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The Effects of Selected Kenyan Herbal Formulations on Glucose, Lipid Levels and Hepatic Function in Alloxan Induced Diabetic Rats

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Abstract
Introduction: Polyherbal formulations used for management of diabetes in Kenya lack studies to determine their efficacy or safety. **Objective:** To evaluate the efficacy and safety of two anti-diabetic polyherbal formulations (LUC and MUI). **Method:** Herbs were collected, dried and formulated. Formulations were evaluated using grouped alloxan induced wistar rats. Effects were compared to conventional drugs; pioglitazone (3mg/kg bw), glibenclamide (100 mg/kg bw), metformin (100 mg/kg bw) and normal control group. Each group received an individual drug/water once daily orally for fourteen days. Blood glucose levels were evaluated every seven days using a glucometer. Liver function tests and lipid profile were measured on day 14. The data was expressed in mean ± SEM. Analysis was by ANOVA and post hoc multi-comparison Turkey test ($p < 0.05$). **Results:** No mortalities reported. Both herbal preparations had hypoglycemic effects. LUC was more potent. MUI increased all lipid levels. LUC caused intestinal gas distention on gross examination. **Conclusion:** The herbal formulations were hypoglycemic at the tested doses.
Key words: polyherbal formulations, diabetes mellitus

1.0 Introduction
Diabetes mellitus is a disease affecting 350 million people worldwide. By 2030, the number is expected to grow to 552 million (Masoud, et al., 2014). It is a group of diseases that results in deranged fat, protein and carbohydrate metabolism due to deficient action of insulin in the body. The β cells of the pancreas normally produce insulin. In diabetes however, there is auto-immune destruction of the β cells so that the hormone is deficient or, there are abnormalities that cause resistance to insulin action (American Diabetes Association, 2004).

When there is an absolute lack of insulin, the patient is said to have type 1 diabetes. In Type 2 Diabetes Mellitus (T2DM), there is resistance to insulin action and insufficient compensatory insulin secretory response. The patients present with hyperglycemia and its clinical manifestations include polyuria, polydipsia, loss of weight, ketonuria, fatigue and blurred vision. In the long term, microvascular complications and marcovasular events occur (Patel, et al., 2012). Tight control of blood sugar can slow the progression of these complications.

Various treatment modalities are available to manage the symptoms of T2DM. These include lifestyle changes such as weight management and diet control, injectable insulin and oral allopathic hypoglycemic agents. These oral agents include among others; sulphonamides, biguanides, α-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase IV inhibitors and glinides. These modern drugs are often perceived as expensive for example the (glinides and dipeptidyl peptidase IV inhibitors), unavailable and having several adverse effects. Examples of adverse effects include weight gain and hypoglycemia in sulphonylureas and hepatotoxicity in thiazolidinediones (Fowler, 2007). As a result, herbs are a popular alternative. Several herbal formulations are in use in some countries for example Diabeta®, Epinsulin®, Diabecon®, Diasulin® and Diacare® (Modak, et al., 2007; Patel, et al., 2012). In Kenya, several non-conventional herbal formulations are in use to manage T2DM. Herbalists claim that their plant based treatments for diabetes mellitus have superior action compared to modern conventional drugs. The herbs are also popular among patients who claim that they lack any adverse effects common in allopathic drugs.

1.1 Objective
To evaluate the hypoglycemic, lipid lowering and hepatic effects of two polyherbal formulations (LUC and MUI) in diabetic rats.

2.0 Materials and Methods
2.1 Ethics
Ethical clearance was obtained from Kenyatta National Hospital/University of Nairobi Ethical committee (KNH/UoN-ERC) (as per the letter references KNH/ERC/A/133).

2.2 Materials
Wistar rats were purchased from the Animal Houses of the Departments of Pharmacology and Pharmacognosy (DoPP) and the National Public Health Laboratories. The following drugs were procured; glibenclamide (Daonil, Sanofi Aventis), pioglitazone (Sun Pharmaceuticals), metformin (Merck) and alloxan (Sigma Aldrich, St. Louis, Mo, USA). A glucometer (Expeeed™ Glucose Monitoring System Vivo GM 100, Hubdic Co. LTD) to measure blood glucose levels

was obtained. Ingredients to prepare two polyherbal formulations (LUC and MUI) were obtained from field visits and markets.

