SOME EFFECT OF CRUDE EXTRACT OF NEEM BARK ON THE PANCREAS OF STREPTOZOTOCIN INDUCED DIABETES OF ADULT WISTAR RATS

ABSTRACT

This study investigates the histological and serum enzymatic activities of *Azadirachta indica*, an Indian medicinal plant, on the pancreas and blood sample of adult wstar rats.

Forty six adult wistar rats weighing 100g to 220g were grouped randomly into four groups; of control group, Diabetes group, the group receiving the low dose of the extract at 250mg/kg/b.w and the group receiving the high dose of the extract at a dose of 500mg/kg/b.w

The control group received water throughout the experiment; the remaining three groups were induced with streptozotocin intra-peritoneally to induced diabetes into the animals. After some days, the animals were confirmed diabetes with the help of a measuring glucometer.

The aqueous neem bark extract was suspended in the drinking water of the treated animals for the period of 42days. The body weights of the animals were weighed at the end of each week and likewise the measurement of the blood glucose level were measured.

The animals were sacrificed at the end of 42days using the cervical dislocation method and the pancreas was removed and weighed immediately using sensitive weighing balance. The blood samples were collected from the sacrificed animals into EDTA bottle for serum enzymatic analysis. The organ pancreas was fixed in a 10% formosaline, processed and stained with Heamatoxylin and Eosin for general histological study.

The microscopic examination of diabetes groups only showed some areas of necrotic cells and the treated tissues of the experimental animals were improved tremendously.

Analysis of the blood serum level showed that the aqueous neem bark extract has lowering effect on the enzymatic activities of the blood sample in the animals. The alanine amino transferase, aspartate amino transferase and alkaline phosphatase, showed a significant reduction in the treated animals while, the diabetes groups which was the untreated group, increases in the enzymatic activities.

B. The mean body weight of the animals were analyzed, the low dose group of the extract and high dose group of the extract showed a significant increase in the body weight.

Blood glucose level of the experimental animals showed a significant increase in their levels, when compared with the diabetes groups.

Key words: *Diabetes mellitus, blood serum, Streptozotocin, Azadirachta indica.*

General Introduction

The medical properties of Neem have been known to Indians since time immemorial. Theearliest Sanskrit medical writings refer to the benefits of Neem's fruits, seeds, oil, leaves, rootsand bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines, and isnow being used in the manufacture of modern day medicine, cosmetics, toiletries andpharmaceuticals. The Neem tree has been known as the wonder tree for centuries in the Indiansubcontinent. Neem has become important in the global context today for its variety of medicinaluses.Neem extracts which have Nimbinin, nimbandiol as active constituents; alcoholic extract ofthe leaves was found to possess a significant blood sugar lowering effect, which is very usefulagainst diabetes. Neem is used in Dermatitis Eczema, Acne, Bacterial, Fungal infections andother skin disorders. It has demonstrated its effectiveness as a powerful antibiotic. Neem also hasshown antiviral, anti-fungal and anti-bacterial properties. It helps support a strong immunesystem and is used in cases of inflammatory skin conditions. Traditionally Neem has been usedfor skin and blood purifying conditions. Perhaps Neem's most touted advantage is the effect ithas upon the skin. Preparations from the leaves or oils of the tree are used as general antiseptics. Biologicalactivity of neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil (Serrano, 2009).

Due to Neem's antibacterial properties, it is effective in fighting most epidermal dysfunctionsuch as acne, psoriasis, and eczema. Ancient ayurvedic practitioners believed high sugar levels inthe body caused skin disease; Neem's bitter quality was said to counteract the sweetness.

Traditionally, Indians bathed in Neem leaves steeped in hot water. Since there has never been areport of the topical application of Neem causing an adverse side effect, this is a commonprocedure to cure skin ailments or allergic reactions. Neem also may provide

antiviral treatmentfor smallpox, chicken pox and warts--especially when applied directly to the skin. Itseffectiveness is due in part to its ability to inhibit a virus from multiplying and spreading. However, apart from these uses, there are several reports on the biological activities andpharmacological actions of neem based on modern scientific investigations. (Ramchandran,2001).

Neemproduces pain-relieving, anti-inflammatory and fever-reducing compounds that can aid in thehealing of cuts, burns, sprains, earaches, and headaches, as well as fevers. Several studies ofNeem extracts in suppressing malaria have been conducted, all supporting its use in treatment.Neem has broad applications to human and animal health, as well as organic farming. Neem is apowerful antiviral and antibacterial. But, it has peculiarities that set it apart from other herbs inthat class of broad antimicrobials. Neem oil is also commonly added to a variety of creams andsalves. It is effective against a broad spectrum of skin diseases including eczema, psoriasis, dryskin, wrinkles, rashes and dandruff. A few drops can be added to hand healing salves andshampoo. Neem oil is highly effective as a mosquito repellent. Because of its unpleasant smell, itis best when it is added to a formula with other essential oils, such as citronella.Neem oil is aneffective and environmentally safe pesticide when it is diluted and sprayed on crops throughirrigation systems. It is a healthier alternative to artificial chemical pesticides. Neem oil does notharm the soil and it increases yields (Debjit Bhowmik, 2010)

Pancreas

The pancreas is a glandular organ in the digestive system and endocrine system of vertebrates. In humans, it is located in the abdominal cavity behind the stomach. It is an endocrine gland producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide which circulate in the blood. The pancreas is also a

digestive organ, secreting pancreatic juice containing digestive enzymes that assist digestion and absorption of nutrients in the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids in the chyme.

