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Effect of Aqueous extract of Azadirachta indica leaf on Some Essential Biochemical Parameters of Streptozotocin-induced Diabetic Rats.

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Abstract

This study was carried out to determine the effect of aqueous extract of Azadirachta indica leaf on essential biochemical parameters of streptozotocin-induced diabetic rats. The 24-hour acute toxicity test of the orally administered aqueous extract was determined using Finney's method. Glucose levels were checked using One Touch Glucometer and test strips. Diabetes was induced intraperitoneally using 50mg/kg body weight of Streptozotocin. The biochemical parameters were analysed using standard diagnostic methods. The acute toxicity study of the aqueous extract revealed that the median lethal dose was 5.5g/kg body weight. The urea, creatinine, ALT, AST, ALP and bilirubin levels increased significantly ($p<0.05$) in the diabetic untreated rats compared with the groups treated with the graded doses of the aqueous extract of A. indica leaf. The serum alpha-amylase activity of the extract treated groups decreased significantly ($p<0.05$) compared with the diabetic untreated group. The diabetic untreated rats showed a significant ($p<0.05$) decrease in their insulin level compared with the insulin levels of rats treated with the graded doses of the aqueous extract of A. indica leaf. The aqueous extract of A. indica leaf has the capability of lowering blood glucose level in STZ-induced diabetic rats without altering essential biochemical parameters of the experimental animals. The findings from the present study suggest that the mechanisms by which the aqueous extract of A. indica leaf exert its antidiabetic activity may be by reducing the activity of alpha-amylase enzyme by slowing the rate at which carbohydrate is being hydrolysed and regeneration of the beta-cells of the pancreas. The study also suggested that the aqueous extract of A. indica leaf is safe as it does not cause any alteration in the essential biochemical parameters of diabetic rats.

Keywords: Glucose, Antidiabetic, A. indica, Streptozotocin, Alpha-amylase, Insulin,

Introduction

Diabetes is a health condition resulting from deficiency of insulin due to autoimmunity of the beta cell of the islet of Langerhans in the pancreas (type 1 diabetes) and/or when the activity of the hormone insulin is been impaired thereby affecting the uptake of blood glucose by glucose transporters (type 2 diabetes) [1]. Type 1 diabetes was reported in about 1.1 million children and juvenile aged 14-19 years [2]. In 2013, it was reported that 382 million have diabetes and approximately 592 million cases by 2035 were predicted [3], and 463 million diabetes cases worldwide was reported as the latest figure as the number keeps increasing and it is accountable for about 760

billion dollars in health expenditure [4]. Type 2 diabetes is prevalent in adult with complications such as kidney failure, stroke, miscarriage in pregnant women, foot ulcer and blindness [5,6]. In Nigeria about 4.3% cases of diabetes was reported in 2016 [7].

Conventional drugs used for the treatment of diabetes are costly with its concomitant side effects. This has impeded its use in the treatment and management of diabetes. A long-term solution to this epidemic problem is needed to relief a greater number of the populace suffering from the disease. Research is currently geared towards the use of medicinal plants due to its accessibility and minimal or no side effects. In Africa, hundreds of plants are used traditionally for the management and control of diabetes mellitus. Unfortunately, only a few of such medicinal plants have been scientifically validated. One of the plants commonly used in Africa traditional medicine for the management of diabetes mellitus is Azadirachta indica. Azadirachta indica (Family: Meliaceae) popularly known as "neem" is a medicinal plant originally grown in India but is now being cultivated in almost every part of the world including Nigeria, where it is called "Dogonyaro". It is one of the most useful medicinal plants. These plants have been used in traditional and modern medicine to treat cases like malaria, colds, gonorrhea, wounds with little or no side effect.

