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Hypoglycemic Effects of Aqueous and Ethanolic Extracts of *Leptadenia hastata* on Streptozotocin-Induced Diabetes Mellitus in Albino Rats

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Abstract: ***Single intra-peritoneal injection of 60 mg/kg body weight streptozotocin is known to induce diabetes mellitus and subsequently damage to the liver due to its hepato-toxic nature. The present study investigated the hypoglycemic and curative effects of Leptadenia hastata plant extracts conducted on 35 healthy male albino rats. The rats with blood glucose level above 200 mg/dl were randomly divided into seven (7) experimental groups of five rats each. Oral administration of 150 mg/kg body weight, 300 mg/kg body weight aqueous extract and 150 mg/kg body weight, 300 mg/kg body weight ethanolic extract were given to the diabetic rats for the period of 21 days. The fasting blood glucose was monitored at 7 days interval while markers of liver and kidney function were assayed at the end of 21 days treatment. The results revealed that oral administration of 300mg/kg body weight ethanolic extract was found to be significant p<0.05 in lowering the blood glucose level (115.60 ± 3.82 mg/dl) as compared to the diabetic control group (420.80 ± 3.68 mg/dl). The studies also revealed a significant decrease p<0.05 in the level of serum AST (30.39 ± 0.66 U/L), ALT (24.07 ± 0.97 U/L) and ALP (153.64 ± 1.00 U/L) as compared with diabetic control group (168.81 ± 1.96 U/L), (85.37 ± 1.23 U/L) and (368.97 ± 3.45 U/L) respectively. The serum level of albumin (36.48 ± 0.33 g/l), total protein (75.20 ± 0.80 g/l) were also significantly decreased p<0.05 following 21 days treatment by oral administration. These results suggest that ethanolic extract Leptadenia hastataposse' shypoglycemic potential and ameliorate indices of hepato-toxicity following more than 14 days oral administration.***

Keywords: Leptadenia hastata, Hypoglycemic effect, Diabetes mellitus, Streptozotocins

1. Introduction

Health professionals have described Diabetes mellitus as a chronic metabolic disorder characterize bydefective and degeneration of carbohydrates, protein and fat metabolism.Such alterations subsequently result in increased blood glucose and if left unchecked, will cause a long-term complication in various organs such as the kidneys, liver, and eyes (Hu, 2003). According to Nash *et al.* (2001), diabetes mellitus is the sixth leading cause of death globally with International Diabetes Federation estimating that this number will grow to 11.5 million by 2025 unless measures are taken to control the disease (Hayat and Shaikh, 2010).The increase in prevalence has accelerated due to the our life style, aging population structure in the developed countries and due to the globally increasing obesity, as well as other contributing factor that may include some diseased conditions, dieting and genetic factors (Etuk, 2010).

According to reports by World Health Organization (2009), the most common type of diabetes is type 2 diabetes as it accounts for 85to 95% of all cases and constitutes the major and growing public health problem. Diabetes mellitus type 2, formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency and sometimes ketoacidosis (Kumar *et al*., 2011).

The most common drugs that are currently being used for the experimental induction of diabetes are alloxan and streptozotocin (STZ). Alloxan and STZ have been extensively documented for the induction of diabetes via free radical generation and depletion of antioxidant defence system. STZ has been reported to significantly decrease the activity of erythrocytes antioxidative enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Ali and Agha, 2009).

Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognized by the other glucose transporters. Intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in 2-4 days and this explains its relative toxicity to beta cells and hepatic cells, since these cells have relatively high levels of GLUT2 (Lenzen, 2008). Moreover, liver failure is associated with chronic diabetes mellitus and usually monitored to assess the progress and effectiveness of treatment (Prakash *et al.*, 2015).

The limitations in efficiency associated with side effects and an exponential increase in the prevalence of diabetes mellitus, motivate researchers to scientifically validate the use of medicinal plants as possible alternative therapies. Plants have always been a very good source of drugs and many of the currently available drugs have been derived directly or indirectly from them (Husanain and Mooradian, 2002).

2. Materials and Method

Plant

Leptadenia hastate plant was collected from Kwanan Kuka Jimeta, along Yola Town Adamawa State and was authenticated by a Botanist at the Department of Plant Science Moddibo Adama University of Technology Yola.

Experimental Animals

A total number of 35 male wister rats weighing between 90-110g were purchased from National Veterinary Research Institute, Vom, Nigeria. The animals were housed in a plastic cage and allowed to acclimatise and feed with standard diet and water *ad libitum*.