Diabetes Mellitus

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [1].Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine [2].The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance.(A Vasudeva Rao, 2012)

Hyperglycemia is involved in the etiology of development of diabetic complications. Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver.

Insulin and oral hypoglycemic agents like sulfonylureas and biguanides are still the major players in the management but there is quest for the development of more effective anti-diabetic agents.For proper investigation and research, experimental animal models play a pivotal role for therapeutic efficacy of candidate drug. Experimental diabetes (ED) can be induced by pancreatectomy, administration of insulin-antagonist hormones or other chemical agents. There are three groups of chemical agents used to induce ED, the first group destroys the beta cells of the pancreatic islets; the second group alters the beta cells but do not destroy them and the third group increases the endogenous insulin requirements weakening the pancreas and producing ED (Mendez *et al.*,1994). Streptozotocin (STZ) and alloxan are the chemical inducers of ED mostlyused in laboratory animals. STZ is a methylating agent for DNA (Bennett,1981) that destroys pancreatic beta cells, inducing permanent diabetes. Alloxan is a toxic agent for pancreas beta cells; its proposed mechanism for diabetes induction includes: sulfhydryl group attack, chelant action, enzyme and metabolic modifications; membrane transport changes on electrolytes (Carrol.,1994) plus increased lipoperoxidation.(Soto *et al.*,1994).

AIMS

The aim of this study was to investigate some effect of aqueous extract of neem bark on streptozotocin induced diabetes mellitus in adult wistar rats..

SIGNIFICANCE OF STUDY

In the light of intake of this decoction by man, it become necessary to study some of the effect it may have on the body. In Nigeria, particularly among the Yoruba's, this decoction is often taken for several ailment or diseases.

It is believe that when taken, it reduces the blood glucose level, and this led to it abuse particularly in absence of acceptable dosage.

Neem grows in the plains and in areas up to an elevation of 1850 m. In its introduced range, Neem is cultivated from sea level to an altitude of 1500 m. Neem is tolerant to most soil types including dry, stony, shallow soils, lateritic crusts, highly leached sands and clays. With an extensive and deep root system, the hardy Neem can grow and flourish even in marginal and leached soils. The Neem tree is noted for its drought resistance. Normally it thrives in areas with sub-arid to sub-humid conditions, with an annual rainfall between 400 and 1200 mm. It can grow in regions with an annual rainfall below 400 mm, but in such cases it depends largely on the ground water levels. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils (pH 6.2-7.0). It is a typical tropical/subtropical tree and exists atannual mean temperatures between 21-32 °C. It can tolerate high to very high temperatures. Itdoes not tolerate temperature below 4 °C (leaf shedding and death may ensue).(Debjit Bhowmik et al 2010)

Health Benefits

Neem needs no introduction in today's world. Neem is known as free tree of India as it is found almost everywhere in India. It is considered as a magic tree, which has properties that not onlyrelieves but also cures from illness.Neem is an herb that has been a great assert to human species since thousands of centuries. Neem is extremely useful to humans and this is the reason it is being worshiped in India and is considered as the place where Gods resides. It is said that no evil spirits dares to come near a neem tree and this is the reason neem is a part of every Indian house.Neem is used for treatment of eye problems such as night blindness and conjunctivitis. In case of night blindness, apply the juice of the neem to the

eyes externally each night. Direct application has better results. This is done by grinding the neem leaves to a fine powder and then making a paste of this with water. Strain this juice through a clean cloth and apply the juice which filters out onto the eyes with an eye rod. In conjunctivitis, apply the neem juice obtained from its leaves directly onto the eyes. Neem has been used as a medicine for more than 5000 years. Neem is especially good for those with skin disorders such as eczema. As a natural eczema remedy, neem when applied on the skin relieves you from itching and the painful symptoms arising from your disorder. You can also take a warm bath with neem leaves in it. In fact, this is a very common custom in India. It is also highly suitable in the instance when you have some minor infections. Acne causing bacteria are killed by neem. Boil some neem leaves in water and use the water to wash your body.(Debjit Bhowmik et al 2010).

Neem is known to have antiallergenic, antidermatic, antifeedant, antiviral, antifungal, anti-inflammatory, antipyorrhoic, antiseabic insecticidal, lavicidal, anti-implantation, nematicidal, spermatocidal, and other biological activities.(Ogbuewu IP *et al,* 2011)

Medicinal Properties

Medicinal properties of neem have been known to Indians since time immemorial. The earliestSanskrit medical writings refer to the benefits of neem's fruits, seeds, oil, leaves, roots and bark. Each of these has been used in the Indian Ayurvedic and Unani systems of medicine.In Ayurvedic literature neem is described in the following manner: 'Neem bark is cool, bitter, astringent, acrid and refrigerant. It is useful in tiredness, cough, fever, loss of appetite, worm infestation. It heals wounds and vitiated conditions of kapha, vomiting, skin diseases, excessive thirst, and diabetes. Every part of the tree has been used astraditional

medicine for household remedyagainst various human ailments, fromantiquity(Chopra,*et al* 1958)

Diabetes mellitus (DM), also known as simply diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (WHO, 2014).

