EFFECTS OF CRUDE EXTRACT OF NEEM BARK ON THE PANCREAS OF STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

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ABSTRACT

This study investigated the histological and serum enzymatic activities of *Azadirachta indica*, an Indian medicinal plant, on the pancreas in streptozotocin induced diabetic adult wstar rats.

Forty six adult wistar rats weighing 100g to 220g were randomly separated into four groups; Group A was regarded as the control , while group B was the diabetic group ,C and diabetic – extract treated group. The control group received distilled water throughout the experiment; the remaining three groups were induced with streptozotocin intra-peritoneally to induced diabetes in the wistar rats. After some days, the animals were confirmed diabetic with the help of a measuring glucometer. Thereafter, group B diabetic rats remained untreated while Group C and D were treated with a low dose (250mg/kg) of the crude neem bark extract and a high dose (500mg/kg) of the extract respectively for 42 days. 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 weekly and likewise the measurement of the blood glucose level was taken. The animals were sacrificed at the end of 42days by cervical dislocation 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% formol saline, processed and stained with Heamatoxylin and Eosin for general histological study.

The microscopic examination of diabetic group showed some degenerative and necrotic cells which made the pancreatic tissue distorted compared with the control that appeared normal. The diabetic group C and D rats treated with the extract showed ameliorative potentials of the extract with improvement in histo-architecture of the pancreatic tissue following recovery from damage. Analysis of the blood serum level showed that the aqueous neem bark extract has ameliorative effect on the enzymatic activities in serum of the treated rats. The alanine amino transferase, aspartate amino transferase and alkaline phosphatase levels were significantly reduced ($P<0.05$) in the crude extract- treated animals compared with the untreated group B and control group A with significantly increased ($P<0.05$) enzymatic activities in wistar rats in these groups. The antioxidant status was compromised in diabetic group B with significantly increased ($P<0.05$) LPO, reduced SOD and GSH compared with significantly reduced($P<0.05$) LPO, increased ($P<0.05$) SOD and GSH in group A- control and Group C and D diabetic-extract treated Wistar rats

Similarly, the blood glucose level increased significantly ($P<0.05$) in group B diabetic group compared with significantly reduced ($P<0.05$) blood glucose level in group A control as well as C and D extract-treated rats . The study concluded that crude extract of

neem has ameliorative potentials on streptozotocin-induced diabetic wistar rats characterized by oxidative damage which reveals improvement in tissue morphology.

Key words: *Diabetes, degenerative, Streptozotocin, Azadirachta .neem, ameliorate, antioxidant*

INTRODUCTION

Report from the literature has described *Azadirachta indica* a large evergreen tree that belongs to the family Meliaceae known in many countries of the world. This plant is believed to have originated from Assan and Burma in South Asia[1] and thrives well in sub-tropical and tropical regions of the world[2] . It has the characteristic potential to withstand many adverse environmental conditions such as, shallow, infertile soil, drought, , stony and acidic soil[2]. Research finding has indicated that the major active constituents of this plant are nimbin, nimbidin and nimbinene [1,3]. Similarly, the leaves possess quercetin (Flavonoid) and nimbosterol (β-sitosteriol) coupled with a number of liminoids ([2]. The trunk bark contains nimbinene (0.001%), nimbin (0.04%), tannins (6.0%), while, the stem bark contains tannins (12-16%) and non-tannins (8-11%)[3] . It has been revealed that the oil that is derived from the seeds contains nimbosterol and flavonoids [3].

Additionally, other investigations have shown that *Azadirachta indica* has a wide range of uses. For instance, the oil extracted from the seeds (Neem Oil) possesses insecticidal properties [4] . Neem cake; obtained from the residue of neem seed after oil extraction is used as fertilizer which enhances soil fertility [4] nematicide [4] and to reduce soil nitrogen loss. Furthermore, It has been also discovered that *Azadirachta indica* could be used as traditional remedies for treatment of various types of diseases from antiquity. Essentially, previous studies has document that all the various parts of neem plant are said to have some medicinal properties[3] . Moreover, the plant extract is traditionally used for treatment of arthritis, typhoid, respiratory disorders, constipation, chronic fatigue, leprosy, cancer, chronic syphilis sores and indolent ulcer. Other findings have revealed that it is used traditionally as tonic and astringent for wounds healing, tooth decay and gum diseases [3] and also generally used as health conditioner. it is also known to be a potent antibacterial ,antimalarial and antifungal[5].

