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Effect of a poly-herbal formulation, *Okudiabet,* on alloxan- induced diabetic rats

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ABSTRACT

The study examined the acute toxicity, hypoglycaemic and hypolipidaemic effects of a poly-herbal drug, *Okudiabet* –a mixture of *Stachytarpheta angustifolia, Alstonia congensis bark* and *Xylopia aethiopica* fruits extract– used in the treatment of diabetes. The median acute toxicity value (LD_{50}) of the drug was determined to be 16.5 g/kg body weight (bwt). A significant increase in the body weight was observed in the diabetic and normo-groups treated with the drug. There were significant reductions ($p<0.05$) in the plasma glucose and low density lipoprotein (LDL)-cholesterol levels, and significant increase ($p<0.05$) in high density lipoprotein (HDL)–cholesterol in the treated diabetic group compared to the control. Furthermore, significant reductions in aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels were observed in the treated diabetic animals compared to the control. Also significant reduction in the creatinine and increase in the protein levels respectively were observed in the treated diabetic group. The results showed that the phytomedicine had both good hypoglycaemic activity and beneficial effects on cardiovascular risk factors. The high LD_{50} value is an indication that the drug has a high safety margin.

Keywords: *Stachytarpheta angustifolia, Alstonia congensis, Xylopia aethiopica*, acute-toxicity, diabetes.

INTRODUCTION

Diabetes mellitus (DM) is now recognized as one of the leading causes of death in the developing countries, where the high prevalence of the disease can be attributed to improved nutritional status coupled with a gross lack of modern facilities for the early diagnosis of the disease (Ogbonnia *et al*., 2008). It has been described as the common metabolic disorder of carbohydrate and fat metabolism, which is due to absolute or relative lack of insulin and is characterized by hyperglycaemia (Walter, 1977). Two main types of diabetes based on their clinical manifestation are identified as type I diabetes- known as juvenile onset or insulin sensitive diabetes and type II diabetes or non insulin dependent diabetes mellitus (NIDDM) (Gale, 2001). Type II diabetes which is the more prevalent form may have as its underlying metabolic causes the combined effects of impairment in the insulin-mediated glucose disposal and defective secretion of insulin by the β-cells of the pancreas (Grundy *et al*., 1999).

Diabetes has been conventionally treated with orthodox medicines that function as hypoglycaemic agents, or insulin production modulators and/or lipoprotein lowering agents (Ogbonnia *et al*., 2008). Sulfonylurea and metformin are valuable in the treatment for hyperglycaemia in NIDDM but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis (Senthilvel *et al*., 2006). Even when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects (El.Nagar *et al*., 2005; Egwim E. 2005). In addition they are not suitable for use during

pregnancy (Senthilvel *et al*., 2006; Sushruta *et al.,* 2006). Since the therapy is life long, therapeutic agents devoid of side effects would be appreciated and one of such approach is the use of alternative system of medicine comprising herbal products (Pari and Saravanan, 2004). For these reasons, therefore, there is a great need for a search of an acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agents that would be effective in the treatment of diabetes and devoid of serious side effects of the currently used oral hypoglycaemic agents. Herbs and marine sources have been considered the best option. *Okudiabet* is a poly-herbal formulation that has been employed by some traditional herbalist for the management of DM and is prepared with *Stachytarpheta angustifolia* (Mill) Vahl aerial part, *Alstonia congensis* Engler bark and *Xylopia aethiopica* (Dunal). Rich dried ripe fruits of *S. angustifolia* was earlier evaluated for its antibacterial activity and acute toxicity effects (Enwuru *et al.*, 2008) while a mixture of *A. congensis* and *X. aethiopica* used locally in the treatment of various diseases has also been evaluated for its sub-acute effects on rodents (Ogbonnia *et al*., 2008).