This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes. (WHO, 2013)

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced.Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes, and "other specific types". The "other specific types" are a collection of a few dozen individual causes. The term "diabetes", without qualification, usually refers to diabetes mellitus(Shoback, 2011).

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin. It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed

"juvenile diabetes" because a majority of these diabetes cases were in children(Rother, KI (April 2007)).

"Brittle" diabetes, also known as unstable diabetes or labile diabetes is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. Still, type 1 diabetes can be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes with serious hypoglycemia. Other complications include an impaired counter regulatory response to hypoglycemia, infection, gastro paresis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). (Merck, April 2010) These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1diabetes.(DornerM .*et al,* may,1977)

Type1diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. There is some evidence that suggests an association between type 1 diabetes and Coxsackie B4 virus. Unlike type 2 diabetes, the onset of type 1 diabetes is unrelated to lifestyle.

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type ((Shoback, 2011)).

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization.(Risérus U.*et al*, January 2009).

Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders (*Williams textbook*). Those who are not obese often have a high waist–hip ratio.

Dietary factors also influence the risk of developing type 2 diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik VS, *et al* 2010). The type of fats in the diet is also important, with saturated fats and trans-fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk. Eating lots of white rice appears to also play a role in increasing risk. (Hu EA*et al 2013*). A lack of exercise is believed to cause 7% of cases.

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management

may include dietary changes, blood glucose monitoring, and in some cases insulin may be required(National Diabetes stastistics, 2011).

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

Other types

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology.Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is

typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The ICD-10 (1992) diagnostic entity, malnutrition-related diabetes mellitus (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999(WHO, 1999).

ORGAN OF STUDY

Gross Anatomy of Pancreas

The pancreas is a glandular organ in the digestive system and endocrine system of vertebrates. In humans, it is located in the abdominal cavity behind the stomach. It is an endocrine gland producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide which circulate in the blood. The pancreas is also a digestive organ, secreting pancreatic juice containing digestive enzymes that assist digestion and absorption of nutrients in the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids in the chyme.

Glucose is measured in whole blood, plasma or serum. Historically, blood glucose values were given in terms of whole blood, but most laboratories now measure and report plasma or serum glucose levels. Because red blood cells (erythrocytes) have a higher concentration of protein (e.g., hemoglobin) than serum, serum has a higher water content and consequently more dissolved glucose than does whole blood. To convert from whole-blood glucose, multiplication by 1.15 has been shown to generally give the serum/plasma level.

Animal sacrifice

- After six weeks of administration all the rats were sacrificed by cervical dislocation

- The dissecting board for the process was kept on the table and a white cardboard was pinned down on it.
- Immediately after the dislocation which weakens the rats and leaves them pliable for dissection, the blood sample was taken and kept in an EDTA bottle and the organ pancreas was harvested from the abdominal cavity using surgical scissors and forceps.
- The extracted tissues were weighed on a peril dish with an electronic sensitive balance.
- After weighing, some part of the tissue was fixed in fixative and some part of the tissue was homogenized in a ceramic mortar and pestle. The ceramic mortal was placed on an ice block so as to prevent the autolysis of the homogenate.
- The pancreas from each rat was fixed in 10% formosaline in a labeled

RESULT ANALYSIS

Table 1; Body weight

WEEK	CONTROL GROUP S.E.M	DIABETES GROUP S.E.M	AQUEOUS LOWDOSE OF NEEM BARK S.E.M	AQUEOUS HIGHDOSE OF NEEM BARK S.E.M
WEEK 0	147.9 ± 5.723	165.5±7.282	160.4 ± 4.825	225.0 ± 6.155
WEEK 1	153.2 ±4.872	160.00 ± 15.0*	186.5 ± 10.33 *	180.0 ± 5.00 b
WEEK 2	133.4 ± 3.563	132.50± 12.39*	170.5 ± 11.59*/ c	155.0 ± 7.265b/ c
WEEK 3	129.2 ± 5.180	120.00 ± 11.06b	156.8 ± 12.20*/ c	142.5 ± 7.500b/ c
WEEK 4	150.0 ± 4.352	105.00± 11.67*	185.0 ± 10.67*/ c	165.0 ± 7.638b/ c

WEEK 5	158.3 ± 3.553	95.00± 8.975*	190.0± 15.46*/ b	175.0 ± 7.457*/ a *
WEEK 6	160.4 ± 4.825	85.00± 7.638*	195.0 ± 17.40*/ b	181.8 ± 8.320b/ c

Values are mean±SEM, where * = P<0.05 when compared to control group, b=ns when compared to control group, a* = P<0.05 when compared with the diabetes group, and c= ns when compared with the diabetes groups.

From the table above, there was a gradual decrease in the body weight of the streptozotocin induced diabetes animals. With the administration of the aqueous extract, the experimental animals show a gradual increase in their body weights.

Table 2;Initial and Final body weight of experimental animals

	CONTROL GROUP S.E.M	DIABETES GROUP S.E.M	AQUEOUS LOWDOSE OF NEEM BARK S.E.M	AQUEOUS HIGHDOSE OF NEEM BARK S.E.M
BEFORE ADMINISTRATION	147.9±5.723	165.5±7.282	160.4±4.825	225.0±6.155
AFTER ADMINISTRATION	160.4±4.825	85.0±7.638	195.0±17.40	181.8±8.320

From the table above, the aqueous extract of neem bark has increasing effect on the body weight of experimental animals, when comparing the weight of the experimental animals before the administration of aqueous extract of neem bark with the weights of the experimental animals after the administration of aqueous extract of neem bark..