Plant Extraction

Fresh plant of *L. hastata*was allowed to dry at room temperature under shed. Dried plant was made into powder using mortar and pestle where 500g of the dried sample was extracted using water and 70% ethanol over 48 hours period. Each extract was then filtered using a filter paper (Whatmann No. 1) and concentrated using water bath at 50°c (Bello *et al.,* 2011).

Induction of Diabetes

The experimental animals were fasted for 16 hours prior to the induction of diabetes. Streptozotocin was freshly prepared 10ml distilled water and was intraperitoneally injected to mice with a single dose of 60mg/kg. Random blood glucose was monitored for five (5) days, and the rats were fasted for 16h after the fifth day. Blood sample was collected from their tails for measurement of blood glucose. Rats with fasting blood glucose higher than 200mg/dl were considered diabetic once and were randomly divided into groups designed. (Jin-yin *et al*., 2012).

Experimental Design

After the induction of diabetes mellitus, the rats, were randomly divided into experimental and control groups. Experimental animals were fasted for 16 hours before treatment and grouping will be done as follows:

1) Group 1. Normal control (standard diet and water).
2) Group 2. Positive control (diabetic + no treatment).
3) Group 3. Positive + synthetic drug (metformin 5mg/kg^{-1}).
4) Group4. Positive + water extract (150, 300mg/kg^{-1}).
5) Group 5. Positive + ethanol extract (150, 300mg/kg^{-1}).

The animals in group 3, 4 and 5 were treated with various doses as indicated in the morning hours for the period of twenty one (21) days, while group 1 and 2 received no treatment. All the animals were on standard diet and water *ad libitum*. The rats were anaesthetized at the time of sacrifice by placing them in inhalation jarsoaked with chloroform in sealed cotton wool. Blood was collected via cardiac puncture from each animal for the determination of fasting blood sugar, kidney functions and liver function (Cheesbrough, 1992 and Bello *et al*., 2011).

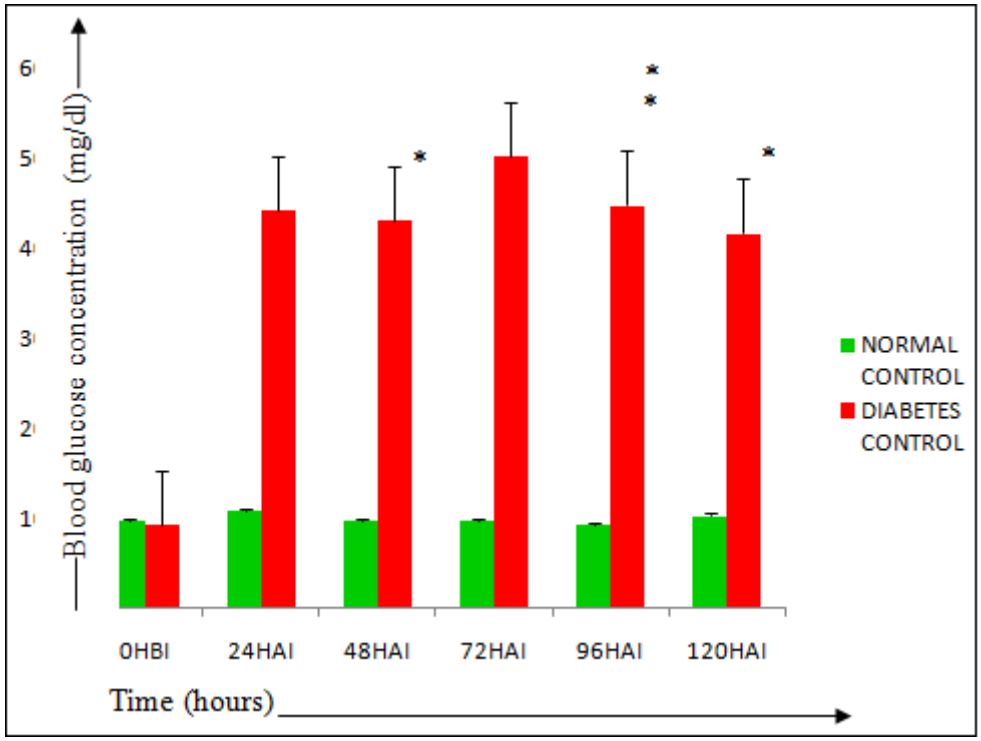
Statistical analysis

Values obtained were expressed as mean ± SEM and data were analysed using Anova with multiple comparison versus control groups with the help of SPSS version 20. The values $p<0.05$ and $p< 0.01$ were considered significant (Duncan *et al*., 1977).

