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Antidiabetic Activity of the Leaves of *Ficus sur* Forssk (Moraceae) on Alloxan Induced Diabetic Rats

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Original Research Article

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Abstract: To investigate the acute toxicity and antidiabetic potentials of various extracts of the leaves of *Ficus sur* Forssk on alloxan-induced diabetic rats. Powdered leaves were successively extracted with n-hexane, chloroform and 70% ethanol for three consecutive days respectively and the obtained extracts were assessed for phytochemicals, acute toxicity and antidiabetic activity on albino rats. The acute toxicity was assessed by Lorke's method. Diabetes was achieved by intraperitoneal injection of alloxan monohydrate (120mg/kg). Blood glucose was determined daily for seven days using a glucometer. On the seventh day of treatment, blood samples were obtained and the serum triglycerides (TG), total cholesterol (TC), glycosylated heamoglobin (GH), urea and high density lipoprotein (HDL) were determined. The active N-hexane fraction was further fractionated by column chromatography packed with silica gel G (60-80mesh) eluting with gradient mixture of n-hexane, dichloromethane and ethanol. The phytochemical screening revealed the presence of terpenoids, tannins, flavonoid and carbohydrates. The lethal dose (LD_{50}) was recorded at 2154mg/kg in the toxicity study. The n-hexane extract showed the highest significant ($p<0.05$) reduction of 77.7% in blood glucose which was comparable to Glibenclamide (78.3%). Further fractionation yielded five fractions (F1-F5) in which fraction F4 and F5 were the most active with 54.7 and 55.9% reduction of blood glucose. The lipid profile of fraction F4 also exhibited decrease in TG, TC, GH, Urea and increase in HDL. This study reports the LD_{50} of F. sur to be2154 mg/kg and further confirms its ethnobotanical use of in the treatment of diabetes mellitus and its potential in preventing cardiovascular diseases.
Keywords: *Ficus sur*, Moraceae, alloxan-induced diabetes, acute toxicity.

INTRODUCTION

All around the world today, one of the major health concerns is said to be diabetes mellitus [1]. Diabetes mellitus is a metabolic disorder in the endocrine system characterized by elevated levels of glucose in the blood called hyperglyceamia over a prolonged time resulting from defects in resistance to the action of insulin and insufficient insulin secretions or by the two characteristics mentioned). In 2014, the international diabetes federation (IDF) estimated that diabetes resulted in 4.9 million deaths [2]. *Ficus sur* is a large growing evergreen tree reaching up to 35m high with large oval, green leaves borne on a massive, spreading crown. All parts exude milky latex. The leaves are large, alternate and spirally arranged. They are ovate to elliptic with irregular serrated margin. The fresh foliage is conspicuously red in color and the papery 1cm long stipules are soon dropped. *Ficus sur* has many uses in traditional medicine. The latex is used for treating wounds, toothaches, eye problems, general body pain, and lung and throat problems and as antiemetic [3]. In Nigeria, *Ficus sur* Forssk is used in traditional medicine for the treatment of epilepsy, pain and inflammation [4]. Other reported uses includes treatment of dysentery and wound dressing [5], circumcision, leprosy, rickets, fertility, edema, respiratory disorders and as an emollient [6]. *Ficus sur* is used to treat diarrhea and anemia as well as sexually transmitted diseases . Several pharmacological studies have also been reported on *Ficus sur* which include gastrointestinal motility effect [7], reduction of blood glucose [8], antiulserogenic and antioxidant and so on [9]. However, the use of the plant in the treatment of diabetes mellitus prompted interest in investigating its acute toxicity and antidiabetic activity.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Ficus sur* Forssk (family, Moraceae) was collected from Omokwa in Abua Odua Local Government Area of Rivers State in August, 2014. The fresh leaves were identified and authenticated by D. E. Esimonekhai and a specimen was deposited at the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt. The leaves

were air dried at room temperature, pulverized and stored in a plastic container for further use.

Phytochemical Screening

Phytochemical screening was performed on the powdered leaves and extracts to detect the presence of secondary metabolites using standard procedures [10, 11].

Extraction and Column Chromatography

The dried and powdered leaves of *Ficus sur* (1kg) was macerated successively with n-hexane, chloroform and 70% ethanol respectively, for a period of three days for each solvent. The combined filtrate for each was evaporated *en vacuo* in a rotary evaporator at 40^{o}C, weighed and tested on alloxan-induced diabetic rats at a concentration of 1g/kg. The bioactive n-hexane extract was fractionated on column chromatography packed with silica gelG (60-120) eluting with gradient mixture of N-heaxane, dichloromethane and ethanol. Fractions were monitored on analytical thin layer chromatography (TLC) silica gelGF_{254}, developing in hexane/dichlioromethane (1:1). Five fractions were obtained and tested separately on alloxan-induced diabetic rats.