Table 3;relative organ Weight

	CONTROLGROUP	DIABETES GROUP	AQUEOUS LOWDOSE OF NEEM BARK	AQUEOUS HIGHDOSE OF NEEM BARK
PANCREASEWEIGHT	0.3708	0.2450	0.2870	0.4070
RELATIVEPANCREATIC WEIGHT	0.2312	0.1329	0.1472	0.2239

The streptozotocin induced diabetes wistar rat's organ (pancreas), show a reduction in weight when compared with the control. When compared with the extract, the organ weight has increased tremendously; hence, the aqueous extract of neem bark has increasing effect on the organ weight of the experimented animals.

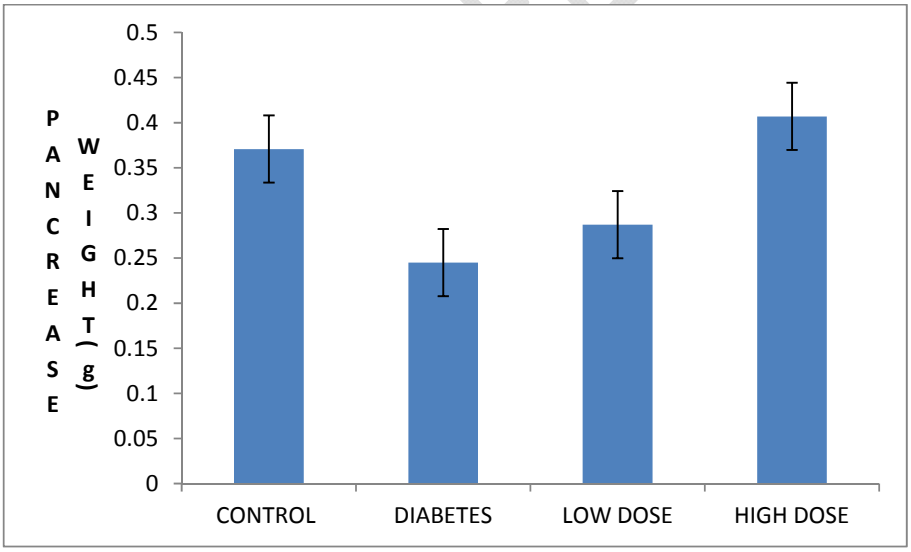


Figure5; graph showing organ weight in streptozotocin induced diabetes

Table4;Effect of Aqueous Neem Bark on Blood Glucose Level

WEEKS	CONTROL GROUP S.E.M	DIABETES GROUP S.E.M	AQUEOUS LOWDOSE OF NEEM BARK S.E.M	AQUEOUS HIGHDOSE OF NEEM BARK S.E.M
WEEK 0	92.42±1.288	93.50± 4.075	88.45± 4.674	82.09±3.706
WEEK 2	92.42±1.288	158.0±33.70*	114.0±12.64*/t*	94.60± 4.895 */t*
WEEK 5	92.42±1.288	149.5±35.55 *	90.50±3.707*/t*	87.50±2.136q/t*
WEEK 6	95.42±1.994	155.9±29.93 *	96.40±1.979q/t*	95.70±3.774q/t*

Values are mean±SEM, where * = P< 0.05 when compared to control group, q= ns when compared to control group, and t* = P<0.05 when compared to diabetes group.

From the table above,

Week 0, showed a fairly constant blood glucose level from the control group to the high dose of the aqueous extract of the neem bark.

Week 2, after induction of streptozotocin into the experimental animals, the experimental animals treated with the aqueous extract of neem bark shows a gradual reduction in blood glucose level.

Week 5, the treated animals showed a further reduction in the blood glucose level.

Week 6, the treated animals showed a fluctuation in the blood glucose level from the low dose extract of aqueous neem bark to high dose of aqueous extract of neem bark.

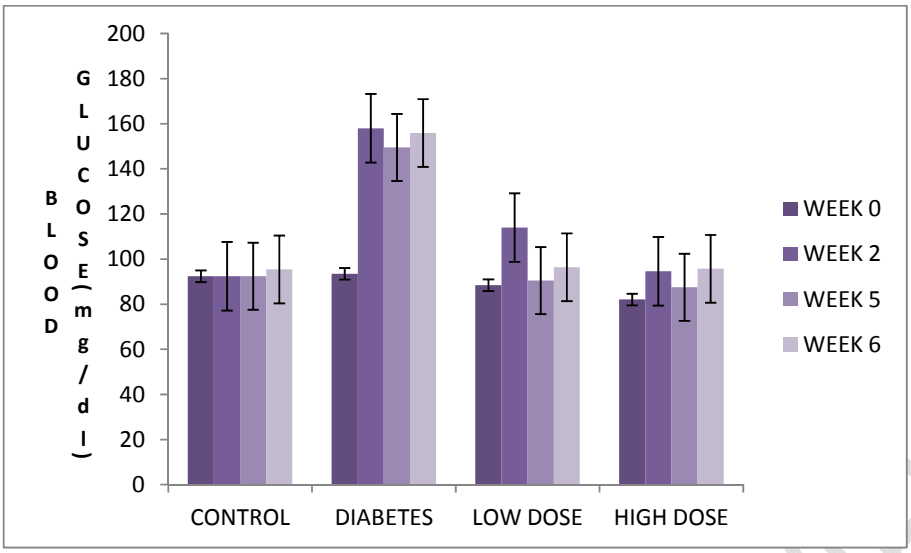


Figure6; graph showing changes in the blood glucose level.