2.2 Plant collection, identification, drying, formulation and extraction

The plants were collected from their natural habitats with guidance from the herbalists. The date and time of collection was noted. Voucher specimens were prepared by pressing the plant flowers and the upper aerial parts between two presses. The voucher specimens were taken to the National Museums of Kenya for identification. The formulations of the combined different plants were prepared under the instructions and supervision of the herbalists.

2.3 Animal models and husbandry

The animal models were adult wistar rats which were bred according to Good Animal and Husbandry Practices (National Academy of Sciences, 2011). They were housed at DoPP in plastic cages with a maximum of 6 animals per cage with proper ventilation. They were exposed to a 12 hour dark and light cycle and the room temperature was maintained between 20-24°C. Humidity was at least 30%. The rats were monitored for general condition daily. Animal beddings were changed once a day and comprised of saw dust. Animals were feed on commercial feeds from a local supplier (Unga Feeds™) and tap water *ad libitum*. They were allowed to acclimatize for one week prior to starting the experiment.

2.3 Induction of diabetes

The rats were weighed and randomly caged in groups. A base line blood glucose level was measured and the rats were fasted overnight. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg b.w.) (Szkudelski, 2001). The alloxan was weighed individually for each animal according to the body weight and then solubilized with cold 0.95% NaCl just prior to injecting them intraperitonealy. They were injected with 20% dextrose intraperitonealy immediately and allowed to feed on normal feeds and 10% glucose solution for four days. Four days after the alloxan injection, rats with plasma glucose levels of more than 160 mg/dL were considered diabetic and included in the study. The glucose was withdrawn on confirmation that the animal had become diabetic and replaced with water.

The rats were divided into six groups. One group consisted of normal non-diabetic rats receiving pellets and water. Diabetes was induced in the remaining five groups. Each of these groups separately received an oral gavage of the following drugs once daily: glibenclamide (100 mg/kg b.w.); metformin (100 mg/kg/b.w.); pioglitazone (3 mg/kg/b.w.); LUC polyherbal formulation (1000 mg/kg b.w.) and MUI polyherbal formulation (1000 mg/kg b.w.). All drugs were dissolved in distilled water.

Care was taken to dose the animals at the same time daily. Random blood glucose levels were taken from tail snips at day 1, 7 and 14 using a glucometer. These procedures were done on both control and treatment groups. On day 14, blood was collected by cardiac puncture and stored in EDTA vaccutainer tubes for immediate biochemical analysis. The animals were sacrificed by cervical dislocation. The pancreas and liver were removed, rinsed in 10% formalin and preserved in 10% formalin solution. The tissues were processed using haematoxylin and eosin for histology examination.

2.4 Biochemical analysis

Selectra Pro Analyzer (Eli Tech Group Clinical Systems, Netherlands) was used for the biochemical tests. Analysis were conducted using manufacturer's protocols. The kinetic method (LiquiUV test) was used to determine Alanine Transaminse (ALT) and Aspartate Transaminase (AST). Direct and Total Bilirubin (D and T bil) were determined using Modified Jendrassik/Grof method. Total protein (T prot) was determined using Biuret method. Gamma glutamyl Transaminase (GTT) was determined using colorimetric test. Albumin (ALB) was determined by BCG method. Alkaline Phosphatase (ALP) was determined using optimised standard method. Total Glycerides (TG) and Total Cholesterol (TC) were determined by CHOD-PAP method. Liquicolour test kit was used to determine High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL)).

2.5 Statistical Analysis

All the values of fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.). Differences across groups were tested using one way analysis of variance (ANOVA) test. The post-hoc multi-comparison Turkey test was used to compare the test and negative control groups. Differences between groups was considered significant at p values of less than 0.05.

3.0 Results

3.1 Physical observations

There were no behavioral or neurological changes observes during the experimental period. However, during necropsy, gas accumulation in the gastro-intestinal tract (GIT) was observed in the group which had been on the LUC formulation (figure 1).