Azadirachta indica from meliaceace family has a therapeutic effect as a result of its active compound like nimbolinin, salannin, azadirachtin, quercetin, nimbidin, and flavonoids. It was reported to possess antioxidant activity, antimicrobial activity, antimalarial activity, hepatoprotective activity and antidiabetic activity, to mention but a few [8,9]. Nimbolide, one of the active compounds of the leave extract, has a hepatoprotective effect against carbon tetrachloride- induced liver damage [10]. Another finding reported a wider area of inhibition of bacteria with the leaf extract of A. indica than with 3% sodium hypochlorite [11]. A study by Nagashayana et al [12] stated that the aqueous extracts of A. indica downregulate hyperglycemia than the drug glibenclamide on 3, 7 and 14 days of administration. Reports by various researchers show that Azadrichta indica leaf extracts lower blood glucose level in streptozotocin-induced diabetic rat [13,14]. Azardirachta indica improves the expression of Akt (serine/ threonine kinase) protein which activates insulin on skeletal muscle [15]. Hence the study was carried out to determine the effect of aqueous extract of Azardirachta indica leaf on some essential biochemical parameters of streptozotocin-induced diabetic rats in order to proffer the possible mechanism by which A. indica leaf exerts its antidiabetic effect.

Methods

Sample Collection/Identification:

The leaves of A. indica were collected from Nnamdi Azikiwe University Awka, Anambra State. The sample was identified and validated by a botanist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra state. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is 14.

Sample Preparation

The leaves were properly washed and air dried at room temperature. The dried leaves were ground into powder using corona manual grinding machine. Exactly 1kg of the ground leaves powder were soaked in 5 litres of distilled water for 24 hrs for complete aqueous extraction. It was sieved and filtered using Whatman no 1 (125mm) filter paper. The filtrate was lyophilized (freeze dried) using freeze dryer. The dried sample was put in a stoppered universal bottle and stored in the refrigerator until needed. The powdered sample was reconstituted with distilled water before use.

Chemicals

Streptozotocin, manufactured by Sigma, Germany. All other chemicals used in this study were of analytical grade.

Experimental Animals

A total of one hundred (100) male rats were purchased from the animal house of Chris Farms, Awka and used for the acute toxicity (LD_{50}) test and antidiabetic study. They were maintained and housed in aluminium cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed water and guinea growers mash pellets obtained from Vital feed distributors, Awka ad libitum.

Acute toxicity test (LD_{50} Determination) of A. indica leaf

The Median Lethal Dose (LD_{50}) was determined using seventy (70) male Wistar Albino rats. Test animals were randomly divided into seven (7) groups of ten (10) rats each and administered graded doses of 0.5, 1, 2, 3.5, 4.5, 5 and 5.5g/kg body weight. The A. indica extracts were administered by oral gavage using an intubation cannula and were monitored for 24 hours for changes in behaviour and mortality. The animals were monitored closely for signs of toxicity. The LD_{50} was determined by plotting a graph of Probit against Log Dose according to Finney's method [16].

Determination of Anti-Diabetic effect of ethanol extract of A. indica

A total of thirty (30) male wistar albino rats were used for the antidiabetic study. Twenty five (25) of the rats were made diabetic and subsequently divided into five groups of five rats each. The remaining 5 non-diabetic rats were used as control subjects. Groups A, B and C were orally administered 100mg, 200mg and 400mg/kg body weight of aqueous extract of A. indica leaf respectively. Group D received 100mg/kg body weight of metformin (a standard drug used in the treatment of diabetes), group E did not receive any treatment and group F was a control group of 5 non-diabetic rats that received 1ml of distilled water in place of treatment regimen. The blood glucose levels of the rats were checked before the administration of Streptozotocin using One Touch Glucometer and test strips. The rats were then fasted for 16 hours, but with free access to water after which they received an intraperitoneal injection of streptozotocin 50mg/kg body weight. The rats were orally given 5ml each of 5% glucose solution 2 hours after administering streptozotocin to prevent hypoglycemia. The animals were allowed free access to food and water after streptozotocin injection. After 48 hours of the streptozotocin administration, blood was collected orbito rectally and the glucose concentrations were determined using One Touch Glucometer (Life Scan, USA) and test strips based on the method of Trinder [17]. Diabetes was confirmed to have been induced when the fasting glucose level was observed to be far much higher than normal (between 60mg/dl to 120mg/dl) to above 200mg/dl. Treatment was done for 28 days. Kidney and liver function tests as well as alpha amylase activity and insulin level were determined at the end of 28 days treatment.