Table 5;CHNGES IN SERUM ENZYMES ACTIVITIES

	CONTROL GROUP S.E.M	DIABETES GROUP S.E.M	AQUEOUS LOWDOSE OFNEEM BARK S.E.M	AQUEOUS HIGHDOSEOF NEEM BARK S.E.M
AST	151.8±0.5478	233.7±20.78 ^{c*}	180.3±3.829$^{c*/ n*}$	156.3±10.26$^{c*/ n*}$
ALT	23.99±4.448	72.06±5.399^{c}	41.11±2.578$^{c/ n}$	29.98±2.501$^{c/ n}$
ALP	12.00±0.3411	26.23±0.3449^{c}	19.85±0.1355$^{c/ n}$	17.07±0.1049 $^{c/ n}$

Values are mean±SEM where ^{c*} is P< 0.005,when compared with control group, c = ns, when compared to control group, n* P< 0.005 when compared to diabetes group, and n= ns when compared with the diabetes group.

Aspartate Amino Transferase (AST)

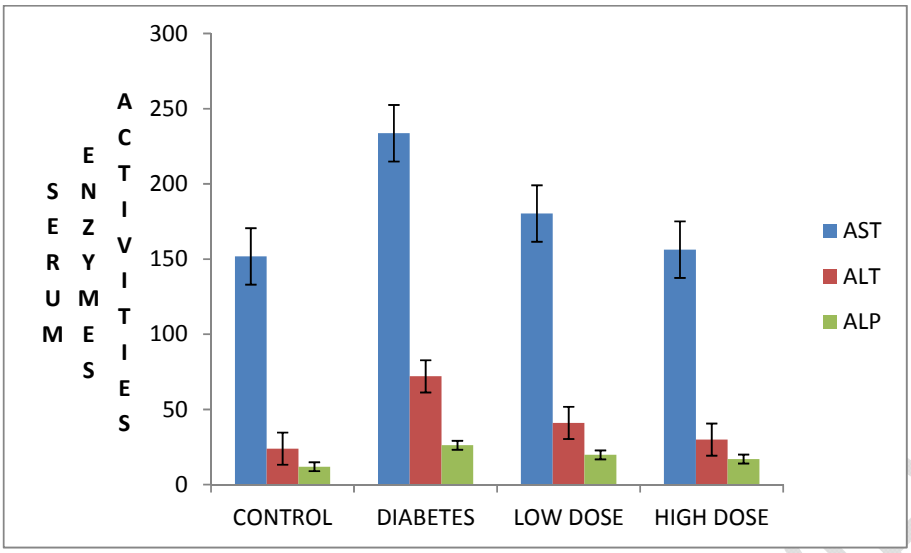
When comparing the enzymatic activities of the control group with the diabetes groups, the enzymatic activities of aspartate amino transferase of diabetes group increases tremendously. When compared with the treated groups, the activity of aspartate amino transferase reduces gradually from the aqueous low dose of neem bark to the aqueous high dose of neem bark respectively.

Alanine Transferase (ALT)

When comparing the control group with the diabetes group, the enzymatic activity of Alanine amino transferase increases significantly. After, the administration of aqueous extract of neem bark, there was a gradual reduction in the activity of the alanine amino transferase from the low dose to the high dose respectively.

Alkaline Phosphatase (ALP):

From the table above, when the control group was compared with the diabetes group, there was an increase in the activity level of alkaline phosphatase. When comparing the diabetes groups with the treated groups, there was a gradual reduction in the activity of the enzymatic activities from the low dose to the high dose respectively.



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373 **Figure 7;showing serum enzymes levels or activities in streptozotocin induced diabetes**

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375 **Table 6;showing changes in Homogenate enzymes**

HOMOGENATE ENZYMES ACTIVITIES	**SOD** **S.E.M**	**GSH** **S.E.M**	**MDA** **S.EM**
CONTROL	52.75±2.181	33.14±1.229	19.10±3.177
DIABETES	20.55±2.010 [e]	7.368±0.3267 [e]	24.30±.698 [e]
LOW DOSE	27.91±0.2633[e]*/ [f *]	12.90±0.1900 [e]*/ [f]	16.11±3.25 [e]*/ **f**
HIGH DOSE	36.85±1.960[e/ f]	9.435±1.905 [e/f]	11.78±0.31[e]*/ [f *]

376 Values are mean±SEM, where [e*] is P< 0.005 when compared to control group, [f *] is P< 0.005
377 when compared to diabetes group, [e] = ns, when compared to control, and [f] = ns, when
378 compared to diabetes group.