Figure 1: Swollen Intestines and caecum from Luc Formulation

3.2 Blood glucose

Blood glucose levels are presented in Table 1. Percentage changes during different periods of the study are in Table 2.

Table 1: Blood glucose averages

Group	Day 0 (blood glucose levels in mg/dL)	Day 1 (blood glucose levels in mg/dL)	Day 7 (blood glucose levels in mg/dL)	Day 14 (blood glucose levels in mg/dL)	Average blood glucose levels in mg/dL
Glibenclamide	352.80±70.00	279.40±68.31	277.00±82.98	181.40±20.68	303.07
LUC	288.57±68.55	194.29±37.26	201.29±54.51	113.00±33.50	228.05
MUI	243.60±38.59	212.40±47.08	199.80±32.73	174.80±25.70	207.60
Pioglitazone	170.25±9.70	127.50±11.06	118.50±9.22	60.50±20.50	162.08
Metformin	245.50±60.57	179.25±46.96	133.25±8.36	70.25±7.14	186.00
Normal Control	99.67±5.21	98.00±5.60	95.00±8.29	92.80±6.30	96.38

Table 2: Percentage change in blood glucose between day 1 and the 15th day

Duration	Normal	Glibenclamide	LUC	MUI	Pioglitazone	Metformin
Day 0-1	-1.672	-20.805	-32.673	-12.808	-25.110	-26.986
Day 1-7	-3.061	-0.859	3.603	-5.932	-7.059	-25.662
Day 7-14	-2.222	-34.513	-43.861	-12.513	-31.927	-47.280

The normal control maintained almost constant levels of blood glucose. After the first dose, the LUC group recorded the biggest drop in blood glucose levels (-32.673%). During the second week, metformin recorded the biggest dose of blood glucose levels (-25.662%). Metformin registered the biggest fall in blood glucose in the final period (47.280%). ANOVA shows that a significant difference exists between the drugs in this test ($p < 0.05$). A plot of average blood glucose levels over the experimental period is found in Figure 2.

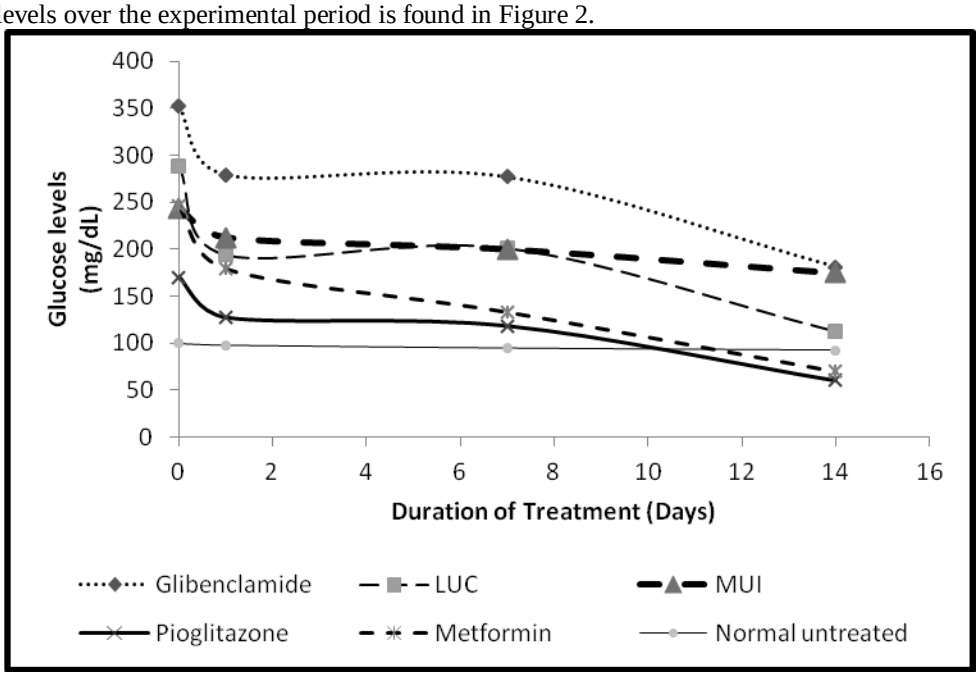

Figure 2: Changes in the glucose levels in alloxan-induced diabetic rats over the treatment period (To indicate the doses, the SD were deliberately omitted)