3. Results and Discussion

The induction of diabetes mellitus is shown in Figure i by intraperitoneal injection of streptozotocin 60 mg/Kg body weight as single dose. The changes in blood glucose was monitored for 120 hours (5days) before commencing treatments by oral administration of aqueous and ethanolic extracts, 150mg/Kg body weight and 300mg/Kg body weight of each of the extracts.

The figure shows that at 48 hours the blood glucose level was at peak in the entire streptozotocin- induced diabetes mellitus rats. The figure indicates that the increase in blood glucose remains significantly increased for as long as 120 hours (5 days) as compared to the normal control group. The figure explains the relative toxicity of streptozotocin to beta cells and hepatic cells.



(Change in blood glucose levels before induction and after induction

Figure 1: Hyperglycemic effects of streptozotocin by intraperitoneal injection of 60 mg/kg body weight
Values are expressed as mean ± SEM; n=5, HBI: Hours before induction, HAI: Hours after induction.
Key: * significantly different among groups $p<0.05$
** highly significant different among groups $p<0.05$

Results obtained as shown in table 2 reveals the comparison of blood glucose concentration in albino rats of normal control that had free access to food and water *ad libitum*, diabetes control group that were not treated but also had free access to food and water *ad libitum* and standard drug control (metfornim) 5 mg/kg body weight. The table shows a significant decrease $p<0.05$ in the levels of blood glucose of diabetes groups treated with various doses of *Leptadenia hastata* plant extract 150 mg/kg bwt ethanolic extract (399.40 ± 8.44), 300 mg/kg bwt (326.40 ± 4.13) ethanolic extract, 150 mg/kg bwt (387.20 ± 3.64) aqueous extract, 300 mg/kg bwt (340.20 ± 2.94) and standard drug control group (312.00 ± 0.55) as compared to the diabetes control group (410.00 ± 1.92) on the 7th day of treatment.

However, the levels of blood glucose of the entire treatment group remained significantly increased $P<0.05$ compared to the normal control group (90.40 ± 1.63) but continues to decrease significantly as compared to the diabetic control group (440.00 ± 2.89) as revealed by the table on the 14th day of treatment. The findings as shown in the table further indicates a significant decrease $p<0.01$ in the blood glucose levels of the diabetes group treated with 300 mg/kg bwt ethanolic extract (147.60 ± 2.34) as compared to the standard drug control group (197.00 ± 1.30) on the 7th day treatment.

The results also shows that on the 21st day of treatment, there is a significantly decrease $p<0.05$ in the levels of blood glucose of groups receiving 150 mg/kg bwt and 300 mg/kg bwt ethanolic and aqueous extracts respectively as compared to the diabetes control group (420.80 ± 3.68) and as compared to the blood glucose levels of the treatment groups on the 7th and 14th day.

The study has revealed that group treated with 300 mg/kg bwt ethanolic extract (115.60 ± 3.82) has significantly decreased the level of blood glucose $p<0.01$ as compared to the standard drug control group (136.60 ± 0.68) on the 21st day of treatment.

Table 1: Effects of *Leptadenia hastata*on blood glucose level (mg/dl) in streptozotocin-induced diabetes and non-diabetic albino rats

TREATMENTS	0day	7days	14days	21days
Normal control	102.60±2.71	90.40 ± 1.63	97.20 ± 1.32	95.20 ± 1.69
Diabetes control	446.20 ± 0.28	410.00 ± 1.92	440.00 ± 2.89	420.80 ± 3.68
Diabetic+ met. 5mg/kg-bwt	420.80 ± 5.65	312.00 ± 0.55*	197.00 ± 1.30*	136.60 ± 0.68*
Diabetic +150mg/kg-bwt EE	427.20 ± 5.85	399.40 ± 8.44*	200.20 ± 0.45*	141.40 ± 1.50*
Diabetic +300mg/kg-bwt EE	431.60 ± 4.64	326.40 ± 4.13*	147.60 ± 2.34**	115.60 ± 3.82**
Diabetic + 150mg/kg-bwtAE	416.20 ± 2.80	387.20 ± 3.64*	288.80 ± 6.39*	175.80± 1.59*
Diabetic + 300mg/kg-bwtAE	428.00 ± 5.12	340.20 ± 2.94*	248.80 ± 2.08*	156.80 ± 1.77*