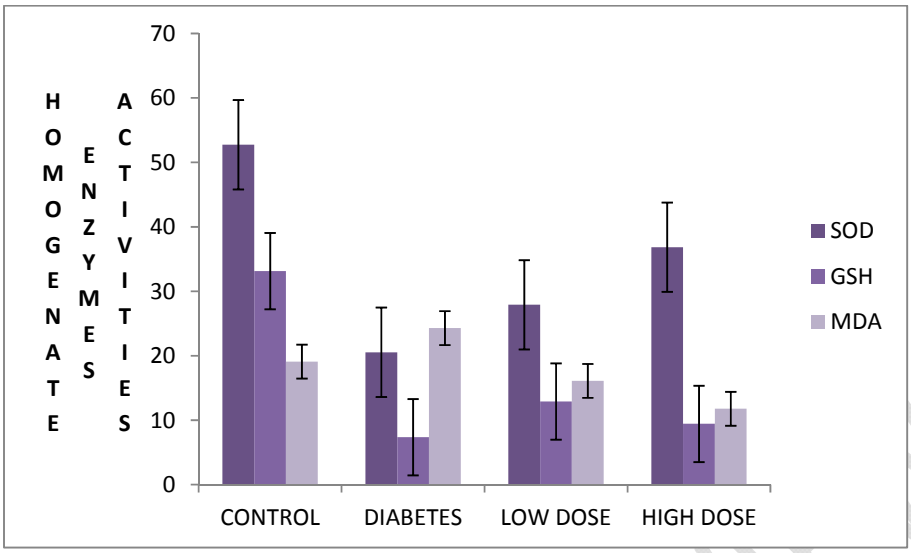
379 **Superoxide dismutase (SOD):** From the table above, the level of superoxide dismutase
380 reduced in the diabetes groups, which can lead to oxidative stress in the tissues of the animals
381 in question. It is well noted from the table above, that aqueous neem bark has the power of

suppressing oxidative stress in the tissues of the animal. Moreover, higher dose of the extract goes a longer way in the enhancement of superoxide dismutase activities, since it is a scavengers which attacks oxygen radicals. Oxygen radicals are the major causes of oxidative stress.

Reduced glutathione (GSH): This enzyme is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds as well as maintaining exogenous antioxidantsuch as vitaminC and E in their reduced form. From the table above, the level of this enzyme reduces drastically in the diabetes groups only, and its shows an increment with the administration of aqueous low dose of neem bark extract, but, with the administration of aqueous high dose of the extract, it shows a reduction in the enzymatic activities of reduced glutathione. This suggests the effectiveness of the aqueous low dose of the neem bark extract rather than the aqueous high dose of the extract in the enhancement of this enzyme.

Malondialdehyde (MDA): This is an organic compound which occurs naturally, and it is the maker for oxidative stress,which means, if this substances increases in the diagnosis of a patient, its mark oxidative stress in the patient i.e the released of excess free radical in the body system. .

From the table above, the administration of aqueous neem bark extract reduces the risk of having free radicals in the body system. Theaqueous low dose extract of neem bark, enhances the effectivess of malondialdehyde and indirectly reduces oxidative stress in the tissues of an organism. While, the aqueous high dose of the extract shows no effectiveness in reduction of oxidative stress.



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406 **Figure 8; graph showing the homogenate enzyme activities.**

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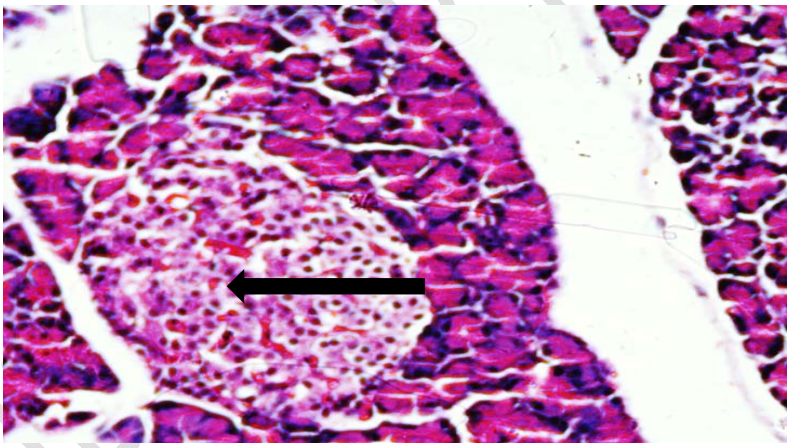


408

409 **PLATE I, CONTROL (X 100) H&E**

410 Photomicrograph showing the normalarrangements of the endocrine cells of the islet of

411 Langerhans in wistar rat.

412

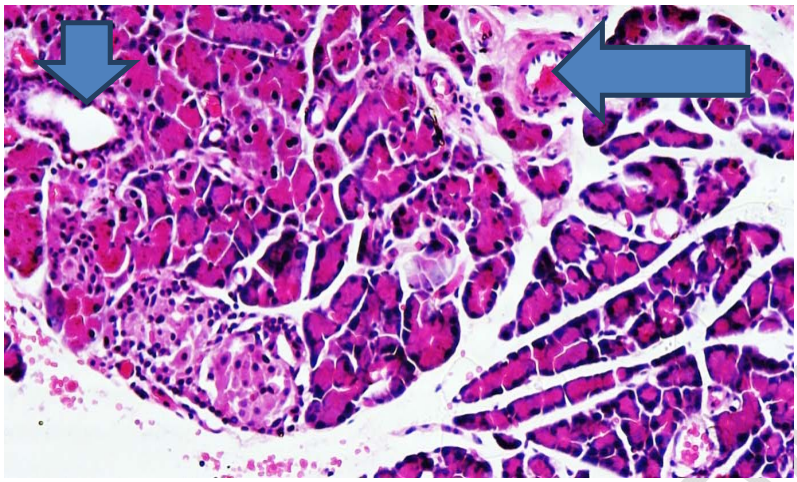


PLATE III, DIABETES GROUP (X100)

Photomicrograph; of islet of Langerhans tissue of streptozotocin induced diabetes ratsshowing some necrotic area of the islets of Langerhans, and the number of cells available have reduced compared to the normal islets of Langerhans tissue. The small arrow shows part of islets of Langerhans tissue and bigger arrow showing the necroticarea.

