Abstract:

This study investigates some of the effects of Mischeland anti-diabetic herb in the kidney of diabetic adult wistar rats. Mischeland is an herbal mixture consisting of alkaloids, anthraquinone, cyclogenetic glycosides, saponin and tannins. Diabetes is a serious lifelong multi-system disorder of glucose metabolism in the body. It is a condition characterized by high blood sugar level that results from defect in insulin secretion, or action, or both.

36 adult wistar rats of both sexes weighing between 195g – 280g were randomly divided into three groups; A, B and C. Diabetes mellitus was induced in group B and C rats by single intraperitoneal injection of streptozotocin (STZ) at a dosage of 70mg/Kg b.w, freshly dissolved in 1ml citrate buffer solution at pH of 4.5. The control rats (group A) were injected with only citrate buffer solution intraperitoneally. At the beginning of the 7th week post STZ injection, mischeland anti-diabetic herb was administered at a dosage of 0.92g/Kg b.w for rats in group C with the diabetic group B rats left untreated. The animals in group C were further subdivided into C1, C2 and C3. Group C1 received a normal dose of 0.92g/Kg b.w, C2 animals were administered additional 100% of C1 dosage (serving as high dose) and group C3 received 50% of group C1 dosage which served as low dose. This mischeland anti-diabetic herb treatment lasted for another six weeks, post STZ induction.

The result shows that the mean body weight of diabetic group (Group B) was significantly reduced compared to the control group A. It was also observed that the treated groups (C1, C2 and C3) regained a significant amount of body weight, following treatment with mischeland herb. In addition, it was also observed that the increased glucose level (in diabetic group B rats) was significantly reversed in the mischeland treated group C rats. G6PDH and LDH levels in the kidney and blood of treated rats were significantly increased, compared to their low levels in diabetic group B rats. This experimental work reveals mischeland anti-diabetic herb as a promising therapy for the reversal of the dreadful conditions caused by diabetes if further researched up on.

All results were expressed as Mean ± Standard Deviation (S.D) for each group. All grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included the independent – samples t–test. Statistical significance was set at p<0.05.

Keywords: Diabetes mellitus, streptozotocin, misheland herb, LDH, G6PDH, wistar rats.
Introduction:

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. In the ancient world, diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss. Hyperglycemia leads to spillage of glucose into the urine, hence the term "sweet urine." When the blood glucose elevates, insulin is released from the pancreas to normalize the glucose level. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia.

When people eat, the pancreas automatically produces the right amount of insulin to move glucose from blood into the cells. In people with diabetes, however, the pancreas either produces little or no insulin, or the cells do not respond appropriately to the insulin that is produced. Glucose builds up in the blood, overflows into the urine, and passes out of the body in the urine. Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose. This causes the cells of the body to lack glucose for energy production and so different disorders or total breakdown are seen in various systems of the body. With regard to this, diabetic patients have been described as people suffering in the face of abundance. Diabetes mellitus is classified into four 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. The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the blood metabolism and does not involve the same disease mechanisms. The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM). Likewise, the term "type 2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and nondiabetes-dependent diabetes mellitus (NIDDM). Beyond these two types, there is no agreed-upon standard nomenclature.

Diabetes can lead to serious complications and premature death, but people with diabetes can take steps to control the disease and lower the risk of complications. Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage may result in microvascular and macrovascular diseases. Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. However, it may be managed, keeping blood sugar levels euglycemic without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Diabetes is the most common cause of kidney failure, accounting for nearly 44 percent of new cases. Even when diabetes is controlled, the disease can lead to chronic kidney disease (CKD) and kidney failure. Nearly 24 million people in the United States have diabetes, and nearly 180,000 people are living with kidney failure as a result of diabetes.

Individuals with diabetes and chronic kidney disease are at high risk for cardiovascular disease. According to the National Kidney Foundation, about 30% of patients with Type 1 diabetes and 10 to 40% of those with Type 2 diabetes eventually will suffer from kidney failure. When kidney disease is diagnosed early, during microalbuminuria, several treatments may keep kidney disease from getting worse. However, when kidney disease is observed during macroalbuminuria, renal disease results.