Experimental Animals

Albino Wister rats weighing 120-180g were obtained from animal house University of Nigeria Nsuka. They were kept in metabolic cages in a well-ventilated room and fed on standard palletized feeds (Vital Feeds Ltd, Jos) and water *ad libitum.*

Acute toxicity evaluation

A total of 18 albino rats of either sex weighing 150-180 g were used in the determination of the acute toxicity of the leaf extract of *Ficus sur*. The rats were randomly divided into six groups of three (3) rats each and the first group was given 10mg/kg, the second group 100mg/kg and the third group 1000mg/kg of the plant extract respectively via the oral route. The rats were observed for signs of toxicity, adverse effects or death. After 24 hours, the second three groups of rats were given 1600, 2900 and 5000 mg/kg of the plant extract respectively and observations were noted as previously described [12].

Alloxan-induced diabetes

The rats were divided into groups of five rats each. Rats in group 2-5 were fasted overnight, given a single intraperitoneal injection of 120mg/kg of alloxan monohydrate in isotonic saline and allowed to rest for three days to stabilize blood glucose level.[13] Rats in group1 consisted of normal (control) rats. Group 2 contained diabetic untreated rats. Group 3 consisted of diabetic rats but treated with glibenclamide (2.5mg/kg) and group 4-6 consisted diabetic rats treated with extracts (1g/kg). Basal blood glucose level at zero time (fasting) was determined prior to oral treatment. The extracts and glibenclamide were administered immediately to rats in appropriate groups and blood sugar levels determined for 7 days as previously described [13].

Sample Collection

At the end of the experiment, rats in the most active group and the controls were sacrificed under anesthesia and blood was collected by cardiac puncture into EDTA bottles and centrifuged to obtain serum.

Lipid profile

The lipid profile of serum samples were performed using reagents kits (Randox Kits, Randox Laboratories, UK). Biochemical parameters measured were serum triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), glycosylated haemoglobin (HBA1c%) and urea.

Statistical Analysis

Data obtained from the animal studies were expressed as mean ± standard error of mean. The significance between the mean of the treated and the control animals were established by student's t-test

RESULTS

Yield of extract and phytochemicals

From 1kg of powdered material, 13.2g, 18.2g and 8.6g of extracts were obtained from hexane, chloroform and aqueous ethanol respectively. The phytochemical screening revealed the presence of triterpenoids, flavonoids, tannins and carbohydrates while alkaloids, saponins and athraquinones were absent from the extract.

Result of acute toxicity test on aqueous ethanol extract of *Ficus sur*

The result revealed that calculated lethal dose of *Ficus sur* leaves is 2154mg/kg body weight of rat.

Effect of crude aqueous ethanol extract of *Ficus sur* on the blood glucose level of alloxan induced diabetic rats

Aqueous ethanol extract (70% ethanol) revealed the inherent antihyperglycemic potential of *Ficus sur* by exhibiting a significant reduction in blood glucose of rats from day 3 to day 7 of treatment, with a peak reduction of 81.79% on day 7 as shown on table 2. A reduction that was higher than the glibenclamide (55.47%) on the same day.

Effect of various extracts of *Ficus sur* on blood glucose level of alloxan induced diabetic rats

From 3 during the acute study of the effect of the extract on day I (0 minute to 180 minutes), there was no significant reduction in the blood glucose of the rats treated with the extracts except those treated with glibenclamide and N-hexane at 180 minutes. From day 2 to day 7, chloroform extract did not show any significant reduction in blood glucose but 70% ethanol showed in day 7alone. Significant blood glucose

reduction manifests in the group treated with N-hexane extract with a peak of 77.7% reduction in day 7.

Effect of N-hexane extract of *Ficus sur* on the lipid profile of diabetic rats on day 7

The biochemical parameters of the rats after day7 showed that glycosylated haemoglobin decreased significantly. The total cholesterol and high density lipoprotein increased significantly with 14.6 and 43.6percent respectively. However, the triglyceride, low density lipoprotein and urea decreased significantly.