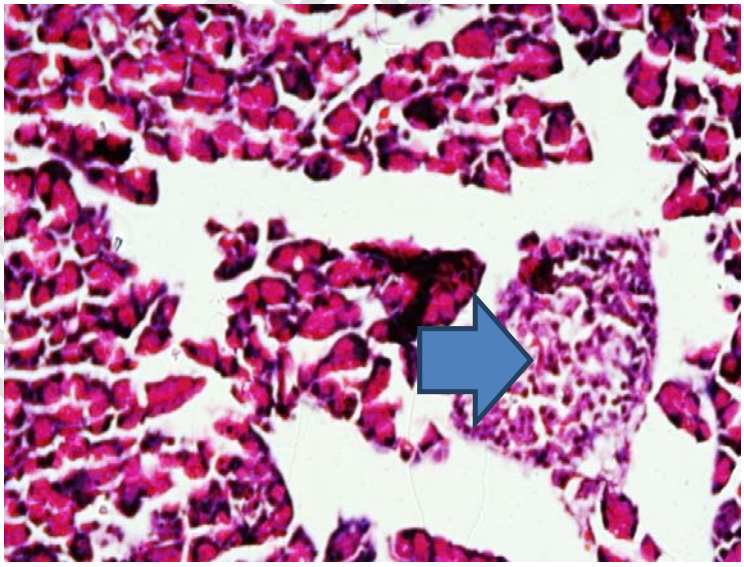
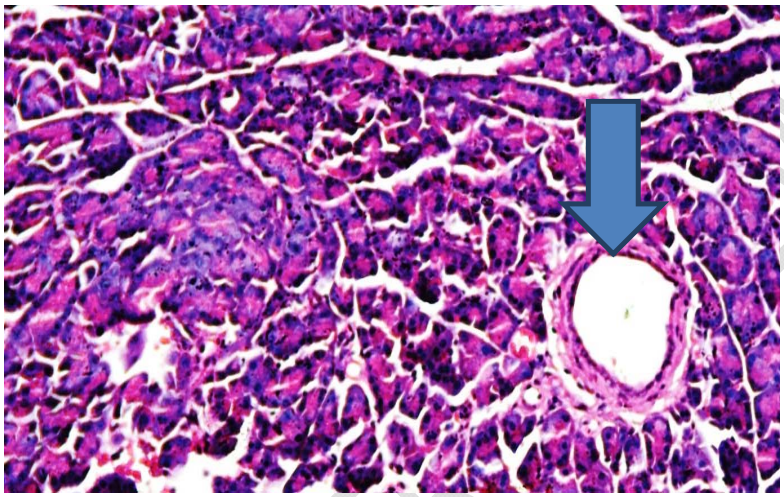


PLATE V, Low Dose neem bark (X 100) H&E

Photomicrograph showing islets of Langerhans in adult wistar ratfollowing treatment with aqueous neem bark extract at a dose of 250mg/kg for a period of six weeks showing tissue recovery.



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Histological findings

The pancreas samples were processed using normal histological techniques of H&E. The following were observed from the photomicrograph of the pancreas.

Control group plate; which was given water and growers mash throughout the experiment. The photomicrograph is showing a normal histological feature of islet of Langerhans. Although, the alpha and beta cellsappeared similarly because of the nature of the staining, the cells are correctly located and the morphological arrangements of these cells arein order. The alpha cells located at the peripheral surface of the islet of Langerhanswhile that of the beta cells are located in the inner part of the tissue.

Diabetes group plate; these groups were given water and feed, and besides they were induced with streptozotocin which were injected intra-peritoneally. The plate shows some part of the islet of Langerhans degenerated or translucent areas wereshown as a result of the streptozotocin induction. There are some vessels which were evident in the plate of the diabetic group, this vessels are the venule and the arteriole. The venule collapsed, while the arteriole seems to be having some clot in it lumen, the arrangement of the cells are not normal.

Low dose group plate; this group shows some normal histological feature of the islet of Langerhans and some of the cells are not in good morphological condition. Some of the cells are necrotic while majority of the cells are showing good morphological features.

High dose group plate; this photomicrograph shows single blood vessel and the histological appearance seems to be normal and having a good orientation. The vessels shows a clear lumen, unlike the clot that was the diabetics group.

Blood glucose level

Control group. The blood glucose level remains fairly constant throughout the whole weeks of experimentation.

Diabetes group. The blood glucose level heightens after streptozotocin induced induction of diabetes.

Low dose. In this group, the blood glucose levelswere reduced gradually.

Highdose. The blood glucose level reduced a little farther than the low dose group.

Organ weight

The organ weight shows a remarkable increase in the weight of the organ of theexperimental animals, after the administration of aqueous extract of neem bark for a period of six weeks, when compared with diabetes group.

Enzymatic Activities analysis

Alanine amino transferase (ALT): From the graph above, the level of the enzyme activity of diabetes group increases gradually when compared with the control group. After the administration of aqueous extract of neem bark, there was a reduction in the enzymatic activities from the low dose aqueous extract to the high dose aqueous extract of neem bark respectively.