Streptozotocin or Streptozocin or bad Izostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classically an anti-tumor antibiotic and chemically is related to other nitrosoureas used in cancer chemotherapy. It may also be a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce an animal model for Type 1 diabetes in large dose as well as Type 2 diabetes with multiple low doses. Each vial of sterilized Streptozotocin powder contains 1g of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[(methylnitrosoamino)-carbonyl]-aminol-D-glucopyranose and 200mg citric acid. Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylation agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself. 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. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2.
Glucose-6-phosphate Dehydrogenase (G6PD) is a cytosolic enzyme in the pentose-phosphate pathway (PPP), a metabolic pathway that supply reducing energy to cells while maintaining the level of co-enzymes Nicotinamide Adenine Dinucleotide Phosphate (NADPH). The NADPH in turn maintains the level of Glutathione in these cells that helps protect the cells against oxidative damage. It is widely distributed in distributed in many species from bacteria to man. Increased utilization of NADPH for fatty acid biosynthesis will dramatically increase the level of NADPH, thus stimulating G6PD to produce more NADPH. Glucose-6-phosphate dehydrogenase deficiency is very common worldwide, and causes acute hemolytic anemia in the presence of simple infection, ingestion of fava beans, or reaction with certain medicines, antibiotics, antipyretics, and antimalarials.

Lactose Dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply and performs the reverse reaction during the cori cycle in the liver. It exists in four distinct enzyme classes. Two of them are cytochrome c dependent enzymes, each act on either d-lactate or l-lactate. The other two are NAD(P) dependent enzyme with each acting on either d-lactate or l-lactate. It is an enzyme found in the cells of many body tissues including brain, heart, liver kidney, skeletal muscle, red blood cells and lungs. This protein may use the morpheein model of allosteric regulation.

The mammalian LDH exists as five tetrametric isometric isoenzymes which are LDH1, LDH2, LDH3, LDH4 and LDH5. Because of its presence in almost all the tissues of body, it is used to detect tissue alteration as an acid in diagnosis involving cellular destruction.

In Africa, Caribbean and China, the extensive uses of the plant include treatment of angina, high blood pressure, conjunctivitis, cough, diabetes, diarrhea, dieresis, dysmenorrheal, dysentery, febrile convulsion, fever, flank pains, food poisoning, fractures, hepatitis, inflammation, jaundice, rheumatism, sore throat, arthritis and tooth ache. Misenland is an herbal drug formulated for the treatment of diabetes and it is composed of the following active ingredients; Garcinia kola, Alstonia boonei, Vernonia amygdalina and Hydrangea macrophylla.

Garcinia kola belongs to the family Guttiferae (Clusiaceae). Its common names include bitter kola, monkey fruit, Orogbo (Yoruba language). It is an evergreen shrub with a pyramidal growth like the Christmas tree. It is widely cultivated in many parts of the world, but native to Asia, Australia, Tropical and Southern Africa. Common biomolecules in G. kola include kolaviron, biflavonoid (ametoflavone) and flavonoids (apigenin). These biomolecules are responsible for some of the healing properties of the plant.

Vernonia amygdalina belongs to the family Asteraceae (Compositae). Its common names include bitter leaf, Ewuro (Yoruba language). The plant is an evergreen shrub with many branches at the ground level. It is widely cultivated in many parts of the ground level, but native to Tropical Africa, especially west and central Africa and South America. Aqueous extract of V. amygdalina contains biomolecules that are effective for treating digestive disorder, fever, dysentery, headache, and diabetes. The bitter taste of V. amygdalina is due to antinutritional biomolecules, which include sesquiterpene lactones (vernodalin, vernolepin and vernomygdin) and steroid glycosides (vernoinosides).

Alstonia boonei belongs to the family Apocynaceae. Common names include stool wood, pattern wood, cheesewood, Awun (Yoruba language) and Ebgu (Igbo language). It contains biomolecules that are potent for treating hypotension, veneral diseases, rheumatism, stiff muscles. Other uses include enhance breast development, fever and worm expellant. Common biomolecules responsible for some of the healing properties include alkaloids (echitamide and echitamine), lactone boonein, triterpenes beta (amyrin and lupenol), saponins and tannins.