Effect of fractions obtained from N-hexane extract of *Ficus sur* on the blood glucose level of alloxan induced diabetic rats

The acute study which was observed within 0 minute to 180 minutes on day 1 showed that fraction F4 exhibited significant percentage decrease of 54% which was higher than the standard drug, glibenclamide (41.9%) at 180 minutes when compared with the diabetic control at the same time. From day 2 to day 5, fraction F4 and F5 showed steady significant percentage decrease in blood glucose when compared to the control group although fraction F4 showed a peak reduction of 57.7% in blood glucose on day 4.

Effect of fraction F4 obtained from N-hexane extract of *Ficus sur* on the lipid profile of diabetics rats on day 7

The glycosylated haemoglobin, triglyceride and urea decreased significantly by 59.9, 58.6 and 98.7percent respectively when compared to the control diabetic rats parameters. The total cholesterol and high density lipoprotein increased while the later was significant.

Table-1: Result of acute toxicity test on aqueous ethanol extract of *Ficus sur*

Dose (mg/kg)	10	100	1000	1600	2900	5000
Survival	3	3	3	3	2	0
Death	0	0	0	0	1	3

Table-2: Effect of crude aqueous 70% ethanol extract of *Ficus sur* on the blood glucose level of alloxan induced diabetic rats

Groups	**DAY 1 0min**	**60min**	**120min**	**180min**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Normal	80.33±6.39	76.23±2.31	82.33±1.33	74.0±0.54	76.67±1.77	78.33±6.01	78.33±2.03	76.67±2.33	88.00±1.73	81.00±3.46
D. control	319.33±18.77	405.0±44.03	526.0±63.00	532.0±4.64	531.66±43.60	556.33±26.67	540.00±35.99	551.00±31.03	543.00±37.15	545.67±35.38
Diabetic + Glibenclamide (2.5mg/kg)	450.3±26.5	544.0±45.00	511.0±59.6 (2.72)	507.0±67.34 (4.99)	507.33±63.85 (4.58)	571.66±7.32 (2.67)	525.66±20.27 (2.65)	425.00±34.41 (22.87)	282.67±72.90 (47.94)	243.00±55.81 (55.47)
Diabetic + 70% ethanol extract (1g/kg)	444.67±22.79	519.33±56.88	456.0±54.13 (13.31)	443.67±48.63 (16.97)	400.00±49.87	*287.33±83.22 (51.11)	*300.66±65.46 (44.32)	*153.00±40.78 (72.23)	*102.00±23.89 (81.22)	*99.33±22.46 (81.79)

Each value represents mean ± SEM from 5 rats; Figures in parenthesis represents the % decrease in blood glucose level
*represent the values significantly different from the control ($p<0.05$)

Table-3: Effect of various extracts of *Ficus sur* on blood glucose level of alloxan induced diabetic rats

Group	Day 1 0 minutes	60 minutes	120 minutes	180 minutes	Day2	Day3	Day4	Day5	Day6	Day7
Normal	118.00 ±3.79	118.00 ±3.78	128.67 ± 4.26	119.00 ±6.11	142.00±3 .84	120.33±4 .03	134.00±6 .23	113.00±1 3.24	118.00±8 .32	127.67±6 .98
Diabetic Control	193.67± 7.32	205.00±7 .83	188.33±1 1.46	355.00±2 0.65	235.67±3 .84	255.00±2 .73	361.33±2 .17	316.67±3. 60	422.67±3 .79	495.67±4 .33
Diabetic+ Glibencla mide (2.5mg/k g)	255.00± 6.10	217.33±5 .89	150.33±4 .85 (20.1%)	*186.00± 7.03 (47.6%)	158.33±7 .35 (32.8%)	124.67±3 .61 (51.1%)	113.67±2 .61 (68.5%)	105.00±3. 96 (41.4%)	88.67±25 .77 (79.0%)	107.33±9 .71 (78.3%)
Diabetic + N- hexane (1g/kg)	248.33± 9.39	161.67±9 .52 (17.66%)	137.67±7 .35	*116.67± 9.76 (35.11%)	*154.67± 4.33 (34.3%)	*127.00± 4.93 (50.2%)	*145.67± 5.44 (59.6%)	*132.67± 13.15 (58.1%)	*101.67± 3.92 (75.9%)	*110.33± 9.71 (77.7%)
Diabetic+ Chlorofor m extract (1g/kg)	248.00± 8.09	255.00±5 3.92	229.00±7 2.95	260.67±1 4.49 (22.87%)	257.67±1 4.50	203.67±9 .04 (20.1%)	236.67±6 .01 (34.5%)	254.33±8. 69 (22.5%)	266.00±1 0.01 (37.0%)	270.33±1 4.54 (45.4%)
Diabetic + 70% Ethanol extract (1g/kg)	218.67± 8.24	215.67±5 .42	208.67±5 3.85	317.67±6 .82 (10.5%)	233.67±1 0.70 (0.8%)	199.00±8 .71 (21.9%)	266.33±7 .03 (26.2%)	395.33±9. 57	355.93±1 3.20 (15.7%)	*163.00± 7.20 (67.1%)