Previous studies have similarly reported that *Azadirachta indica* is generally used worldwide in the treatment of diabetes mellitus [6, 7]. It has been shown that its use for the treatment of diabetes in Ayurveda (India) was based on the ancient belief that when an excess of one taste causes a disease, the opposite taste is introduced into diet to counteract the effect of the other[8]. Findings from both traditional and clinical trials have supported the fact that *Azadirachta indica* is a potent antidiabetic agent, consequently, based on the numerous successful clinical trials, the Indian Government approved the manufacture and sales of neem tablets by pharmaceuticals bodies for diabetes treatment [3]. Findings from previous studies have demonstrated the effect of neem extract on **blood glucose** levels both in humans and animal diabetic models [9,10,8]. Although the mechanism of antidiabetic properties of neem extract is not fully understood. However, Some proposed modes of action of the plant reported include; the presence of insulin-like substances or substances which interfere with carbohydrate absorption, inhibition of insulinase activity and/or increase in number of

functional beta cells in the pancreas of the diabetics [11]. The present study was designed to determine the effect of crude extract of the bark of *Azadirachta indica* on blood glucose level, antioxidant status, and histo-architecture of pancreas of streptozotocin - induced diabetic Wistar rats

MATERIALS AND METHODS

Forty-six adult Wistar rats weighing between 125g-250g, were used for this experiment.

The animals were housed in a plastic cage having wooden shavings, to serve as shock absorber and to provide them with a micro environment. The wooden shaving were gotten from Anuoluwapo sawmills industry in Ogbomosho, Oyo state**.** The rats were housed in their respective cages under natural light and dark cycles at room temperature and were given growers mash for water and acclimatize them to their new environment for two weeks. The rats were maintained in the Departmental Animal Holdings and were housed in standard animal cages, fed with standard rat chow and distilled water was given to the Wistar rats *ad libitum*. The rats were maintained under standard laboratory conditions. They were also given adequate care in accordance with the principles of laboratory and animal care as indicated and published by Institute of Laboratory Animal Resources 1996[12]

Neem bark was gotten from under-G Lautech Ogbomosho, Ogbomoso Oyo state. The neem bark was then air dried for approximately four weeks under laboratory condition. Later, the

Neem bark was pounded by a wooden mortar and a wooded pestle. The sieved substance of the Neem bark was approximately 500g. The 500g of the resulting pounding was used for the decoction. The extract was prepared by soaking the resulting substance in a 4.5litres of water and heated for several hours, and was sieved and allowed to cool.

The control group was given water throughout the experiment, Diabetic groups were induced with streptozotocin and given water throughout the experiment, Group B was diabetic –induced , group C was induced with streptozotocin and was later treated with a dose of neem bark extract of 250mg/kg of body weight, while high dose group D was induced with streptozotocin and similarly treated with a high dose of neem bark extract of 500mg/kg per body weight. The extract was administered to them daily for six weeks or 42days by suspension in water. The blood sugar concentration or blood glucose level was evaluated during the study

The measurement of the blood glucose level was done by the blood glucose monitoring meter or Glucose meter. The procedure for the measurement was done by taking sample of blood from fasting animal for a period of 8hours before meal. The extreme tail of the wistar rat was pinched with a needle to take sample of blood, the blood sample from the tail of the wistar rat was quickly transferred on the strip (a slender electronic part of the measuring machine) attached to a glucometer [13]. (Chiaohsin Yang *et al*, 2012*)*

After six weeks of administration all the rats were sacrificed by cervical dislocation and blood sample was taken and kept in an EDTA bottle following dissection of each rat and the pancreas was harvested from the abdominal cavity using surgical scissors and forceps. The extracted tissues were weighed using a sensitive balance. After weighing, some parts of the tissue were 10% formol saline to be processed for light microscopy and the other part of the tissue was homogenized for biochemical analysis specific parameters.