Hydrangea macrophylla belongs to the family Hydrangeaceae. Common names include big leaf, and mop head. It is widely cultivated in many part of the world, but native to southern and Eastern Asia and South America. H. macrophylla blossoms with shades of colours depending on the pH of the soil and it makes a good garden plant because of its beautiful blossom colours. Leaf-extracts of Hydrangea macrophylla are being investigated as a possible source of new chemical compounds with antimalarial activity. Hydrangeic acid from the leaves is being investigated as a possible anti-diabetic drug as it significantly lowered blood glucose, triglyceride, and free fatty acid levels in laboratory animals.

**MATERIALS AND METHODS**

**Management**

A total number of 36 adult wistar rats of both sexes weighing between 195g and 280g were used in this study, with the experiment lasting for a period of thirteen...
weeks. The animals were procured from the breeding stock of the department of anatomy, Ladoke Akintola University, Ogbomoso having observed to be all physically healthy. Upon procurement, the rats were kept at the animal house provided by the Department of Anatomy, Olabisi Owanbano University and divided into three groups of 12 animals each (groups A, B and C) for a period of one week acclimatization. They were fed with standard commercial rat pellet. Food, water and air were given ad libitum. The animal room was well ventilated with a temperature range of 25-27 °c.

Induction of Diabetes Mellitus and Treatment with Mishenland Herb

Group A served as the control group, group B; diabetic untreated group and group C; treated group. Diabetes mellitus was induced in group B and C rats by single intraperitoneal injection of streptozotocin (manufactured by Zao Sigma Scientific Company; representative of Sigma Aldrich, St. Louis, Mo, USA) at a dosage of 70mg/Kg b.w, freshly dissolved in 1ml citrate buffer solution at pH of 4.5. The control rats (group A) were injected with only citrate buffer solution intraperitoneally. The group C animals were further subdivided into 3 groups (C1, C2 and C3) according to the Mishenland dosage administered for six weeks following the initial six weeks post STZ induction. Group C1 received a normal dose of 0.92g/Kg b.w, C2 animals were administered additional 100% of C1 dosage (serving as high dose) and group C3 received 50% of group C1 dosage which served as low dose. The total body weights of all animals were measured at alternate days for the entire 12 weeks of induction and treatment.

Diabetes mellitus was confirmed induced by accessing the effect of STZ on the circulating blood glucose level with the aid of a glucometer, following blood samples taken via the animals’ tail region.

Histology and Biochemical Analysis

All animals in the three groups were anaesthetized with chloroform in closed chamber at the end of the 6 weeks of herbal treatment (for the group C animals), making a total period of 12 weeks for this work (excluding the one week acclimatization). The thoracic vertebrate was opened under aseptic condition; the same procedure was performed throughout with kidneys removed accessed for the level of LDH and G6PDH, then blood samples were taken for biochemical (LDH- lactate dehydrogenase and G6PDH- glucose-6-phosphate dehydrogenase) assays as well. Kidneys were fixed in 10% formal saline, blocks embedded in paraffin and sections cut at 5 micron which was then stained with H&E and mounted in Canada balsam. Microscopic examination of the sections was then carried out under a light microscope.

All results were expressed as Mean ± standard deviation (S.D) for each group. All grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included the independent – samples t-test. Statistical significance was set at p< 0.05.

RESULTS

Table 1: Variation in the weight of the rats (g)

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>No of rats</th>
<th>Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (NORMAL)</td>
<td>12</td>
<td>211.10 ± 02.25</td>
</tr>
<tr>
<td>Group B (DIABETIC)</td>
<td>12</td>
<td>129.50 ± 06.77</td>
</tr>
<tr>
<td>Group C (TREATED)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1 (Normal Dose)</td>
<td>4</td>
<td>163.80 ± 3.16</td>
</tr>
<tr>
<td>C2 (High Dose)</td>
<td>4</td>
<td>181.60 ± 2.45</td>
</tr>
<tr>
<td>C3 (Low Dose)</td>
<td>4</td>
<td>142.60 ± 1.08</td>
</tr>
</tbody>
</table>

The means of the groups were compared using T-test at p< 0.05.
Table 2: Variation in the glucose level of experimental adult wistar rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>No of rats</th>
<th>Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (NORMAL)</td>
<td>12</td>
<td>5.90 ± 0.56</td>
</tr>
<tr>
<td>Group B (DIABETIC)</td>
<td>12</td>
<td>27.60 ± 1.13</td>
</tr>
<tr>
<td>Group C (TREATED)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1 (Normal Dose)</td>
<td>4</td>
<td>12.40 ± 1.72</td>
</tr>
<tr>
<td>C2 (High Dose)</td>
<td>4</td>
<td>7.01 ± 0.99</td>
</tr>
<tr>
<td>C3 (Low Dose)</td>
<td>4</td>
<td>15.10 ± 0.11</td>
</tr>
</tbody>
</table>