Kidney Function Test

Kidney function parameters Na^+, Cl^-, K^+ and HCO_3 were measured using routine diagnostic techniques by autoanalyser, Selectra Junior manufactured by Vital Scientific B. V. Netherlands. The procedure is according to the manufacturer's instruction. Urea and creatinine were analysed using Randox test kits.

Liver Function Test

Serum biochemical indices routinely estimated for liver functions including aspartate aminotransferase (AST),

alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Bilirubin were determined using Randox diagnostic kits.

Alpha-Amylase Activity

Alpha-amylase (Single Reagent) – GALG2-CNP Test Kit was used to assay for the inhibitory effect of the extract on the alpha-amylase enzyme. Alpha amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-1-galactopyranosyl-maltoside (GALG2-CNP) to glucose polymers and p-nitrophenyl oligosaccharide at short chain producing 2-chloro-4-nitrophenol (CNP). The increased extinction was measured by spectrophotometry at 405nm. Alpha-amylase was calculated using the following formular: $\Delta E=A2-A1$Alpha amylase (U/L) = ΔEx 765.

Insulin Assay

Insulin (Enzyme Immunoassay Test Kit) was used to assay for the regeneration of the β-cells by the extract and production of insulin. Immunospec Insulin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the insulin antibody coated microtiter wells. Then anti-Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If Insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 1 hour incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The colour development is stopped with the addition of stop solution. The colour changed to yellow and was measured spectrophotometrically at 450nm. The concentration of insulin is directly proportional to the colour intensity of the test sample.

The desired number of coated wells in the holder was secured. 50µl of insulin standard, specimens and controls were dispensed into the appropriate wells. This was gently but thoroughly mixed for 10 seconds. 100µl of enzyme conjugate reagent was dispensed into each well. They were mixed gently for 30 seconds and then incubated at room temperature for 60 minutes. The incubation mixture was removed by emptying the plate content into a waste container. The microtiter plate was rinsed and emptied 5 times with 1 x washing buffer (300µl each well). The microtiter plate was stroke sharply onto absorbent paper to remove all residual water droplets. 100µl of TMB substrate reagent was dispensed into each well. It was gently mixed for 10 seconds and then incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100µl of Stop solution to each well. This was gently mixed for 10 seconds until the blue colour completely changed to yellow. The optical density was read at 450nm with a microtiter plate reader within 15 minutes.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results obtained were performed by using ANOVA and POS-HOC tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

Results

Acute Toxicity (LD_{50}) Test

The result of a 24-hour acute toxicity test of orally administered aqueous extracts of A. indica in male albino rats is represented in table 1. The groups that were administered 0.5g/kg, 1g/kg and 2g/kg body weight did not show any sign of toxicity. The group that was administered 3.5g/kg body weight was slightly weak with one death recorded. The group that was administered 4.5g/kg body weight showed some signs of weakness with three deaths recorded. The groups that were administered 5g/kg and 5.5g/kg body weight were very weak with four and five deaths recorded respectively. The percentage mortalities were transformed to probit according to Finney's method [16] in order to extrapolate the LD_{50} from the graph of probit against log dose (figure 1). The results obtained showed that the LD_{50} of the aqueous extract of A. indica leaf is 5.5 g/kg body weight.

Table 1: Result of the acute toxicity study (LD_{50}) of the aqueous extract of A. indica.