This study, therefore, was aimed at evaluating the hypoglycaemic and hypolipidaemic effects of aqueous ethanol (80%) extracts of a mixture (3:2:1) of *S. angustifolia* aerial parts, *Alstonia congensis* bark and *X. aethiopica* dried ripe fruits. The effect on plasma glucose level, total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-cholesterol), low density lipoprotein-cholesterol (LDL-cholesterol), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and plasma creatinine levels in alloxan–induced diabetic albino rats (a model of type II diabetes) were evaluated as index of toxicity, hypoglycaemic and hypolipidaemic activities.

MATERIALS AND METHODS

The poly-herbal formula composed of *Stachytarpheta angustifolia*, *Alstonia congensis* and *Xylopia aethiopica* used in the ratio of 3:2:1 was ceded by a traditional practitioner, Agu Titus Iwuala Clinic, of No 106 Mushin- Itire Road, Itire, and Lagos, Nigeria.

1. *Stachytarpheta. angustifolia* 300 g
2. *Alstonia congensis* 200 g
3. *Xylopia aethiopica* 100 g

Stachytarpheta angustifolia (Mill) Vahl (Fam. Verbanaceae) is a seasonal plant growing mostly in the farmlands at the banks of River Niger and streams, in southern Nigeria, during the rainy seasons. The plant called "Ncha aji" meaning crocodile's soap in the local language was collected from Atani, a town located at the bank of River Niger in Anambra State, Nigeria and was authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan and voucher specimen (FHI 81324) deposited at the Institute's herbarium. *A. congensis* bark and *X. aethiopica* fruits were bought from Mushin market in Lagos State, Nigeria and were authenticated by Prof. Dele Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Lagos. The voucher specimens LUH 2054 and LUH 2079 respectively were deposited at the Department of Botany Herbarium. The plant materials were dried at an ambient temperature between 35 – 40 °C in an oven for five days and powdered to coarse particles. Six hundred grams (600 g) of the poly-herbal powder materials in the ratio of 3:2:1 respectively was macerated with 2.5 L ethanol (80 %) for seven days with frequent stirring. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50 °C and dried to a constant weight of 37.45 g (6.24 %) yield

Animals: Swiss albino mice (20 – 25 g) and wistar rats (160 ± 20 g) of either sex were obtained from the Laboratory Animal Center, College of Medicine, University of Lagos, Idi-Araba and were kept under standard environmental condition of 12/12 hr light/dark cycle. They were housed in cages (5 animals per cage), maintained on standard animal cubes (Livestock Feeds Nigeria Ltd) and provided with water *ad libitum.* They were allowed to acclimatize for seven days to the laboratory conditions before the commencement of the experiment. The use of the animals and the experimental protocol was approved by the Experimental Ethics Committee on Animals Use of the College of Medicine, University of Lagos, Nigeria.

Acute toxicity study: The toxicity study was carried out using thirty- five (35) male and female Swiss albino mice. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. After depriving them food overnight, the control group received 0.3 ml of 2 % Tween 80 orally while each treated group received orally solution of the extract in 2 % Tween 80 in the doses of 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg body weight respectively. They were closely observed in the first 4 hours and then hourly for the next 12 hours followed by 6 hourly intervals for the next 56 hours (overall 72 hr) after the drug administration to observe any death or changes in

general behaviour and other physiological activities (Shah *et al.,*1997; Bürger *et al*.,2005).

Diabetic study: After overnight fast, diabetes was experimentally induced on the animals by administering intraperitoneally (i.p.) alloxan monohydrate dissolved in normal saline (150 mg/kg). The blood sugar levels was monitored with a glucometer (Accu-Chek, Roche Diagnostics) after 72 hours and the rats with plasma glucose level > 200 mg/dl were classified as diabetic and were used for the study. A total of six groups containing five animals per group were used. Four groups were diabetic while the remaining two groups were used as different controls and were treated daily for 30 days as follows:

Group I: Induced diabetic rats treated with the drug 500 mg/kg bwt
Group II: Induced diabetic rats treated daily with the drug 250 mg/kg bwt
Group III: Normal rats treated daily with the drug 250 mg/kg bwt
Group IV: Induced diabetic rats treated daily with Glibenclamide 600 µg/kg bwt (Mahdi *et al*., 2003),
Group V: Diabetic rats not treated
Group VI: Control given 0.5 ml 2 % Tween 80 solution