In all the treated groups, there was a gradual decline in glucose levels. At treatment induction, the glucose levels were substantially greater than levels of a normal non-diabetic mice. By the 14th day of treatment, the glucose levels of pioglitazone and metformin treated animals were slightly below that of non-diabetic animals. By Day 14, the mean glucose levels of the group treated with LUC was slightly greater than that of the non-diabetic animals. At Day 14, glucose levels of animals treated with Glibenclamide and MUI were substantially greater than those of non-diabetic untreated animals (≥175 mg/dL vs 92.8 mg/dL in the non-diabetic group).

Given that the mean glucose levels at the start of treatment varied considerably, the percentage reduction in glucose levels was computed to obtain a better idea of the hypoglycemic efficacy of each of the treatments. The percentage reductions in glucose levels on Days 7 and 14 are presented in Figure 3. ANOVA showed that there was a statistically significant difference in glucose levels across the groups (P=0.008). The Tukey multiple comparison test showed that on

Day 14 there was no statistically significant difference in the glucose levels of the normal rats and diabetic rats treated with LUC, MUI, Pioglitazone and Metformin. However, there was a statistically significant difference in the glibenclamide treated group (P=0.01).

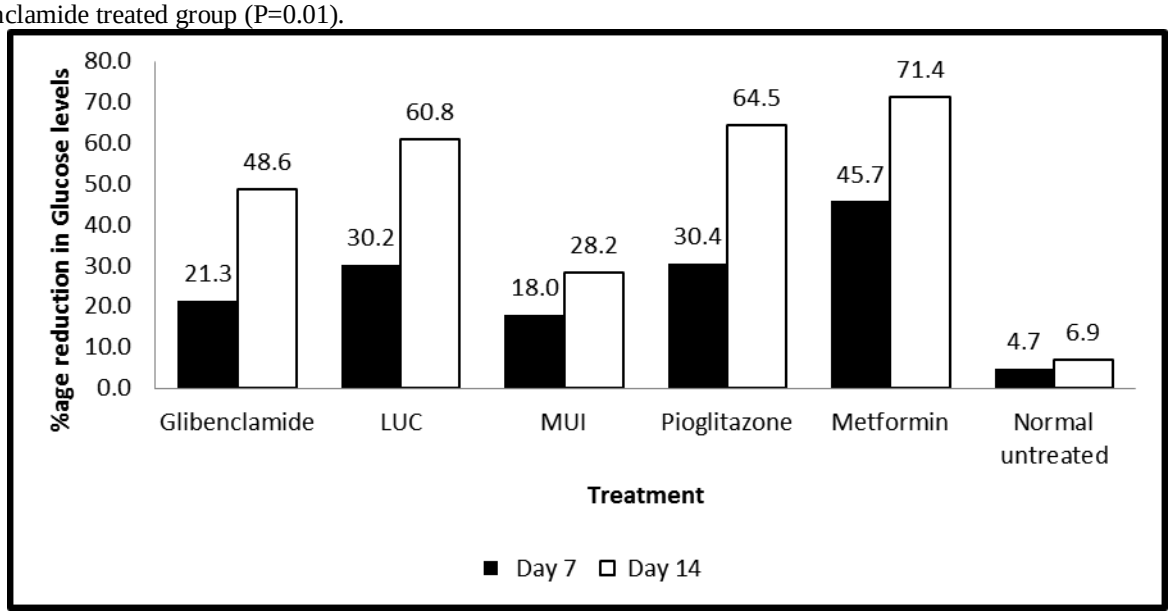

Figure 3: Percentage reduction in mean glucose levels in alloxan-induced diabetic animals

By Day 14, Metformin (71.4 %) caused the greatest reduction from the mean value at baseline, followed by pioglitazone (64.5%). Glibenclamide caused a 48.6% reduction in the baseline glucose values. The LUC herbal formulation seemed to have a greater efficacy than MUI formulation. It caused a 60.8% reduction from the mean baseline value compared to MUI which reduced glucose levels by 28.2% by Day 14. The effects of the all treatments increased with time. Fasting glucose levels in the normal animals reduced slightly by a maximum of 6.9%. This was attributed to random variation.