Each value represents mean ± SEM from 5 rats; Figures in parenthesis represents the % decrease in blood glucose level
*represent the values significantly different from the control ($p<0.05$)

Table-4: Effect of fractions obtained from N-hexane extract of *Ficus sur* on the blood glucose level of alloxan induced diabetic rats

Groups	DAY 1 0 minute	60 minutes	120 minutes	180 minutes	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Normal	88.00±4 .50	100.0±7. 72	100.0±5. 66	110.0±2. 49	101.33±6 .13	125.33±3 .93	129.67±7 .34	179.67±8 .44	115±2.16	105.33±7 .91
Diabetic control	313.00± 0.00	318.67± 126.3	327.0±11 8.05	320.0±12 1.70	377.00±9 9.90	304.00±1 9.40	437.67±7 3.50	394.67±9 4.67	442.67±4 2.50	336.00±1 01.8
Diabetic+ glibencla mide (2.5mg/k g)	255.00± 6.10	*217.33 ±5.89 (31.8%)	*150.33± 4.85 (54.0%)	*186.0±7 .03 (41.9%)	*258.33± 7.35 (58.0%)	*224.67± 3.61 (58.9%)	*213.67± 2.67 (74.0%)	*205.00± 3.960 (73.4%)	*188.67± 4.44 (79.9%)	*107.33± 9.71 (68.1%)
Diabetic +F1 (100mg/k g)	532.67± 2.33	558.33± 4.56	548.33±6 2.20	559.0±2. 68	527.00±1 .25	569.00±0 .94	586.00±2 .63	546.00±2 .06	541.00±2 .06	570.00±4 .54
Diabetic +F2 (100mg/k g)	554.33± 8.67	538.33± 20.33	465.33±1 3.50	428.67±1 8.55	427.33±4 1.9	472.67±5 9.90	411±53.1 0 (6.1)	400.67±5 6.30	*314.00± 61.50 (29.1)	*225.67± 76.90 (32.8)
Diabetic +F3 (100mg/k g)	559.67± 3.33	422.0±3 1.50	415.33±2 9.42	419.67±1 8.47	453.33±2 3.80	332.00±3 5.17	453.33±2 3.78	501.00±2 1.70	467.00±4 0.10	386.67±1 7.85
Diabetic+ F4 (100mg/k g)	217.33± 90.50	301.0±2 4.80 (5.5%)	*181.00± 49.40 (44.6%)	*145.33± 47.80 (54.6%)	*261.67± 41.30 (30.6%)	*179.67± 39.50 (40.9%)	*185.33± 35.60 (57.7%)	*230.33± 36.37 (41.6%)	*263.33± 47.95 (40.5%)	*186.33± 36.40 (44.5%)
Diabetic+ F5 (100mg/k g)	375.67± 104.95	322.67± 12.67	282.67±1 1.86	250.67±1 5.30 (21.7%)	*219.00± 26.98 (41.9%)	*199.33± 15.20 (34.4%)	*207.33± 25.90 (52.5%)	*257±18. 50 (34.9%)	*195.00± 25.04 (55.9%)	*210.67± 11.65 (37.3%)

Each value represents mean ± SEM from 5 rats; Figures in parenthesis represents the % decrease in blood glucose level
*represent the values significantly different from the control ($p<0.05$)