RESULTS

TABLE 1: PANCREATIC WEIGHTS AFTER TREATMENT

Groups	PANCREATIC WEIGHT	RELATIVE PANCREATIC WEIGHT
Group A-Control	0.370	0.231
Group B-Diabetic group	0.245	0.133
Group C- Diabetic –Treated	0.287	0.147
Group D-- Diabetic –Treated	0.407	0.224

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.

Table 2: Effect of Aqueous Neem Bark on Blood Glucose Level

WEEKS	GROUP A	GROUP B	GROUP C	GROUP D
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±2.136$^{q/\,t*}$
WEEK 6	95.42±1.994	155.9±29.93 *	96.40±1.979$^{q/\,t}$*	95.70±3.774$^{q/\,t\,*}$

Values are Mean ±SEM, where * = P< 0.05 Significant when compared to Control group, q= ns when compared to Control group, and t* = P<0.05 Significant when compared to Diabetic group.

The above table shows that Week 0, indicates a fairly constant blood glucose level in the control group to the high dose of the aqueous extract of the neem bark.

Week 2, after induction of diabetes using streptozotocin in 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.

Table 3: CHNGES IN SERUM ENZYMES ACTIVITIES AFTER TREATMENT

	GROUP A	GROUP B	GROUP C	GROUP D
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, Significant when compared with Control group, c = ns, when compared to control group, n* P< 0.005 Significant when compared to diabetic 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.

Table 4; SHOWING CHANGES IN HOMOGENATE ENZYMES

PARAMETER	SOD	GSH	MDA
CONTROL	52.75±2.181	33.14±1.229	19.10±3.177
DIABETIC	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$^{e*/f*}$

Values are Mean ±SEM, where ^{e*} is P< 0.005 significance when compared to control group, ^{f*} is P< 0.005 significance when compared to diabetic group, ^{e} = ns, when compared to control, and ^{f} = ns, when compared to diabetes group.

Superoxide dismutase (SOD): From the table above, the level of superoxide dismutase reduced in the diabetes groups, which can lead to oxidative stress in the tissues of the animals 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.

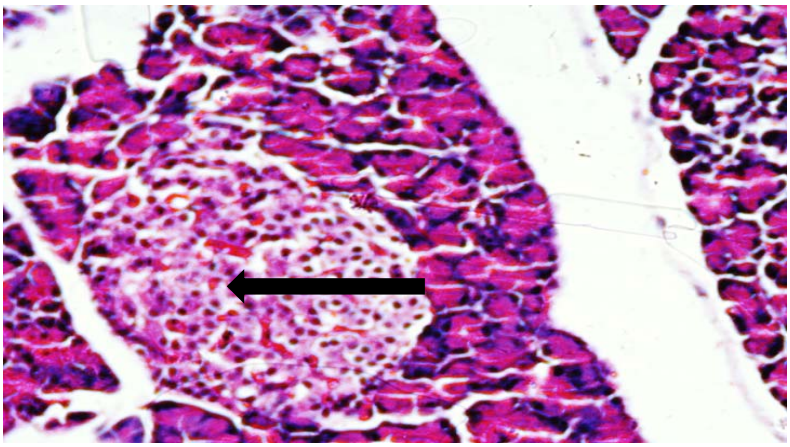

FIGURE I; CONTROL (X 100) H&E

Photomicrograph showing the normal morphology of the endocrine cells of the islet of Langerhans in wistar rat.