3.3 Effects of drugs on various lipids

Table 3: Effects of drugs on triglyceride and total cholesterol levels

	Triglycerides			**Total cholesterol**		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	1.04±0.08	1	-	1.99±0.44	1	-
Glibenclamide (100 mg/kg)	1.48±0.24	1.42	0.949	1.99±0.35	1	1
LUC (1 g/kg)	1.63±0.21	1.52	0.801	2.8±0.62	1.41	0.995
MUI (1 g/kg)	2.78±0.91	**2.66**	0.056	8.37±3.6	**4.21**	**0.037**
Pioglitazone (3mg/kg)	2.30±0.12	**2.21**	0.264	4.38±1.40	**2.20**	0.817
Metformin(100 mg/kg)	2.01±0.24	1.93	0.467	2.7710.26	1.40	0.998
F-test result	0.055			**0.021**		

The mean TG levels of all the diabetic animals were greater than the mean of the normal arm. ANOVA indicated that these increases were almost statistically significant (p = 0.055) (Table 3). The Tukey pairwise multi-comparison test showed the MUI group almost had a statistically significant value (p = 0.056). Increasing the sample sizes may give significant values.

All drugs except glibenclamide had elevated TC values. TC was found to be statistically significant across the groups (ANOVA, p= 0.021). The MUI group was found to have statistically significant TC values (p = 0.037).

Table 4: Effects of drugs on LDL and HDL levels

	HDL			**LDL**		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	1.07 ± 0.13	1	-	0.43 ± 0.14	1	-
Glibenclamide (100 mg/kg)	0.45 ± 0.14	0.42	0.832	0.87 ± 0.30	**2.00**	0.999
LUC (1 g/kg)	1.1 ± 0.20	1.03	1	1.04 ± 0.43	**2.39**	0.992
MUI (1 g/kg)	2.91 ± 1.04	**2.71**	**0.046**	4.19 ± 2.20	**9.67**	0.070
Pioglitazone (3mg/kg)	1.38 ± 0.33	1.28	0.994	1.95 ± 1.03	**4.50**	0.831
Metformin(100 mg/kg)	0.84 ± 0.06	0.78	0.998	1.02 ± 0.12	**2.35**	0.996
F-test result	**0.004**			0.063		

Metformin and glibenclamide treated arms had lower HDL levels compared to the normal controls but the differences were not statistically significant. HDL levels of the LUC and pioglitazone treated arms were similar to those of the control. The only group that had statistically significant elevated HDL levels was the arm treated with pioglitazone (P=0.046).

The mean LDL levels of all the diabetic animals were greater than the mean of the normal arm. However the Tukey pairwise multi-comparison test showed that differences between the test and negative control group were not statistically significant (Table 4).

3.4 Effects of drugs on liver function

Table 5: Effects of drugs on albumin levels and total proteins

	Albumin			**Total protein**		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	60.07±1.19	1	-	108.00±7.78	1	-
Glibenclamide (100 mg/kg)	29.78 ± 1.38	0.50	**0.000**	88.14±5.34	0.82	0.964
LUC (1 g/kg)	50.86 ± 3.95	0.85	0.462	114.06±16.13	1.06	1.000
MUI (1 g/kg)	60.23 ± 3.72	1.00	1.000	102.10±5.11	0.95	1.000
Pioglitazone (3mg/kg)	62.17 ±2.31	1.03	0.999	159.50±10.47	1.48	0.452
Metformin(100 mg/kg)	64.68 ±2.03	1.08	0.961	180.95±23.52	1.68	0.095
F-test result	**0.000**			**0.006**		

Glibenclamide and LUC treated rats had lower levels of albumin compared to the normal negative control group. The albumin levels of all other groups were similar to that of the normal group. Albumin levels of the glibenclamide treated arm were about half that of the control group and the difference in levels was statistically significant. Glibenclamide is known to be hepatotoxic. +The effects of LUC on albumin levels may indicate it was hepatotoxic. The concentrations of total proteins and albumin were closely correlated with glibenclamide treated animals having the lowest levels.

