New England Journal of Medicine* 329 14: 977–86...

Venkataraman LV. Spirulina 1998global reach of a health care product. Souvenir, IFCON '98, 4th International Food Convention;175.

Whistler W. Tongan1992 herbal medicine. Isle Botanica, Honolulu, Hawaii, 1992. p 89-90.

Wild S, Roglic G, Green A, Sicree R, King H May 2004. "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030". *Diabetes Care* 27 5: 1047–53.

Wild S, Roglic G, Green A, Sicree R, King H May 2004. "Global prevalence of diabetes: estimates for 2000 and projections for 2030". *Diabetes Care* 27 5: 1047–53..

World Health Organisation Department of Noncommunicable Disease Surveillance 1999. "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications" http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf.

Wu MS, Liang JT, Lin YD, Wu ET, Tseng YZ, Chang KC. 2008. Aminoguanidine prevents the impairment of cardiac pumping mechanics in rats with streptozotocin and nicotinamide-induced type 2 diabetes. *Br J Pharmacol.* 1544:758-64.

Yanardag, R., Simmon ,Wolf 1999. Hypoglycemic activity of insulin in STZ induced diabetic rats.

http://www.diabetes.org/diabetes-statistics.jsp

Zhou SS, Li D, Sun WP, Guo M, Lun YZ, Zhou YM, et al. 2009. Nicotinamide overload may play a role in the development of type 2 diabetes. *World J Gastroenterol.* 7;1545:5674-84.