Values are expressed as mean ± SEM; n=5, bwt-Body weight, EE- Ethanol extract, AE-aqueous extract, Met- metformin (standard drug control)
Key: * Significantly different as compared with diabetic control p<0.05
** Significantly different as compared with standard drug control p<0.01

Results indicated in Table 2 shows a significant increase p<0.05 in the levels of serum enzyme markers of diabetes control group AST (168.81 ± 1.69), ALT (85.37 ± 1.23) and ALP (368.97 ± 3.45) as compared to the normal control group AST (17.76 ± 0.20), ALT (24.41 ± 0.33) and ALP (85.79 ± 3.56) respectively. However, the level of enzyme markers AST, ALT and ALP of the diabetic groups receiving treatment of 150 mg/kg bwt, 300 mg/kg bwt of both aqueous and ethanolic extract respectively has significantly increased p<0.05 as compared to the normal control group but significantly decreased p<0.05 as compared to the diabetic control group.

The findings as shown in the table indicates that diabetes group treated with 300 mg/kg bwt ethanolic extract (30.39 ± 0.20) has significantly different P<0.01 levels of serum AST as compared to the standard drug control group (38.94 ± 1.44) and also the same applies to the levels of serum ALT (24.07 ± 0.97). The findings in this research work as shown in the table reveals that the serum level of ALP of group that received 300 mg/kg bwt ethanolic extract (153.64 ± 1.00) treatment and standard drug control group (142.08 ± 0.62) has significantly P<0.05 decrease as compared to the diabetes control group. Results from the findings has shown that the level of the serum enzyme marker ALT serum level of diabetes group treated with 300 mg/kg bwt (24.07 ± 0.97) has no significant difference as compared with normal control group (24.41 ± 0.33).

The results from the research as shown in Table 3 reveals a significant decrease p<0.05 in serum albumin of diabetes control group (18.62 ± 0.52) as compared to the normal control group (39.74 ± 0.34), standard drug control group (37.69 ± 0.71), diabetes groups treated with 150mg/kg bwt (33.99±0.48), 300mg/kg bwt (36.48±0.33) ethanolic extract and diabetes groups treated with 150 mg/kg bwt (28.22 ± 0.88), 300mg/kg bwt (32.14 ± 0.83) aqueous extract. Results obtained has also revealed that the level of serum albumin of all the experimental groups are significantly different P<0.05 as compared to the normal control group (39.74 ± 0.34).

However, the results as shown by the table reveals that the levels of serum albumin of diabetic groups treated with 150 mg/kg bwt ethanolic extract (33.99 ± 0.48), 150 mg/kg bwt aqueous extract (28.22 ± 0.88) and 300 mg/kg bwt aqueous extract (32.14 ± 0.83) are significantly different p<0.05 as compared to the standard drug control group (37.69 ± 0.71).

The results from this research revealed a significant decrease p<0.05 in the level of serum total protein of diabetic control group (55.20 ± 1.39) as compared diabetes group treated with 150 mg/kg bwt (76.60 ± 1.60) and group treated with 300 mg/kg bwt (75.20 ± 0.86) ethanol extract. From the finding, the table reveals that diabetes group treated with 150 mg/kg bwt and 300mg/kg bwt (61.80 ± 0.37) and (66.40 ± 1.17) respectively are significantly p<0.05 different as compared to the diabetes control group (55.20 ± 1.39) but shows that there is no significant p<0.05 difference between the groups treated with 150mg/kg bwt (76.60 ± 1.60) and 300 mg/kg bwt (75.20 ± 0.86) ethanol extract.

The values as shown in the table shows a significant p<0.05 increase in the level of serum total bilirubin (2.90 ± 0.03) and direct bilirubin (0.72 ± 0.04) in diabetes control group as compared to the values obtained in the treatment groups. The levels serum of total bilirubin of diabetes group treated with 300 mg/kg bwt (0.36 ± 0.02) ethanolic extract has significantly decreased p<0.05 as compared to the diabetic control group (2.90 ± 0.03) and significantly different p<0.05 as compared to the normal control group (0.28 ± 0.02) and direct bilirubin.