(N=10) Groups	Dose (g/kg)	Log dose	No of death	% mortality	Probit	Remarks
A	0.5g	2.70	0	0	0	Normal
B	1g	3.00	0	0	0	Normal
C	2g	3.30	0	0	0	Normal
D	3.5g	3.54	1	10	3.72	Slightly weak
E	4.5g	3.65	3	30	4.48	Weak
F	5g	3.70	4	40	4.75	Very weak
G	5.5g	3.74	5	50	5.00	Very weak

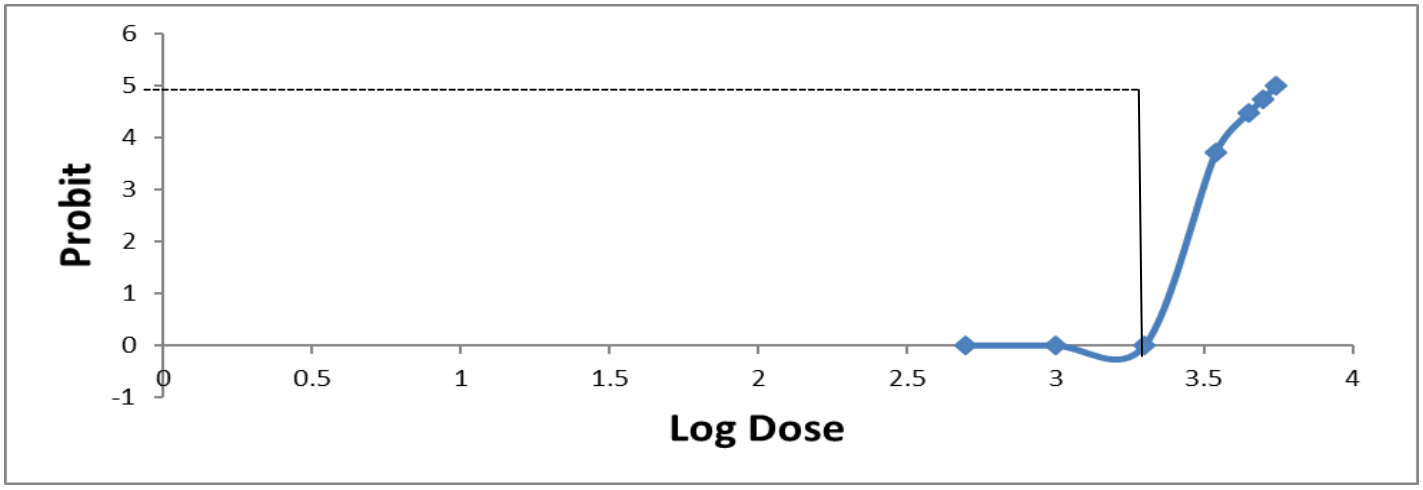

Fig. 1: LD_{50} for orally administered aqueous extract of A. indica leaves.

Log dose at 50% = 3.74.

LD_{50} was derived by determining the antilog of log dose.

Therefore $\log^{-1} 3.74 = 5.5$g/kg.

LD_{50} = 5.5 g/kg

Kidney Function test

The result of the effect of treatment with aqueous extract of A. indica leaves on the kidney function tests (potassium, chloride ion, HO_3, urea and creatinine) is in table 2. There was a significant ($p<0.05$) reduction in the

potassium and chloride ion value in the diabetic untreated compared with the normal control, but a significant ($p<0.05$) increase in the potassium ion and chloride ion value in diabetic treated with aqueous extract of A. indica with respect to diabetic untreated. There was a significant ($p<0.05$) increase in the sodium ion, HCO_3 and urea values in diabetic untreated control with respect to normal control. There was a significant ($p<0.05$) increase in the urea concentration of the diabetic untreated group compared with the groups treated with the graded doses of the aqueous extract and the normal control group.

Table 2: The effect of treatment with different doses of aqueous extract of A. indica leaf for a period of twenty-eight (28) days on the kidney function parameters expressed as mean ± SD.

