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Blood Coagulation Tests and Platelets Counts in Diabetic Rats Treated with *Ficus sur*, *Jatropha tanjorensis*, *Mucuna pruriens* and *Chromolaena odorata* Leaf Extracts

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: This study was designed to study the effects of leaves extracts of four indigenous Nigerian medicinal plants namely- *Ficus sur, Jatropha tanjorensis*, *Mucuna pruriens* and *Chromolaena odorata* on platelets counts and blood coagulation tests (bleeding and clotting times) in alloxan induced diabetic rats with a view to further assess their safety in the management of diabetes mellitus.
Design: Animal experiments were carried out on whole animals in the Physiology Laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.
Methodology: Forty five diabetic rats were divided into 9 groups of 5 rats each (groups 2-9), while group 1 comprised of 5 normal rats. Treatment was assigned to each group with a specified extract and dosed. At the end of treatment period bleeding and clotting times as well as platelets counts was determined for each animal.
Results: All doses of the extracts significantly (P<.05) lowered the observed elevated platelets counts in the diabetic rats with 150 mg/kg of *Ficus sur, Jatropha tanjorensis, Mucuna pruriens* and *Chromolaena odorata* lowering elevated platelets by 48.50, 47.26, 62.15 and 32.54% respectively.

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Same dose increased bleeding time by 460.6, 431.8, 430.3 and 213% respectively. Clotting time was also raised by 86.8, 63.57, 48.06, and 43.41 respectively.
Conclusion: The results show that the leaf extracts of *Ficus sur, Jatropha tanjorensis,* and *Mucuna pruriens* contain principles with anti-haemostatic and fibrinolytic and could be of value in the prevention of blood coagulation diseases often associated with diabetes mellitus but leaves that of *Chromolaena odorata* for further evaluation.

Keywords: Bleeding time; Chromolaena odorata; clotting time; Ficus sur; Jatropha tanjorensis; Mucuna pruriens; platelets.

1. INTRODUCTION

Hematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health [1]. Coagulation tests such as bleeding time and clotting time have over the years been used to assess platelet functions. These two parameters, although considered by many as obsolete provides enough information about platelets activation and function and may serve as a means of accessing clinical conditions such as disseminated intravascular coagulation, Von willebrand disease, thrombocytopenia, end stage liver failure and uremia. Diabetes mellitus is a metabolic and endocrine system disorder characterised by hyperglycaemia resulting from defects in insulin secretion or insulin action or both. It is reported that chronic disruption of membrane fluidity, protein denaturation, lipid peroxidation, alteration in platelets functions coupled with hyperglycaemia are associated with long term damage, dysfunction and eventually the failure of organs especially the eyes, kidneys, nerve, heart and blood vessels among diabetics [2], and that about 80% of people with diabetes mellitus die a thrombotic death due to enhanced activation of platelets and clotting factors [3].

Several green leafy vegetables and weeds abound in tropical Africa that could be used in the management of hematological abnormalities [1]. These medicinal plants in addition to their healing potentials give clues in towards the development of new agents whose physiological and pharmacological dynamics can be properly harnessed for the promotion of the health of man and other animals. Little wonder, it was reported that the primary aim of sourcing for plants drug through any of the known strategies is mainly to detect the active ingredients in plants that exert definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery [4]. *Ficus sur, Jatropha tanjorensis, Mucuna pruriens* and *Chromolaena odorata* are among plants under study whose hypoglycaemic activities have been reported [5,6,7].