The animals were initially weighed and then weighed every seven days from the beginning of the treatment. On the 31st day, after overnight fast, were made unconscious by cervical dislocation and blood was obtained via cardiac puncture into fluoride oxalate and heparinized containers. The blood collected with fluoride oxalate tube was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma used to determine the blood glucose level. The TC, TG and HDL-cholesterol levels were estimated with heparinized blood using precipitation and modified enzymatic procedures from Sigma Diagnostics (Wassan *et al.,* 2001). LDL-cholesterol level was calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for ALT, AST and creatinine by standard enzymatic assay method. The plasma protein content was determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002).

Statistical Analysis: Student's t- test was used and differences were considered significant at $p<0.05$ or $p<0.01$. All data are expressed as mean ± standard error of the mean.

RESULTS

In the acute toxicity study (Table 1), three out of the five animals that received 20.0 g/kg bwt of the extract died within 4 hr (60 % death) while the animals that received 10 g/kg bwt survived beyond 24 hr. The LD_{50} of the drug was therefore calculated to be 16.5 g/kg bwt.

The effect of the poly-herbal medicine on the bwt of the diabetic and normal rats and also the effect of the reference drug, glibenclamide, on the diabetic rats are shown in Table 2 and Fig. 1. There was no significant difference in bwt of the diabetic animals treated with poly-herbal medicine compared to the normal control. Also, no significant decrease ($p<0.05$) in bwt was observed in diabetic untreated compared to the normal control.

The result of the effects of the drug and glibenclamide on the organs of the diabetic animals and on the normal rats is presented in Table 3. The macroscopic examinations of the organs of the animals treated with the drug and glibenclamide did not show any changes in colour while the organs of untreated diabetic animals showed some changes compared to the normal control.

Table 4 summarized the results of the phytomedicine and glibenclamide effects on the biochemical parameters. The plasma glucose levels of the diabetic rats treated with the drug and glibenclamide were significantly reduced ($p<0.05$) compared to the diabetic control. The drug proved to have a better plasma glucose lowering effect than glibenclamide.

There was a significant increase ($p<0.05$) in the plasma AST and ALT levels in the untreated diabetic animals. On the other hand, the two enzymes were observed to have decrease markedly in levels in the diabetic animals treated with the drug. A significant decrease ($p<0.05$) in the plasma TC level was observed in all the diabetic animals treated with the drug or glibenclamide compared to the diabetic control.

There was also a significant decrease ($p<0.05$) in both TG and LDL-cholesterol levels while significant increase in HDL-cholesterol level was observed in all diabetic animals treated with the drug or glibenclamide. In contrast, the untreated diabetic animals showed significant increase in both TG and LDL-cholesterol levels and a significant decrease in HDL-cholesterol level.

There was no significant change observed in the protein levels in all the animals treated with different doses of the phytomedicine and glibenclamide while

a significant change (p≤ 0.1) was observed in untreated diabetic animals compared to normal control. A significant increase in the creatinine level was observed in the diabetic animals treated with highest dose of the phytomedicine and the untreated diabetic rats compared to the normal control. On the other hand, marked decrease was observed in the diabetic animals treated with glibenclamide – the reference drug.