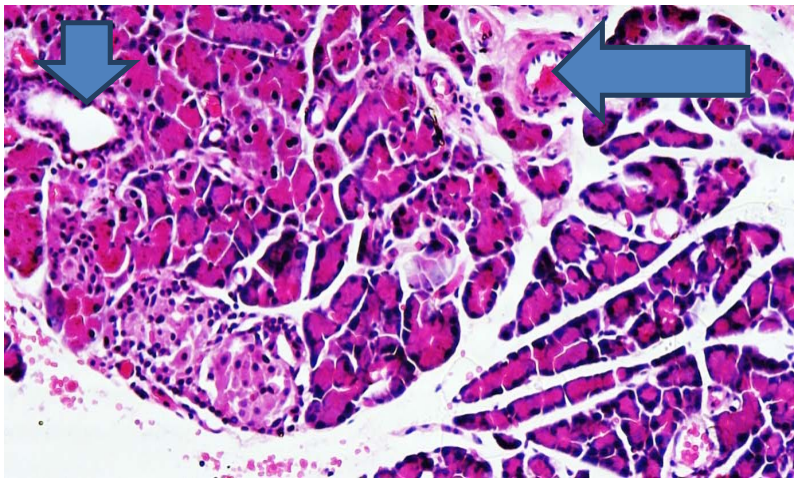

FIGURE 2; DIABETIC GROUP (X100)

Photomicrograph; of islet of Langerhans tissue of streptozotocin induced diabetic rats showing 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.

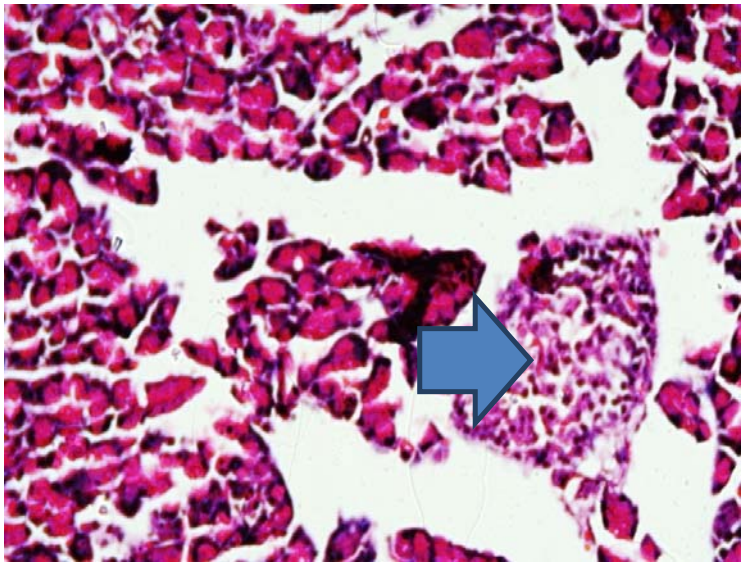

FIGURE3; Low Dose neem bark (X 100) H&E

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

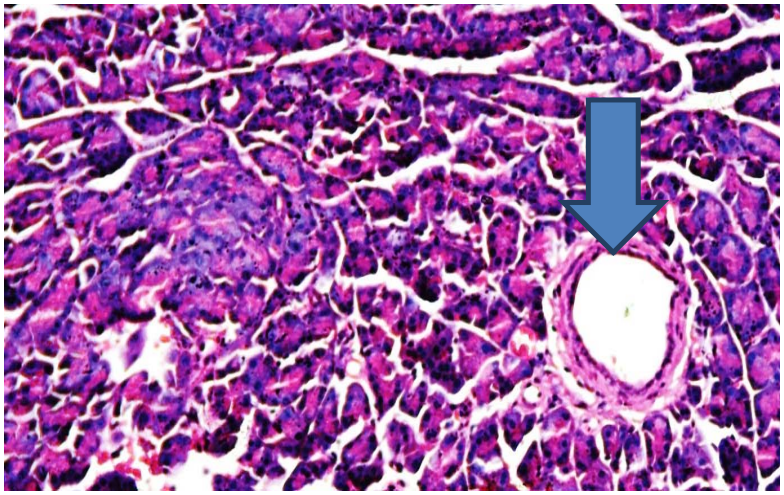

FIGURE4; High Dose of Neem Bark (X100) H&E. Photomicrograph section of islet of Langerhans of adult wistar rats showing clear arteriole with relative normal section after the administration of aqueous extract of neem bark at a dose of 500mg/kg for a period of six weeks.

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 cells appeared similarl

y 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 Langerhans while 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 were shown 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.

Diabetic Group. The blood glucose level heightens after streptozotocin induced induction of diabetes.