The means of the groups were compared using T-test at p < 0.05
Table 3: Variations in LDH and G6PDH levels in the blood of experimental rat groups

<table>
<thead>
<tr>
<th>Groups/Dosage</th>
<th>Group A (Control)</th>
<th>Group B (Diabetic)</th>
<th>Group C1 (Normal Dose)</th>
<th>Group C2 (High Dose)</th>
<th>Group C3 (Low Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (Mean ± SD)</td>
<td>1.5±0.07</td>
<td>0.5±0.07</td>
<td>1.1±0.08</td>
<td>1.3±0.10</td>
<td>0.8±0.08</td>
</tr>
<tr>
<td>G6PDH (Mean ± SD)</td>
<td>1.2±0.02</td>
<td>0.4±0.04</td>
<td>0.8±0.03</td>
<td>1.1±0.02</td>
<td>0.7±0.07</td>
</tr>
</tbody>
</table>

The means of the groups were compared using T-test at p < 0.05

Table 4: LDH and G6PDH variations in the kidney of experimental adult wistar rats

<table>
<thead>
<tr>
<th>Groups/Dosage</th>
<th>Group A (Control)</th>
<th>Group B (Diabetic)</th>
<th>Group C1 (Normal Dose)</th>
<th>Group C2 (High Dose)</th>
<th>Group C3 (Low Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (Mean ± SD)</td>
<td>1.6±0.05</td>
<td>0.7±0.06</td>
<td>1.1±0.01</td>
<td>1.2±0.04</td>
<td>0.9±0.02</td>
</tr>
<tr>
<td>G6PDH (Mean ± SD)</td>
<td>1.8±0.09</td>
<td>0.8±0.08</td>
<td>1.1±0.07</td>
<td>1.4±0.06</td>
<td>1.0±0.03</td>
</tr>
</tbody>
</table>

The means of the groups were compared using T-test at p < 0.05
DISCUSSION

The result of this study showed that Mishenland reduced the adverse effects of STZ-induced diabetes mellitus on the kidney as evident by the reversal of the various adverse conditions caused by diabetes. The changes in the histoarchitectures of the renal tissues, affecting essentially the appearance of the glomerulus and tubular epithelium, are suggestive of cellular degeneration in the latter, caused by STZ-induced diabetes. The changes were however significantly ameliorated (at p<0.05) by Mishenland anti-diabetic herb.

In addition, rats in groups B and C were confirmed diabetic due to the high blood glucose level, loss in weight, hypoactivity, muscle wasting, hair loss etc. However, from the tables and figures in the results section; the following inferences may be drawn following Mishenland administration:

i. A regain of weight in the treated group C rats after it has initially declined significantly (at p<0.05) in the untreated diabetic group B

ii. A significant approach of the blood glucose level towards normal

iii. Marker enzymes; G6PDH and LDH were significantly reversed (at p<0.05) to about their normal level following an initial depreciation caused by diabetes
CONCLUSION

Following the aforementioned statistically and histologically significant effects observed, we may infer that Mishenland anti-diabetic herb is a promising therapeutic herb; containing substances that can significantly reverse the effects of diabetes mellitus on circulating blood glucose level, LDH & G6PDH as well as restoring normal histoarchitectural features of a diabetic-deformed kidney. Further research upon this herb might be huge in solution to diabetes mellitus in man.

Fig. 7: Micrograph of a low dose treated kidney cortex from group C3 stained with H & E. Arrow pointing at regenerating glomerulus, taken at lower magnification

Fig. 8: Micrograph of a normal/medium dose treated kidney cortex from group C1 stained with H&E with arrows pointing at glomerulus (Bowmans capsule thicker than that shown in low dose), taken at lower magnification

Fig. 9: Micrograph of a high dose treated kidney cortex from group C2 stained with H&E, Arrows pointing at the glomerulus with best defined Bowman’s capsule with other structures in good histoarchitecture, taken at a lower magnification

REFERENCES


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