Ficus sur commonly called wild fig is a medium sized tree of 6-9 meters high with large alternate and spirally arranged leaves with regularly serrated margins. The leaves have found relevance in traditional medicine in the treatment of diarrhoea, anaemia, wounds, stomach problems, infertility, peptic ulcer and gonorrhea. *Jatropha tanjorensis,* belonging to family *Euphorbiaceae,* is a common weed of field crops in the higher rainfall forest zones of West Africa. In Nigeria, it is commonly called hospital too far or catholic vegetable. The leaves are commonly consumed vegetable in many parts of southern Nigeria [8], where it is considered a natural remedy against diabetes mellitus. Extracts from the leaves of the plant have also been used to treat malaria infection and hypertension in some parts of Nigeria [8,9]. The leaves were initially and popularly consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume and hence employed in anemia treatment. *Mucuna pruriens* is a tropical legume known as velvet bean or cowitch. It is found in Africa, India and the Caribbean. The plant is notorious for the extreme itchiness it produces on contact, particularly with the young foliage and seed pods. The plant is an annual climbing shrub with long vines that can reach over 15m in length. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost completely free of hairs. The leaves are tripinnate, ovate, reverse ovate, rhombus shaped or widely ovate. *Mucuna pruriens* bears white, lavender or purple flowers. Its seeds pods are about 10cm long and are covered in loose, orange hairs that cause a severe itch if they come in contact with the skin. The chemical compounds responsible for the itch are a protein, mucunain and serotonin. The seeds are shiny black or brown drift seeds [10]. The plant's extract have been long used in tribal communities to treat snakebites, edema,

intestinal worms, diabetes, high blood pressure, high cholesterol, intestinal gas, muscle pain, rheumatism, abortions, cancer, catarrh, cholera, cough, diarrhea, dysentery, impotency, kidney stones, menstrual disorder, nervousness, scorpion stings, sterility, tuberculosis, asthma, burns, cancer, cholera, coughs, cuts, diarrhea, dog bites, insanity, menstrual problem, mumps, paralysis, ringworm, sores, syphilis, tumors, and as a diuretic agent [10]. *Chromolaena odorata* on the other hand, is a rapidly growing perennial herb with multi-stemmed shrub and grows up to 2.5 m tall in open areas. Available literature reveals that the plant contains carcinogenic pyrrolizidine alkaloid and can cause toxicity and allergic reactions in cattle. However there is report that the extract from the leaves is used traditionally to treat skin wounds [11].

This study was designed to investigate the effects of these four indigenous Nigerian medicinal plants on bleeding times, clotting time and platelets counts in alloxan induced diabetic rats with a view to complementing their reported usefulness in the management of diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Extracts

Fresh leaves of *Ficus sur*, *Mucuna pruriens*and *Chromolaena odorata* were collected from a farm settlement in Isiegbu - Ozuitem, Bende Local Government Area of Abia State while *Jatropha tanjorensis* was collected from Owerri-Aba in Ugwunagbo Local Government Area of Abia State in the month of March, 2014. The leaves of were dried under shade for seven days, after which they were pulverished to fine powder using a manual blender. Thirty five (35) grams of each powdered sample was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as a solvent. Temperature was maintained at 70ºC through-out the extraction period of 48 hours. At the end of the period, the extract was dried in a laboratory oven at 40ºC to obtain dried extracts of each sample.

2.2 Animals

Fifty albino rats (130-160g), obtained from the Animal house unit of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike were used for the study. All animals were fed with standard animal feed and water *ad libitum* and handled in accordance with the NIH guidelines for Care and Use of Laboratory Animals (Pub. No.85-23, Revised 1985), as expressed by Akah et al. [12].

2.3 Induction Diabetes

Sixty five rats of both sexes weighting 140-180g were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Sigma Chemical Co., USA) 10% (W/V) in normal saline at a dose of 160 mg/kg body weight. 8 days later blood was obtained from the tails of each rat and tested for glucose level to confirm the development of diabetes using a glucose meter (Roche Co.Germany). The rats with fasting glucose levels above 190 mg/dL were considered diabetic. Forty five of the diabetic rats were used for the study while five normal rats constituted the normal control group.