Metformin treated animals had the highest levels of both albumin and total proteins. There was a statistical significant difference in the concentration of total proteins and serum albumin across groups (Table 5).

Table 6: Effect of treatments on levels of bilirubin

	Total bilirubin			**Direct bilirubin**		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	43.45±9.92	1	1.000	28.03 ± 5.78	1	-
Glibenclamide (100 mg/kg)	41.76 ± 6.52	0.96	0.534	30.14 ± 5.33	1.08	1.000
LUC (1 g/kg)	247.56 ± 89.12	**5.70**	1.000	25.69 ± 4.86	0.92	1.000
MUI (1 g/kg)	44. 39 ± 9.21	1.02	0.663	27.30 ± 2.68	0.97	1.000
Pioglitazone (3mg/kg)	259.23 ±12.14	**5.97**	0.281	39.20 ± 1.89	1.40	0.814
Metformin	337.62 ±117.63	**7.77**	1.000	34.40 ± 4.92	1.23	0.972
F-test result	0.078			0.537		

Total bilirubin levels were almost correlated with albumin levels. Metformin had the highest total bilirubin levels with glibenclamide having the least. LUC treated rats had a very high total bilirubin level which was rather unexpected given that rats that received this treatment had low albumin levels. In addition LUC treated animals had the lowest direct bilirubin levels. In general there seemed to be an inverse correlation between direct bilirubin and albumin levels.

Table 7: Effects of drugs on the transferase levels

	ALT			**AST**		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	134.90 ± 63.54	1	-	393.70± 200.10	1	-
Glibenclamide (100 mg/kg)	326.58 ± 90.24	2.42	0.217	316.22± 90.48	0.80	0.993
LUC (1 g/kg)	132.28 ± 27.12	0.98	1.000	304.80± 61.01	0.77	0.982
MUI (1 g/kg)	106.60 ± 40.75	0.79	1.000	353.30± 1.46	0.90	1.000
Pioglitazone (3mg/kg)	78.73 ± 19.75	0.58	0.988	287.13± 141.98	0.73	0.983
Metformin	124.73 ± 23.55	0.92	1.000	472.15± 31.15	1.20	0.994
F-test result	**0.039**			0.750		

Glibenclamide was the only drug in which the ratio of ALT of test group verses the normal was higher. In addition, in the Glibenclamide treated arm, the levels of ALT were greater than AST level. ALT changes across groups were found to be statistically significant (p =0.039). All AST levels were lower than the normal control except for metformin. Changes AST levels were not statistically significant (Table 7).

Table 8: Effects of treatments on GGT and ALP

	GGT			ALP		
	Mean ± SD	**Fold increase**	**P-value**	**Mean ± SD**	**Fold increase**	**P-value**
Normal	3.30±0.55	1	-	696.10±161.58	1	-
Glibenclamide (100 mg/kg)	5.92±1.51	1.79	0.740	550.54±39.31	0.79	0.998
LUC (1 g/kg)	4.24±0.70	1.28	0.994	441.79±72.96	0.63	0.841
MUI (1 g/kg)	4.63±0.75	1.40	0.988	801.93±91.55	1.15	0.998
Pioglitazone (3mg/kg)	5.70 ±3.00	1.73	0.864	260.67±95.69	0.37	0.562
Metformin	5.68±0.57	1.72	0.835	663.40±345.89	0.95	1.000
F-test result	0.689			0.310		

All the diabetic animals showed elevated GGT serum levels. However, none of the elevations were statistically significant. ALP was elevated in MUI only. ALP changes were also not statistically significant (Table 8).