Table 2: Effects of *Leptadenia hastata* on enzyme markers of liver damage (U/L) in streptozotocin-induced diabetes and non-diabetic albino rats

TREATMENTS	AST	ALT	ALP
Normal control	17.76 ± 0.20	24.41 ± 0.33	85.79 ± 3.56
Diabetes control	168.81 ± 1.96	85.37 ± 1.23	368.97 ± 3.45
Diabetic+ met. 5mg/kg-bwt	38.94 ± 1.44[a]	27.67 ± 0.84[a]	142.08 ± 0.62[a]
Diabetic +150mg/kg-bwt EE	66.96 ± 1.15[a]	35.91 ± 0.64[a]	175.31 ± 1.86[a]
Diabetic +300mg/kg-bwt EE	30.39 ± 0.66[ab]	24.07 ± 0.97[ab]	153.64 ± 1.00[a]
Diabetic + 150mg/kg-bwt AE	105.25 ± 1.02[a]	74.11 ± 1.09[a]	252.51 ± 3.48[a]
Diabetic + 300mg/kg-bwt AE	92.77 ± 2.09[a]	36.26 ± 1.00[a]	192.54 ± 2.23[a]

Values are expressed as mean ± SEM; n=5, bwt-Body weight, EE- Ethanol extract, AE-aqueous extract, Met- metformin (standard drug control)

Key: [a] significantly different as compared to diabetic control p<0.05
[b] Significantly different as compared to standard drug control p<0.01

Table 3: Effects of *Leptadenia hastata* on non enzyme markers of liver damage in streptozotocin- induced diabetes and non-diabetic albino rats

TREATMENTS	Albumin(g/l)	T.P (g/l)	T.B (mg/dl)	D.B (mg/dl)
Normal control	39.74 ± 0.34	79.20±0.49	0.28 ± 0.02	0.20±0.02
Diabetes control	18.62±0.52	55.20 ± 1.39	2.90 ± 0.03	0.72 ± 0.04
Diabetic+ met. 5mg/kg-bwt	37.69 ± 0.71[a]	74.20 ± 0.58[a]	0.66 ± 0.05[a]	0.27 ± 0.03[a]
Diabetic +150mg/kg-bwt EE	33.99 ± 0.48[ab]	76.60 ± 1.60[a]	0.80 ± 0.03[a]	0.25±0.04[ab]
Diabetic +300mg/kg-bwt EE	36.48 ± 0.33[a]	75.20 ± 0.80[a]	0.36 ±0.02[ab]	0.28±0.02[a]
Diabetic + 150mg/kg-bwt AE	28.22 ± 0.88[ab]	61.80 ± 0.37[ab]	0.86 ± 0.02[a]	0.36±0.01[a]
Diabetic + 300mg/kg-bwt AE	32.14 ±0.83[ab]	66.40 ± 1.17[ab]	0.52±0.04[ab]	0.29±0.02[a]

Values are expressed as mean ± SEM; n=5, bwt-Body weight, T.P- Total protein, T.B- Total bilirubin, T.D- Direct bilirubin, EE- Ethanol extract, AE- Aqueous extract, Met- metformin (standard drug control)
Key: [a] significantly different as compared to the diabetic control group $p<0.05$
[b] Significantly different as compared to the standard drug control group

4. Discussion

Medical plants play an important role in the management of diabetes mellitus and many other ailments especially in developing countries where resources are meagre and the larger population mostly affected cannot afford the synthetic drug. In chronic disease such as diabetes, it is more relevant to test the maintenance of lower blood glucose level with long-term treatment rather than the acute hypoglycemic effect after a single administration. In this study, it was found that repeated administration of *Leptadenia hastata* extracts continually decreases blood glucose level in diabetic albino rats after induction of hyperglycemia with 60 mg/kg body weight streptozotocin.

Animal induced model of diabetes has provided considerable approach and clear knowledge about the physiologic and biochemical derangement of the diabetic state and have demonstrated that intra-venous injection of 60mg/kg dose of streptozotocin in adult albino rats, makes pancreas swell and causes degeneration inlangerhans islet beta cells. Streptozotocin selectively destroyed the pancreatic insulin secreting β –cells and induces experimental diabetes mellitus in 24 hours (Elobeid and Ahmed, 2015).

This high increase (hyperglycemia) in blood glucose of all the experimental animals was monitored for about 5 days before commencing treatment with doses designed for the research in which the increase in blood glucose was observed to be significantly high and stable for over 120 hours and this explains why streptozocin is relatively toxic to beta cells, since these cells have relatively high levels of GLUT2 as explained by Ali andAgha, (2009).Since streptozotocin-induced diabetes is accompanied by insulin resistance, *Leptadenia hastata* plant extract improved insulin sensitivity. It was reported that a traditional herbal medicine improved insulin action in streptozotocin-induced diabetic mice via enhancing insulin signalling (Prakash *et al.,* 2015).