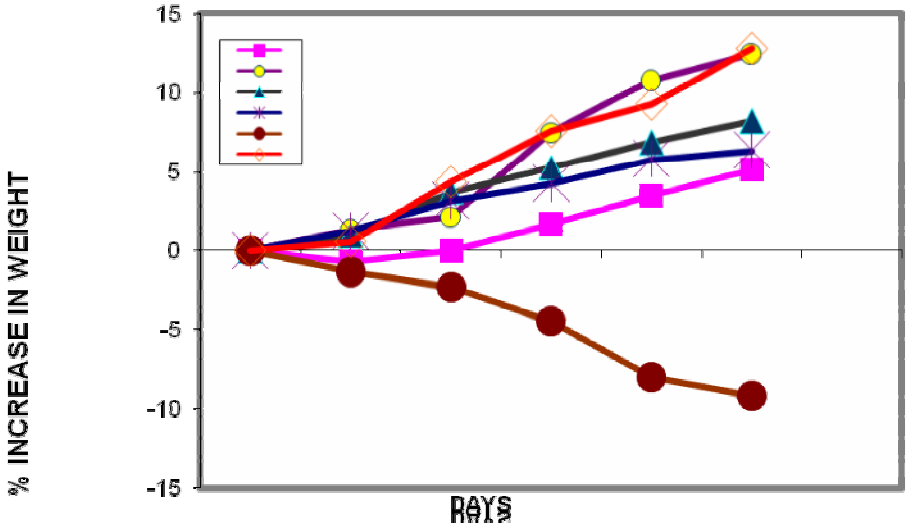

Fig.1 Percentage increase in weight of diabetic animals treated with 500, 250mg kg-^{1} body weight of the phytomedicne and glibenclamide respectively and normorats treated with phytomedicine and diabetic but not treated animals.

●untreated diabetic rats■ diabetic treated with 500mgkg-1 body weight○ control ◊ diabetic treated with 250mg Ж diabetic treated with glibenclamide and ▲normal but treated animal

Table 1: Acute toxicity of the poly-herbal formulation – Okubetic - in mice

Doses of the Drug g/kg	Number of mice used	Number of mice dead	% Cumulative mice dead
1.0	5	0	0
2.5	5	0	0
5.0	5	0	0
10.0	5	1	16.7
15.0	5	1	33.3
20.0	5	4	100.0

Control group received 0.3ml each of Tween 80 (2%) solution.

Table 2: Weight variation of the control, untreated diabetic rats, diabetic rats and normal rats treated with the polyhedral formulation and diabetic rats treated with glibenclamide doses for 30 days.

Group	Day 1	Day 7	Day 14	Day 21	Day 28	Day 31
I	145.3 ± 2.5	144.0 ± 0.7	145.1 ± 2.5	147.5 ± 0.8	150.0 ± 1.3	152.1 ± 3.1
II	130.3 ± 2.5	132.0 ± 1.5	132.8 ± 0.1	140.0 ± 2.3	144.0 ± 2.3	146.2 ± 1.8
III	142.5 ± 0.7	143.9 ± 0.5	147.8 ± 0.6	150.0 ± 3.1	152.3 ± 0.7	154.2 ± 2.5
IV	158.1 ±7	160.0 ± 0.8	163.1 ± 1.5	164.8 ± 1.6	167.3 ± 2.6	168.1 ± 2.7
V	130.5 ±0.2	129.0 ± 1.5	127.5 ± 0.0	124.7 ± 0.9	120.1 ± 0.2	123.1 ±0.1
V1	140.1 ± 3.5	140.9 ±2.3	147.1 ± 2.7	150.7 ±1.2	153.0 ± 2.5	157.9 ± 2.5

Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs control group. .

Group I: Diabetic rats treated with 500mg extract/kg bwt; Group II: Diabetic rats treated with 250mg extract/kg bwt; Group III: Normorats treated with 250mg extract /kg bwt; Group IV: diabetic rats treated with Glibenclamide 600µg bwt ; Group V: Diabetic not treated; Group VI Control rats received 0.5ml Tween 80 (2%) solution.

Table 3: The effects of the extract and glibenclamide on kidney, heart, liver and brain of the diabetic rats and also the effect of the extract on normal rats compared with the control.

ORGAN	Group I	Group II	Group III	Group IV	Group V	Group VI
Heart (g)	0.7 ± 0.3	0.5 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.2
Kidney (g)	0.9 ± 0.1	0.8 ± 0.2	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.4 ± 0.2
Liver (g)	3.1 ± 0.7	2.8 ± 0.5	5.1 ± 0.6	4.1 ± 0.6	3.0 ± 0.4	4.9 ± 0.4
Brain (g)	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	1.2 ± 0.3

Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs control group. .
Group I: Diabetic rats treated with 500mg extract/kg bwt; Group II: Diabetic rats treated with 250mg extract/kg bwt; Group III: Normorats treated with 250mg extract /kg bwt; Group IV: diabetic rats treated with Glibenclamide 600µg bwt; Group V: Diabetic not treated; Group VI Control rats received 0.5ml Tween 80 (2%) solution.