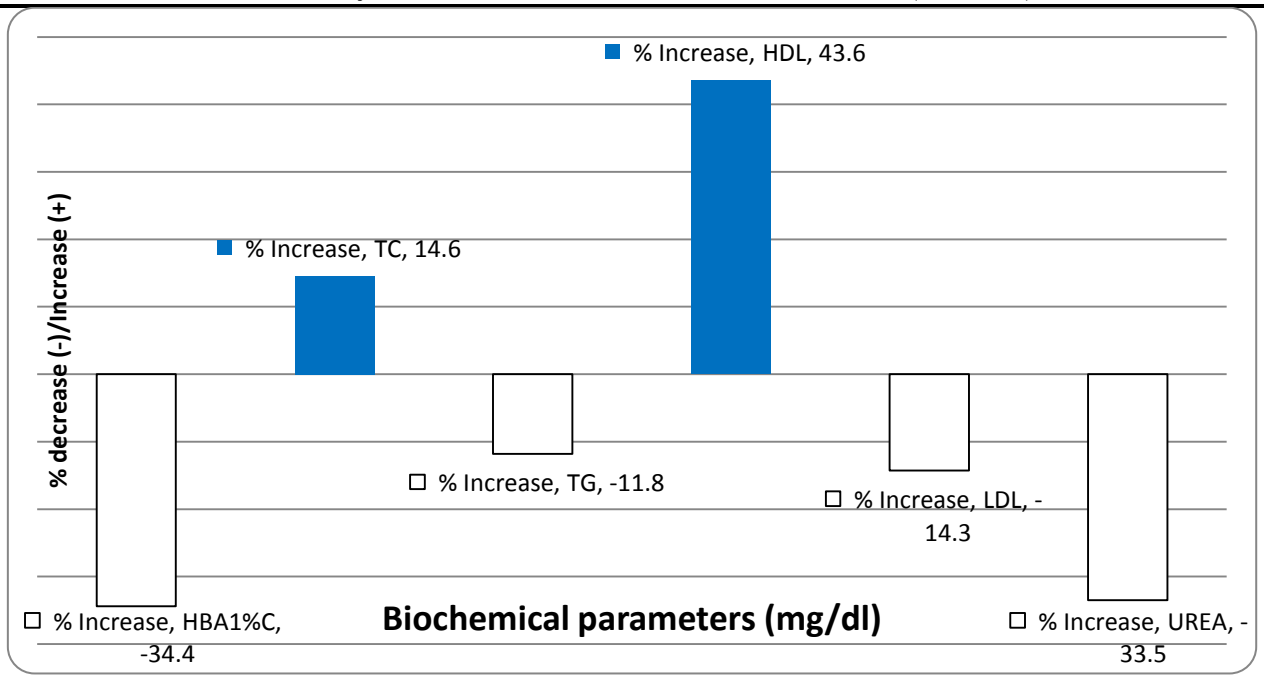

Fig-1: Effect of N-hexane extract of Ficus sur on the lipid profile of diabetics rats on day 7

TC= Total cholesterol, TG= Triglycerides, HDL= High density lipoprotein, LDL= Low density lipoprotein