Aspartate amino transferase (AST); from the graph above, aspartate amino transferase activities increases steadily when comparing control group with diabetes group. After the administration of the aqueous neem bark extract, there was reduction in the enzymatic activities from the low dose to the high dose respectively.

Alkaline phosphatase (ALP); When comparing enzymaticactivityof alkaline phosphatase of control group with the diabetes group, there was an increase in the enzymatic activities. After, the administration of aqueous extract of neem bark, there was a reduction in the activities of the alkaline phosphatase from low dose to the high dose respectively.

Hence, the administration of the aqueous neem bark extract was effective on,

1. The body weight of the experimental animals,
2. The organ weight of the experimental animals,
3. The reduction of blood glucose level in the experimental animals and
4. The enzymatic activities in the blood samples of the experimental animals.

5. The pancreas homogenate.

DISCUSSION

Some of the effect of suspended aqueous neem bark extract on the experimental animals was investigated to explore the possible histological implications,relative index of organ weight, possible changes in the blood glucose level and levels of serum enzymatic activities that could follow its use.

Effects of Aqueous Neem Bark on BodyWeight

The effect of aqueous neem bark extracton body weight instreptozotocin induced diabeteswistar rats is shown in Figure 3.

Weight of wistar ratsin the diabetes group, were shown to have reduced tremendously starting from the first week to the six week of streptozotocin induction of diabetes groups only.

When comparing the treated with the diabetes group, it was shown that neem has a potential effect in the increment of the body weight from the dose of 250mg/kg per body weight – 500mg/kg per body weight.

This result has similarities with the findingsof Bopanna *et al.,* 1997; Akpan *et al.,* 2012who found that body weight of all thetreated groups were significantly (P<0.05)increased with neem treatment compared todiabetesrats. They suggested that this may

be due to some constituents of the neemextract which may have mimicked orstimulated the actions of growth factorshence its ability to enhance the repair andregeneration of damaged pancreatic tissue.

Effects of Aqueous Neem Bark on Organ Weight

The organ (pancreas) from the diabetes group only, show a reduction in weight when compared with the control. When compared with the treated, the organ weight has increased tremendously; hence, the aqueous extract of neem bark has increasing effect on the organ weight of the experimented animals. It was shown that no researcher has review the effect of neem on organ weight.

Effects of Neem on Blood Glucose Level

Wistar rats that were treated with streptozotocin aloneremarkably showed high blood glucose level, whereas, wistar rats treated with aqueous neem bark extractsignificantly ($P<0.05$) reducedblood glucose levels (Figure 6). It was reported that the hyperglycemiceffect of streptozotocin may be due to damagethe cells of pancreas that interfered thesynthesis of insulin which might beresponsible for the metabolism of glucose(Bopanna *et al.,* 1997).
The mechanism of the anti-diabetes properties of the extract was not well known.Jelodar *et al.,* (2005) had suggested that theanti-diabetes properties of the extract may berelated to the ability of the extract tostimulate sufficient production of insulin by the pancreas, that aided in the peripheralutilization of glucose in the cells or apossible ability of the extract to regeneratethe cells to carry out its functions.

Effect of Aqueous Neem Bark Extract on Histology of Pancreas

From the histological platesabove, plate I and plate II are the control group at the magnification of 100 and 400. These plates showed a normal histology of the pancreas of the adult wistar rat.

Plates III and IV are the histological plates of the diabetes group only at a magnification of 100 and 400 respectively.These plates show necrotic areas which was as a result of streptozotocin induction into the experimental animals. From plate III, there were two different vessels, which were the arteriole and the venule. The arteriole was shown to have some areas of blood clot which is indicated by a bigger arrow. Theplates of the diabetes group did not show a normal histological arrangement of the cells in the tissue of the pancreas.

Plates V and VI, were the histological plate of the low dose treated with the aqueous neem bark extract which show a normal histology of the tissue of the islet of Langerhans. The cells of the tissue were arranged properly and the cells are in order.

Plates VII and VIII were the histological plates of treated high dose of aqueous neem bark extract. The plates showed a normal histology of the pancreas when compared with the control group.

When comparing the histological plate III and the histological plates VII and VIII, the arteriole blood vessel being affected in plate III by a stainof blood or a clot in the lumen, has been cleared or the blood clot has been removed.

This is in accordance with Sarwar .N et al 2010, thatmajor long-term complications of diabetes relate to damage ofblood vesselsand it doubles the risk of cardiovascular disease and about 75% of deaths in diabetes are due to coronary artery disease (O'Gara PT et al 2013).

CONCLUSION

The results obtained from the present study show that Azardirachta indica aqueous extract had beneficial effects on blood glucose levels in streptozotocin induced diabetes rats. It confirms to be an attractive material for further studies, leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming. However, the results from this study give scientific support to the use of Azardirachta indica.

RECOMMENDATION

From the research work carried out on the hypoglycemic effect of aqueousneem bark on streptozotocin induced diabetes, it was shown that aqueous neem bark has significant lowering effect on streptozotocin induced diabetes.

Thus, the intake of this decoction is recommended for people with diabetes mellitus. It is an advantage for people who are slender; since this has increasing effect on body weight from the research work carried out, and can help to add weight, because being slender in stature or light in body weight can be very dangerous for a woman who has not had experience of child birth.

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