Kidney Function Parameters	**Normal (Non-diabetic)**	**Diabetic Untreated Rats**	**100mg/kg Metformin**	**100mg/kg Aqueous Extract**	**200mg/kg Aqueous Extract**	**400mg/kg Aqueous Extract**
K^+(mmol/L)	137.6±0.5477	133.5±2.121	140.8±1.924	136.7±2.082	139.3±1.155	140.3±3.096
Cl^-(mmol/L)	101.0±0.7071	99.50±0.703	101.0±0.701	100.7±0.577	100.7±1.528	100.8±0.9574
Na^+(mmol/L)	6.644±0.7014	8.055±0.219	6.840±0.316	6.687±1.124	6.883±0.7912	7.580±20.77
HCO_3^- (mmol/L)	25.80±0.8367	27.50±0.707	26.20±0.837	27.00±1.000	25.33±0.5774	25.75±1.500
Urea (mmol/dl)	26.60±0.8944	34.50±6.36b	26.20±1.30c	27.00±1.00c	26.33±1.528c	28.00±1.41c
Creatinine (mg/dl)	1.100±0.707	1.250±0.071	1.100±0.100	1.233±0.116	1.167±0.1155	1.175±0.125

[a]significant reduction with respect to normal control

[b]significant increase with respect to normal control

[c]significant reduction with respect to diabetic untreated control

[d]significant increase with respect to diabetic untreated control.

Liver Function Test

There was a significant ($p<0.05$) increase in the aspartate transaminase (AST) activity of the diabetic untreated group compared with the groups treated with the aqueous extracts of A. indica leaf and the normal control group (table 3). The group that was administered 100mg/kgbw of metformin did not show any significant difference in the AST level compared with the normal control. There was a significant ($p<0.05$) increase in the level of alanine transaminase (ALT) activity of the diabetic untreated group compared with the groups treated with the graded doses of aqueous extracts of A. indica leaf and the normal control group. The group that was administered 100mg/kgbw of metformin did not show any significant difference in the ALT level compared with the normal control. The ALT level of the diabetic untreated group increased ($p<0.05$) significantly when compared with the normal control group.

There was a significant ($p<0.05$) increase in the level of alkaline phosphatase (ALP) activity of the diabetic untreated group compared with the groups administered graded doses of aqueous extract of A. indica leaf. The ALP level of the diabetic untreated group increased ($p<0.05$) significantly when compared with the normal control group. There was a significant ($p<0.05$) increase in the serum bilirubin level of the diabetic untreated group compared with the groups administered graded doses of aqueous extract of A. indica leaf and the normal control group. The bilirubin level of the diabetic untreated group increased ($p<0.05$) significantly when compared with the normal control group.

Table 3: The effect of treatment with different doses of aqueous extract of A. indica leaf for a period of twenty-eight days on the liver function parameters expressed as mean ± SD.

Liver Function Parameters	**Normal (Non-diabetic)**	**Diabetic Untreated rats**	**100mg/kg Metformin**	**100mg/kg Aqueous Extract**	**200mg/kg Aqueous Extract**	**400mg/kg Aqueous Extract**
AST(U/L)	57.60±4.506	122.0±24.0b	56.00±11.4c	87.67±7.371	77.33±4.04c	70.75±5.31c
ALT(U/L)	32.20±4.919	52.50±7.77b	34.00±8.06c	38.67±2.517	33.67±8.02c	27.00±7.87c
ALP(IU/L)	134.4±5.595	416.0±69.3b	137.0±5.91c	251.7±29.8c	284.3±89.2bc	180.0±65.82c
Bilirubin (mg/dl)	0.630±0.078	1.8050±777b	0.942±0.15c	1.120±0.2bc	1.280±0.240b	0.6800±0.19c

[a]significant reduction with respect to normal control

[b]significant increase with respect to normal control

[c]significant reduction with respect to diabetic untreated control

[d]significant increase with respect to diabetic untreated control.

Alpha amylase

The serum alpha-amylase activity of the groups treated with 200 and 400mg/kg b.w decreased ($p<0.05$) significantly compared to the normal control and diabetic untreated group (table4). diabetic untreated group increased compared with the treatment groups. However, the increase was not statistically ($p<0.05$) significant. The group that was administered 100mg/kgbw of metformin also showed a significant ($p<0.05$) reduction in the alpha amylase activity compared with the normal non-diabetic group and the diabetic untreated group.