The study has demonstrated a significant decrease in the blood glucose levels of the diabetes group treated with both ethanolic and aqueous extract at the end of 21 days treatment. However, there was a higher significant decrease in group with 300 mg/kg body weight ethanolic extract on the 14th day as compared to the standard drug and diabetic control groups on the 14th day treatment and on the 21st day of treatment. This has suggested that the dose 300 mg/kg body weight ethanolic extract is more effective in treatment of streptozotocin-induced diabetes mellitus in rat mode than all other dose of aqueous and ethanolic extracts. This decrease in blood glucose could be attributed to the presence of anti-diabetic bioactive components of the plant and this suggests that plant extract may have produce an effect similar to that of metformin mechanism, and thus could serve as good adjuvant to other oral hypoglycaemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus as reported in a similar studies by (Ahmed *et al.,* 2015).

Proper assertion of diabetic derangements does not depend on blood glucose assessment only but also on other biochemical parameters that are associated with defective metabolism of carbohydrate and other underlying contributing factors to the development of the disease (World Health Organization, 2009).

It is of clinical significance to determine the functionality of liver in monitoring the progress of treatment in chronic cases of diabetes mellitus. The study has demonstrated that the plant extracts was helpful in recovery of normal function of the lever as compared to the diabetic control group. The raise in the serum level of the enzymes was due to the toxic nature of streptozotocin (Qin *et al.,* 2003). Both aminotransferase enzymes are good markers of damage to liver cells that occurs in chronic disorders such as diabetes mellitus. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevations favour liver cell necrosis as a mechanism over cholestasis (Bainbridge, 2008). When AST and ALT are both over 100 U/I, the differential can include acetaminophen toxicity, shock, chronic diseases or fulminant liver failure (Sharma *et al.,* 2010).

One of the most important differential diagnosis of liver function is to determine the levels of serum albumin. The reason for decreased serum albumin is usually renal loss and therefore determination of serum albumin helps in understanding efficiency of drug treatment as albumin is responsible for maintaining osmotic balance between intravascular and interstitial spaces, therefore a deficiency in albumin ordinarily results in edema as water is redistributed to tissues. Glomerular membrane permeability

is partially a function of size but also is related to charge; the negative charge on albumin inhibits its filtration because the membrane likewise is negatively charged (Vandijik *et al*., 2009). Disease that causes damage to the glomerular membrane increases its permeability to all proteins. However, its permeability to albumin may be particularly affected if the negatively charged groups on the membrane surface are neutralized. This appears to be the principal mechanism of albuminuria associated with diabetic nephropathy (American Diabetes Association, 2014).

The study has shown significant improvements in the levels of serum albumin and scientifically support the therapeutic potential of *Leptadenia hastata* plant extract in rats treated as compared to the diabetic control group.

A decrease in serum total protein in diabetic control group as shown by the study may reflect decreased protein synthesis or increased protein loss. Treatment with *Leptadenia hastata* plant extracts showed a significant improvement in the serum total protein. Nearly all proteins are synthesized in the liver hence, hepatic failure is a cause of decreased serum protein but alone, not a good indicator of liver damage. A decrease in total protein is also observed when hepatic function is normal but the proteins are lost in the urine, loss of proteins in the urine results in a decrease in total serum protein (Etuk, 2010).

The study has also shown an improvement in the serum level of bilirubin in the treated groups. Clinical significance of bilirubin determination is essential in provisional diagnosis of livers injury and other diseases such as diabetes. The bilirubin normally presented in serum reflects a balance between production and clearance. Thus, elevated serum bilirubin concentrations can be due to three causes which can sometimes coexist; Overproduction of bilirubin, impaired uptake, conjugation, or excretion of bilirubin and backward leakage from damaged hepatocytes or bile ducts (Yoshinari and Igarashi, 2010).

5. Conclusion

Plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive because, because therapies developed along the principles of western medicine (synthetic drugs) are often limited in efficacy, associated adverse effects, and are often too costly, especially for the developing world.

It is therefore reasonable to suggest that the results clearly demonstrate that long term administration *Leptadenia hastata* plant extracts provides beneficial hypoglycemic and curative effects on the damaged organs associated with diabetes mellitus especially in streptozotocin-induced diabetes in albino rats. These findings represent an experimental confirmation of the traditional use of *Leptadenia hastata* plant extract for diabetes mellitus and amelioration of disease condition associated with chronic diabetes.

This evidence may be useful to the health professionals, scientists and scholars working the field of pharmacology and therapeutics to develop evidence-based alternative medicine to cure different kinds of diabetes in man and animal induced model.

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