Table 4: Plasma glucose level and other biochemical profiles of untreated diabetic rats, diabetic but treated with the extract and glibenclamide respectively and the normal rats treated with extract and the control

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
Glucose (mg/dl)	36.8 ± 1.2*	43.3 ± 20.1*	93.1 ± 1.2	48.0 ± 0.5 *	362.2 ± 1.6*	82.3 ± 2.5
Cholesterol (mg/dl)	32.2 ± 2.1*	62.5 ± 5.2	43.2 ± 1.6	56.1 ± 3.1	600.7 ± 0.5*	61.2 ± 2.5
Triglyceride (mg/dl)	24.9 ± 0.8*	35.4 ± 3.6	60.3 ± 0.7*	78.6 ± 1.5*	113.8 ± 2.1*	44.9 ± 0.8
HDL (mg/dl)	43.3 ± 1.6*	26.5 ± 2.9	37.3 ± 2.2	47.5 ± 0.6	21.7 ± 4.1**	56.3 ± 1.6
LDL (mg/dl)	11.0 ± 2.6	39.5 ± 7.4*	27.9 ± 0.6*	36.3 ± 1.5*	52.5 ± 2.2*	6.8 ± 0.8
Protein (g/dl)	6.8 ± 0.5	7.2 ± 0.1	8.5 ± 0.2	7.1 ± 0.3	14.8 ± 0.7**	7.5 ± 0.5
Creatinine (mg/dl)	8.9 ± 0.0*	3.6 ± 0.2	3.3 ± 0.1	1.0 ± 0.0	7.1 ± 0.5*	3.5 ± 0.2
AST (I.U/L)	7.2 ± 1.2	8.0 ± 2.1	6.7 ±2.5	13.1 ± 0.0	41.8 ± 0.9*	10.8 ± 1.2
ALT (I.U/L)	15.6 ± 1.5	16.8 ± 4.5	18.6 ± 2.8	17.7 ± 3.5	28.7 ± 3.5*	12.3 ± 1.3

Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs control group. .Group I: Diabetic rats treated with 500mg extract/kg bwt; Group II: Diabetic rats treated with 250mg extract/kg bwt; Group III: Normorats treated with 250mg extract /kg bwt; Group IV: diabetic rats treated with Glibenclamide 600µg bwt ; Group V: Diabetic not treated; Group VI Control rats received 0.5ml Tween 80 (2%) solution.

Table 5. The haematological parameters of the control and alloxan induced diabetic animals treated with the poly-herbal.

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
RBC (10^6 /mm^3)	5.5 ±0.0	5.6 ± 0.1	6.1 ± 0.1	5.8 ± 0.1	3.7 ± 0.6*	5.9 ± 0.6
WBC (10^3 /mm^3)	8.8 ± 0.0	6.4 ±1.7	8.2 ± 0.0	4.9 ± 0.0	10.6 ± 0.5*	6.1 ± 0.3
Hb (g/dl)	13.2 ± .3	13.2 ± 1.1	14.9 ± .3	14.7 ± .2	9.8 ± 0 .5*	14.5 ± 0.7
PCV %	40.0 ± 2.2	41.1 ± 1.5	45.1 ± 2.5	43.1 ± 0.5	27.8 ± 0.1*	43.0 ± 0.2
Calcium (mg/dl)	10.0 ± 0.0	8.5 ± 0.1	10.0 ± 0.0	8.9 ± 0.0	19.1 ± 0.2*	9.8 ± 0.3
Phosphorus (mg/dl)	18.9 ± 0.3	13.3 ± 1.0	15.1 ± 0.0	16.0 ± 0.0	17.5 ± 0.4	16.5 ± .1

Mean ± sem, (n=5) *p<0.05; ** p<0.01 vs control group. .Group I: Diabetic rats treated with 500mg extract/kg bwt; Group II: Diabetic rats treated with 250mg extract/kg bwt; Group III: Normorats treated with 250mg extract /kg bwt; Group IV: diabetic rats treated with Glibenclamide 600µg bwt; Group V: Diabetic not treated; Group VI Control rats received 0.5ml Tween 80 (2%) solution.