Table 4: The effect of treatment with different doses of aqueous extract of A. indica for a period of twenty-eight days on the alpha-amylase activity expressed as mean ± SD.

α-amylase activity(U/L)	**Normal (Non-diabetic)**	**Diabetic Untreated rats**	**100mg/kg Metformin**	**100mg/kg Aqueous Extract**	**200mg/kg Aqueous Extract**	**400mg/kg Aqueous Extract**
α-amylase	79.40±6.878	47.00±12.7a	33.60±9.0a	42.00±6.00a	37.04±9.53ac	35.00±6.9ac

[a]significant reduction with respect to normal control

[b]significant increase with respect to normal control

[c]significant reduction with respect to diabetic untreated control

[d]significant increase with respect to diabetic untreated control.

Insulin Test

It was observed that the insulin level of the rats administered the aqueous extract of A. indica leaf at a dose of 400mg/kgbw increased ($p<0.05$) significantly compared with the diabetic untreated group. However, a dose-dependent increase was observed for the groups that were administered graded doses of the aqueous extract of A. indica leaf (table 5).

Table 5: The effect of treatment with different doses of aqueous extract of A. indica leaf for a period of twenty-eight days on the insulin level.

Insulin level (ng/ml)	**Normal (Non-diabetic)**	**Diabetic Untreated rats**	**100mg/kg Metformin**	**Aqueous Extract**	**Aqueous Extract**	**Aqueous Extract**
Insulin level	35.00±11.01	25.00±2.82a	48.80±4.382	54.67±19.66d	59.00±16.8d	68.75±4.9d

[a]significant reduction with respect to normal control

[b]significant increase with respect to normal control

[c]significant reduction with respect to diabetic untreated control

[d]significant increase with respect to diabetic untreated control.

Discussion

The present study was conducted to determine the effect of aqueous extract of A. indica on serum biochemical parameters of streptozotocin–induced diabetic rats. Upon administration of streptozotocin, diabetes was confirmed to be induced with high blood glucose level above 200mg/dl. The LD_{50} value of aqueous extract of A. indica leaf was determined to be 5.5g/kg of body weight. This result is in line with Raizada et al., [18] which stated that A. indica did not show any sign of toxicity even at high doses. According to Lorke, [19], LD_{50} value of 5g/kg and above suggests that the extract is not toxic. The antidiabetic property of the aqueous extract of A. indica leaf following twentyeight days treatment on streptozotocin-induced diabetic rats has earlier been reported [14].

The result of the effect of treatment with aqueous extract of A. indica on the kidney function tests (potassium,

chloride ion, HO_3, urea and creatinine) is shown in table 2. There was a significant ($p<0.05$) reduction in the potassium and chloride ion value of the diabetic untreated with respect to normal control, but a significant ($p<0.05$) increase in the potassium ion and chloride ion value of diabetic group treated with aqueous extract of A. indica with respect to diabetic untreated. One of the intracellular ions need for extremely important cellular processes in the body is potassium and insulin [20]. Diabetic patients are deficient of insulin or there is resistance to insulin activity, thereby restricting the movement of potassium from the extracellular to intracellular compartment. Several researchers have reported low potassium level in the body as a potent risk agent for diabetes [21,22]. This explains the low potassium level in the diabetic untreated rats. Likewise Parmar et al., [23] reported a decrease of chloride ion level in diabetic patients. Alif et al stated that movement of potassium from the extracellular membrane to the intracellular membrane is promoted by insulin which can cause hypokalemia and therefore insulin should be declined if the potassium level is less than 3.3mmol/l. likewise Preston [24] reported that diabetic individuals constantly have kidney disease due to inability to excrete potassium.