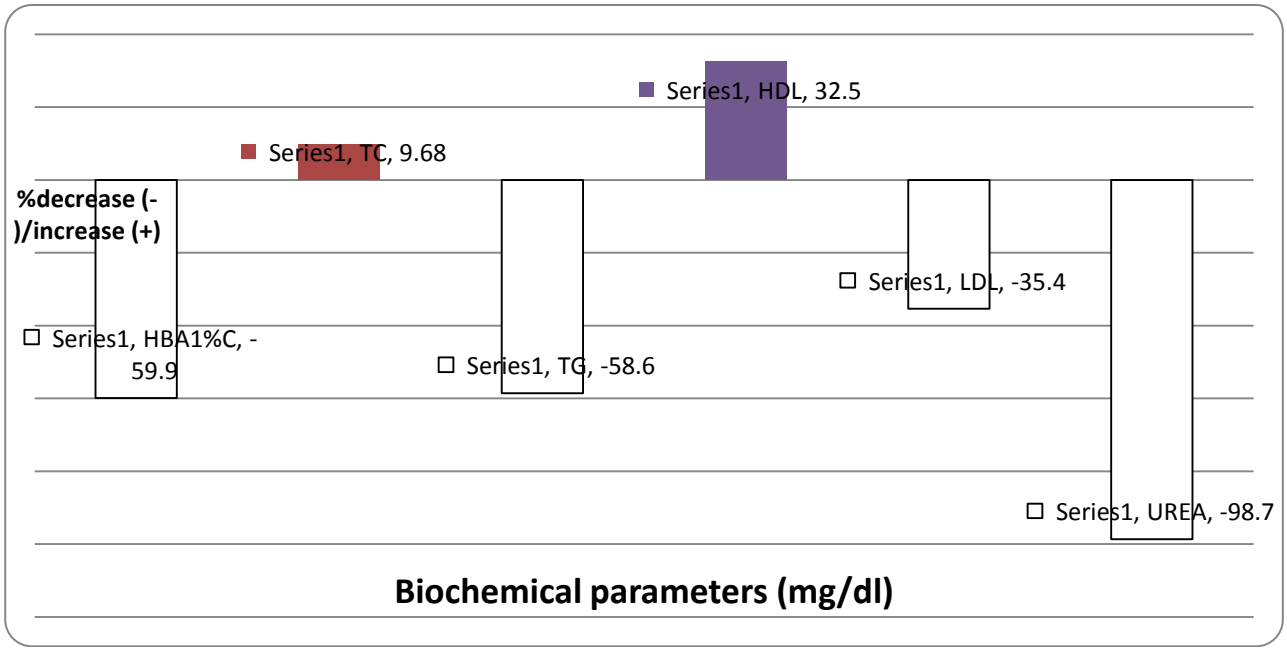

Fig-2: Effect of fraction F4 obtained from N-hexane extract of Ficus sur on the lipid profile of diabetics rats on day 7

TC= Total cholesterol, TG= Triglycerides, HDL= High density lipoprotein, LDL= Low density lipoprotein

DISCUSSION

The unprecedented increase in diabetes has led to unstoppable search for potent and cost effective drug for treatment from the plant kingdom. Many preliminary studies that showed good antidiabetic potentials have lost attention due to failure in isolating their antidiabetic principles. This study however tend to investigate the various extract and column fractions of active extract of *Ficus sur in vivo* as a step to isolation of the active principle.

The leaves of Ficus sur contain triterpnoids, flavonoids, tannins and carbohydrates as was revealed from the phytochemical screening though contrary to the report of Eluka *et al*. 2015, which reported the presence of alkaloid and absent of flavonoid [9].

The acute toxicity study showed that at a dose above 2154 mg/kg, the leaves of ficus sur could be unsafe and toxic. At the preliminary studies of

antidiabetic effect of the leaves, crude aqueous ethanol extract in Table 2 exhibited significant ($p < 0.05$) reduction in blood glucose which is in consonant with the report of Akomas *et al*., [8]. This effect however, prompted successive extraction of the plant material with the three solvents; N-hexane, chloroform and 70% ethanol to yield three extracts. Evaluation of the three extracts showed that chloroform and aqueous ethanol extract did not exhibit significant reduction in blood glucose while the N-hexane had a steady significant ($p < 0.05$) antihyperglycemic activity as exhibited in the blood glucose of the rats. This therefore suggest that the extract could have effected antihyperglycemia through insulin mimetic action or stimulation of beta cells for production of insulin or other mechanisms since diabetes was achieved by the destruction of the beta cells through induction of alloxan although subject to investigation.

Diabetes as an endocrine disorder that is just characterised by hyperglycemia also lead to secondary complication that results to cardiovascular diseases from the risk of elevated plasma cholesterol and triglyceride level [14]. Correction of lipid abnormality by the existing antidiabetic drugs has been a challenge especially hypertriglyceridemia. Lipid profile of the rats treated with the hexane extract (Figure-1) indicated the ameliorative effect of the extract by a significant increase in HDL, reduction of LDL and minor increase in TC when compared to the diabetic untreated control. A significant reduction in blood urea was also recorded for the extract which is also an indication of kidney prevention potential. The sharp reduction in HbA1c% also confirmed the reduction in blood glucose recorded in the study.

Further fractionation of the bioactive N-hexane extract yielded another potent fraction F4 out of the five fractions obtained from the column. However, fraction F5 had activity but not as high as F4 which showed 57.7% significant ($p < 0.05$) reduction of blood glucose on day 4 when compared to the diabetic untreated group while fraction F1, F2 and F3 were not active. This fraction also showed an enhanced lipid profile from the extract with a high percentage reduction of 58% in TG compared to 11.8% recorded in the extract, 35.4% in LDL compared to 14.3% in the extract and the blood urea of 98.7%.

Consequently, the fraction F4 reacted positive to Liebermann Buchard test which detects the presence of triterpenoids. However, triterpenoids have been implicated to be the antidiabetic principle in some plants [13].

CONCLUSION

This study shows that the aqueous ethanol extract of *Ficus sur* leaves is safe at 2154 mg/kg. The N-hexane extract and one of its column fractions (fraction F4) have potent antidiaberic principles that have potentials of preventing cardiovascular diseases as revealed from the antihyperglycemic activity and amelioration of the lipid and urea profile of the diabetic rats.

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