DISCUSSION

Diabetes is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives world over. Oral hypoglycaemic agents especially the sulphonylureas and biguanides have been commonly used in the disease management especially type II diabetes but are not without serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of diabetes. The poly-herbal preparation, *Okudiabet*, is one of such herbal remedies prepared from the bark, fruits and herbs used locally in the treatment of diabetes.

The median acute toxicity value (LD_{50}) of the drug preparation was determined to be 16.5 g/kg bwt. According to Ghosh (1984) and Klaasen *et al.*, (1995), the poly-herbal medicine can be classified as being non-toxic, since the LD_{50} by oral route was found to be 15 g/kg which was much higher than WHO toxicity index of 2 g/kg.

Although increase in appetite and water consumption was observed in the diabetic and normal animals treated with the poly-herbal medicine, there was no significant weight gain by the animals. The non significant weight gain observed in the diabetic animals treated with the drug clearly suggested that the poly-herbal medicine might not have the obesity forming tendency, which is one of the undesirable side effects normally encountered when treating diabetics with sulphonylureas. There were also no changes observed in the macroscopic examinations of the organs of the diabetic animals treated with the poly-herbal medicine or glibenclamide.

The poly-herbal drug showed to be effective in decreasing plasma glucose levels on the diabetic rats and proved to have a better plasma glucose lowering effect than glibenclimade. By decreasing blood glycaemia the risk of other disease complications associated with people suffering with diabetes is minimized. The effective lowering of blood sugar level demonstrated by this drug supports its local use as a hypoglycaemic agent.

The hepatic and cardiac tissues release AST and ALT and the elevation of plasma concentrations of these enzymes is an indicator of hepatic and cardiac damage (Crook, 2006). The decrease in ALT and AST levels in the diabetic animals treated with different doses of the poly-herbal medicine implied that the drug at the doses used did not produce any harmful effects on either the cardiac or the hepatic tissues. However, in the untreated diabetic animals, the two enzymes showed marked increase which suggested that hepatic and cardiac problems occurred.

The lowering of plasma TC, TG and LDL-cholesterol levels and significant increase in HDL-cholesterol level in the treated animals clearly demonstrated the presence hypolipidaemic agents in the poly-herbal medicine. The ability of the poly-herbal medicine to manage dyslipidaemia is a potential beneficial effect on cardiovascular risk factors which is a major cause of death in DM (Valli and Giardina, 2002; Zhou *et al.,* 2006). An increase in plasma creatinine levels coupled with decrease in the protein levels could be a sign of impaired renal function (Tietz, 1982). The poly-herbal drug, at the dose of 500 mg/kg bwt, led to an increase in creatinine and decrease in the protein levels respectively which suggested impairment in the renal system. However, it was observed that treatment at lower concentration did not exhibit deleterious effect. The calcium level was not affected in all the treated animals while a significant increase in the level of phosphorus was observed only in the animals treated with the highest dose of the extract. It had been documented that the most important cause of elevated phosphate level is chronic glomerular disease, and is associated with elevated blood urine nitrogen and creatinine (Tilkian *et al*., 1979).

Appreciable recovery in RBC, PCV and Hb levels were recorded in the diabetic animals treated with the poly-herbal drug while WBC count showed a slight increase. According to a report, WBC usually show increase in activity in response to toxic environment (Robins, 1974).

CONCLUSION

The high LD_{50} value (16.5 g/kg) obtained was a clear indication that poly-herbal preparations could be safe for use. The study showed that the poly-herbal preparation has some hypoglycaemic and hypolipidaemic activities and good reducing effects on the cardiovascular factors. The study also revealed that the drug at doses investigated did not provoke toxic effects to the animals' heart and liver. However, there is a strong likelihood that at a high dose in diabetic treatment, there may be kidney damage which might lead to renal failure.

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