There was a significant ($p<0.05$) increase in the sodium ion, HCO_3 values in diabetic untreated control with respect to normal control but a significant ($p<0.05$) reduction of sodium ion, HCO^-_3 values with respect to diabetic untreated. Hyperglycemia causes osmotic diuresis leading to dehydration and loss of electrolytes [25]. These results do not support the study carried out in Nepal that observed a low sodium level in patient with high fasting blood glucose [23]. A study in Beijing observed a low bicarbonate level in the serum to be related with higher diabetes mellitus risk [26]. There was a significant ($p<0.05$) increase in urea and creatinine level of diabetic untreated with respect to normal control but a significant ($p<0.05$) reduction in urea and creatinine of the diabetic rats treated with aqueous extract of A. indica with respect to diabetic untreated rats. The present result is in agreement with the findings of Madhusudan et al., [27] and Shrestha et al., [28] that reported an increased urea and creatinine level in diabetic patients with respect to normal control when there is kidney damage. The United States Renal Data System 2007 reported that diabetes is one of the main causative agent of kidney failure. A study by Yan et al., [29] stated a high urea level in relation with increased risk of insulin usage.

There was a significant ($p<0.05$) increase in the liver enzyme (aspartate transaminase AST, alanine transaminase ALT, alkaline phosphatase ALP) activity of the diabetic untreated group compared with the groups treated with the graded doses of aqueous extracts of A. indica leaf. High levels of alanine transaminase can be related to type 2 diabetes risk. Thanpari et al., [30] reported significant increase in ALT levels in diabetic patients compared to a control group. There was a significant ($p<0.05$) increase in the serum bilirubin level of the diabetic untreated group compared with those treated with aqueous extract of A. Indica leaf. Mohamed et al., [31] stated that increased bilirubin level in diabetic rats was as a result of uptake due to liver disease. Similarly, a study in Spain reported an increased bilirubin level in a short-term streptozotocin–induced diabetic rats when compared with control [32]. A Mendelian Randomization study of 3381 participant investigated that increased bilirubin level is occasionally related with type 2 diabetes risks and agrees to its functions as a protective agent [33]. These reports explain the high bilirubin level in the diabetic untreated rats.

The serum alpha-amylase activity of the diabetic untreated group increased ($p<0.05$) significantly compared with the

group treated with 400mg/kg b.w. of aqueous extract of A. indica leaf. The Alpha-amylase enzyme activity of the groups treated with the graded doses of the aqueous extract of A. indica leaf decreased in a dose-dependent manner. Dai et al., [34] stated that type 2 diabetic patients have lower alpha amylase level while individuals with high alpha amylase levels are not in the risk of developing type 2 diabetes mellitus. This enzyme catalyzes the hydrolysis of starch to glucose. This result suggests that one of the mechanisms by which the extract of A. indica exert its antidiabetic effect in streptozotocin-induced diabetic rats may be by slowing down the activity of the alpha-amylase enzyme thereby reducing the rate by which carbobydrate is being hydrolysed.

It was observed that the insulin level of the rats treated with the graded doses of the aqueous extract of A. indica significantly ($p>0.05$) increased compared with the diabetic untreated group. The increase in the insulin level was in a dose-dependent manner. This is in agreement with Anggit et al., [35] and Menakshi et al., [36] as the immunohistochemistry analysis show an increase of insulin when A. indica and G. procumbent were combined. A. Indica contains 2.8% rutin, a flavonoid that possess antioxidant activity that scavenges free radical thus enhancing the production of insulin [37]. In hyperglycemia state, the oxidative stress increases which affect insulin action and production [38].

Conclusion

It is evident from preliminary studies that the aqueous extract of A. indica leaf possesses antidiabetic activity against streptozotocin-induced diabetes mellitus. The present study suggested that the mechanisms by which the aqueous extract of A. indica leaf exert its antidiabetic activity may be by slowing down the activity of alpha-amylase enzyme by reducing the rate by which carbohydrate is being hydrolysed and regeneration of the beta-cells of the pancreas which was evident in the significant increase observed in the insulin level of the groups treated with the graded doses of A. indica leaf. Subsequent work should focus on the elucidation of molecular mechanisms by which A. indica leaf exerts its activity.

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