4.0 Discussion

Alloxan is a cytotoxic glucose analogue that causes diabetes by inhibiting the glucose sensor of the β cell hence preventing glucose induced insulin secretion. It also induces reactive oxygen species in a cyclic redox reaction with its reduction product (dialuric acid). This results in selective necrosis of β cells in the pancreas (Ashish & Swapnil, 2011). Marked elevations of blood glucose were recorded after its administration. Treatment with alloxan has been observed to increase the serum enzymes levels of cholesterol, LDL, and ALP and decrease HDL levels (Ahmed, et al., 2010). Hypertriglyceridemia has also be known to occur in alloxan diabetic rats (Kanthlal, et al., 2014; Valezquez, et al., 1991).

It is not known if the herbal formulations show a dose dependent action compared to standards but, they exacted a hypoglycemic effect. LUC showed superior hypoglycemic action compared to MUI. Glibenclamide was the only standard to show a statistically significant blood glucose reduction when compared to the normal control.

LUC's pattern of lipid control was almost similar to metformin but it was more favorable compared to MUI. MUI had marked increase in all lipid levels including HDL. Metformin should have improved the lipid profile (Mazza, et al., 2012), however, it marginally elevated TC, TG and LDL. Glibenclamide had the lowest lipid profile increases indicating that it reversed alloxan induced lipid changes. Pioglitazone was not protective of the LDL or TC.

Albumin levels are considered a true measure of liver function because it is synthesized only by the liver. It is reduced in diabetics due to decreased synthetic rates (Nicholson, et al., 2000). Depressed levels of albumin by LUC and glibenclamide may indicate depressed liver function or that the other drugs improved insulin function.

Levels of ALT and AST are used as indicators of hepatocellular damage. Elevated levels indicate leakage from damaged heptocellular cells. Damage could arise due to inflammation or cell death. ALT is considered to be a more reliable measure of liver damage as opposed to AST which can be found outside the liver (Ozturk & Kadayifci, 2014). The test formulations (MUI and LUC) had AST and ALT levels below those of the normal control. Increased ALT is the most common abnormality in type 2 diabetes although in this case, it only occurred in the glibenclamide treated group (Harris, 2005). In the Glibenclamide treated arm, the levels of ALT were greater than AST levels which was indicative of established cirrhosis

All drugs had increased GGT values compared to the normal control but LUC and MUI had the lowest elevations. Raised GGT values are common in diabetes but when other liver enzymes are raised, a hepatobiliary source is suspected (Limdi & Hyde, 2003). All the diabetic animals showed elevated GGT serum levels. It was difficult to determine whether this could have been due to liver disease or diabetic condition. All types of liver conditions may show GGT elevation. ALP elevation in absence of pregnancy and bone disease may indicate hepatobiliary disease (Grover & Bafna, 2013). MUI formulation marginally increased ALP values while all other test groups registered levels below the normal control. This formulation may not have been able to reverse alloxan associated increases in ALP.

Bilirubin elevation could indicate defects in hepatic uptake, defects in hepatic excretion and hemolysis among other causes (Grover & Bafna, 2013; Limdi & Hyde, 2003). In the present study, metformin, pioglitazone and LUC formulation registered marked increases of more than fivefold in total bilirubin. This could indicate moderate hepatocellular damage from alloxan or the test drugs. Glibenclamide and MUI may have reversed this damage since they did not increase bilirubin. All the elevations (apart from total bilirubin in LUC, metformin and pioglitazone) were however below 5 fold increases and may be considered mild (Giannini, et al., 2005).

The LUC formulation caused gas accumulation in the GIT. This adverse effect is observed in alpha-glucosidase inhibitors. These anti-diabetic agents hinder intestinal enzymes that cleave polysaccharides to monosaccharides. This slows down the absorption of carbohydrates leading to GIT symptoms such as flatulence (Fowler, 2007).

Metformin should have no significant effect on liver histology (Ozturk & Kadayifci, 2014). In this study however, metformin and glibenclamide showed some degree of hepatocellular degeneration. The herbal formulations (LUC and MUI) appeared more protective of the hepatocytes with far less degeneration observed.

5.0 Conclusions

The herbal formulations ameliorated blood glucose levels with LUC showing superior control to MUI. Liver function tests presented a mixed picture. The herbal formulations indicated suggested mild hepatotoxicity. Chronic toxicity testing may be more confirmatory.

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