Low Dose In this group, the blood glucose level was reduced gradually.

High Dose The blood glucose level reduced a little further than the low dose group.

Enzymatic Activities analysis

Alanine amino transferase (ALT): From Table 3 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); Similarly, Table 3 above reveals that aspartate amino transferase activities increased steadily when comparing control group with diabetic 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 enzymatic activity of alkaline phosphatase of control group with the diabetic 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.

DISCUSSION

The findings from this study has shown that crude extract of *Azadiracnta indica* administered orally to streptozotocin (STZ) induced diabetic wistar rats significantly reduced blood glucose level. Similarly, the report of some previous investigations are in agreement with the result of this study [14,3,15]

The principles and mechanism of the anti-diabetic properties of the neem extract is not fully understood. However, it has been suggested that the antidiabetic characteristic properties. of the extract may be associated with potentials and ability of the extract to initiate and stimulate substantial production of insulin by the pancreatic cells which invariably supports the peripheral utilization of glucose in the cells or a possible ability of the extract for regeneration of the β-cells for the performance of its function [15]. This study observed a significantly increased blood glucose level in the diabetic group B Wistar rats which subsequently became significantly reduced in a dose dependent manner following treatment of the diabetic rats with crude extract of neem in group C and D. It is not impossible that the crude extract of neem bark has ameliorative potentials which induced the rebuilt and recovery of the damaged islets and acini cells enabling them to perform their functions. It has been similarly reported that induction of diabetics by Alloxan is known to mediate the destruction of β-cells by establishing a redox-cycles resulting in the formation of reactive oxygen species which in known to primarily induce cellular damage which results in diabetics in rats[16].

The photomicrograph of the pancreatic sections from diabetic rats in this study revealed regenerative changes in β- islets and acinic cells which resulted in improvement in histo-architecture of pancreatic tissue. Report from the literature has indicated that pancreatic β-islet and acini cells are associated with insulin synthesis while the acini produce α-amylase enzyme [17,16]

Furthermore, STZ administration to wistar rats in this study resulted in significant elevation of pancreatic lipid peroxidation while the pancreatic SOD and GSH were significantly reduced in diabetic wistar rats. The significant increase in the lipid peroxidation {LPO} in diabetic wistar rats in association with significant increase in blood glucose level may be occasioned by oxidative stress with resultant effect of oxidative damage. Lipid

peroxidation activities and cellular damage has been described in relation with oxidative stress [18].

STZ is a commonly employed substance for induction of type-I diabetes [19]. It has been shown that STZ induces diabetes by a mechanism which favours rapid depletion of β-cells which ultimately results in reduction in insulin release, in addition, hypoglycemia causes oxidative damage by generation of ROS[20] and development of diabetic complications[21,22] . The significant elevation in ALP, ALT and AST in the diabetic rats as observed in this study might be due to oxidative damage in association with increased lipid peroxidation which became significantly reduced in group C and D diabetic rats that were treated with the neem extract.

GSH and SOD are known primarily as antioxidant substance and enzyme respectively that are involved in direct elimination of ROS[23]. The enzymatic activities of CAT and SOD in diabetic rats were significantly reduced in this study in association with oxidative damage. However, there was a reversal of the increased SOD and GSH levels coupled with inhibition of LPO or reduction in lipid peroxidation following treatment with neem extract in group C and D. These changes may be due to free radical scavenger potential of the neem extract administered in this study. The improvement in the antioxidant status of the diabetic induced rats subsequently treated with neem extract may be responsible for recovery and improvement in pancreatic tissue morphology in extract treated diabetics rats.

This study concluded that administration of crude extract of neem bark exhibits ameliorative potentials on pancreatic tissue following streptozotocin induced oxidative damage with significantly increased blood glucose level.

Disclaimer regarding Consent and Ethical Approval:

CONSENT; Not applicable

Ethical Approval; All authors hereby declare that the principles of laboratory animal care [NIH publication No. 85-23 revised 1985] were followed as well as specific national laws where applicable. All experiments have been examined and approved by the relevant ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

No competing interest exist

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