2.4 Bleeding and clotting times

The animals were grouped and assigned daily oral treatments as indicated below:

Group 1: Normal control which received normal saline
Group 2: Untreated diabetic rats (diabetic control)
Group 3: Diabetic rats treated with 150 mg/kg *Ficus sur.*
Group 4: Diabetic rats treated with 300 mg/kg *Ficus sur.*
Group 5: Diabetic rats treated with 150 mg/kg *Jatropha tanjorensis.*
Group 6: Diabetic rats treated with 300 mg/kg *Jatropha tanjorensis.*
Group 7: Diabetic rats treated with 150 mg/kg *Mucuna pruriens.*
Group 8: Diabetic rats treated with 300 mg/kg *Mucuna pruriens.*
Group 9: Diabetic rats treated with 150 mg/kg *Chromolaena odorata.*
Group 10: Diabetic rats treated with 300 mg/kg *Chromolaena odorata.*

The rats were kept in Aluminum cages and allowed access to feed and water while ensuring that highest level of hygiene was maintained. Treatments were done daily via the oral route and lasted for 21 days. At the end of the period, bleeding time was determined for each animal using Duke's method while clotting time was

determine by Ivy's method as reported by Ibu and Adeniyi [13]. For bleeding time, the tip of the tail of each rat was cut to cause bleeding. A stopwatch was started as soon as animal began to bleed. A blotting paper was used to wipe off blood every 15 seconds. As soon as bleeding ceased the stopwatch was stopped and the time recorded as bleeding time for that particular animal. For the clotting time, a drop of blood from the tail of each rat was placed on a clean glass slide and a stopwatch was started at the same time. A pin was passed across the drop of blood once every 15 seconds. As soon as threads of fibrin were noticed, the stopwatch was stopped and the time recorded as the clotting time for that particular rat. Percentage increase in bleeding and clotting times were calculated using the formula:

% Increase = [(Time in test - Time in diabetic control)/ (Time in diabetic control) x (100/1)]

2.5 Platelets Counts

After 21 days of treatment, the rats in all groups were sacrificed on the 22nd day and blood samples collected by cardiac puncture for platelets counts using an Automated Haematology Analyser, following standard procedures stipulated by the producer, Mindray Company, China. Percentage reductions in platelets counts were calculated using the formula:

$$\% \text{ Reduction} = \frac{A - B}{A} \times 100$$

Where

A = Counts in diabetic control
B = Counts in test

3. RESULTS

3.1 Effects of Plant Extracts on Platelet Counts in Alloxan Induced Diabetic Rats

The diabetic control rats showed significantly ($p< 0.05$) elevated platelets counts when compared to the normal control rats at the end of 21 days (Table 1). All doses of *F. sur, J. tanjorensis, M. pruriens* and *C. odorata* significantly ($p< 0.05$) lowered these elevated platelets values in all treated diabetic rats with 150mg/kg body weight of *M. pruriens* achieving the highest platelets lowering effect of 62.15% (Table 1).

3.2 Effects of Plant Extracts on Bleeding and Clotting Times in Alloxan Induced Diabetic Rats

All doses of *F. sur, J. tanjorensis*, and *M. pruriens* significantly ($p< 0.05$) increased bleeding times in the treated diabetic rats except 300 mg/kg *C. odorata* which did not significantly affect same (Table 2). The clotting time was also significantly increased by all doses of the extracts except 300 mg/kg *C. odorata* which increased same by only 6.20% (Table 3).

Table 1. Effects of extracts on platelets counts in alloxan induced diabetic rats

Group	Treatment (mg/kg)	Platelets counts X 10^9/L	% Reduction in platelets counts
1.	Normal control	909± 227.5	
2.	Diabetic control	1611.8±102.8	
3.	150 *F. sur*	830±30.7*	48.50
4.	300 *F. sur*	694.3±160.9*	56.92
5.	150 *J. tanjorensis*	850±95.3*	47.26
6.	300 *J. tanjorensis*	744±82.9*	53.84
7.	150 *M. pruriens*	610±488.5*	62.15
8.	300 *M. pruriens*	737.7±16.2*	54.23
9.	150 *C. odorata*	1087.3±265.5*	32.54
10.	300 *C. odorata*	909±32.96*	43.60

**P< 0.05 versus diabetic control*

Table 2. Effects of extracts on bleeding time in alloxan induced diabetic rats

Group	Treatment (mg/kg)	Bleeding time (Min)	% Increase in bleeding time
1.	Normal control	4.76±0.10	
2.	Diabetic control	1.32±0.19	
3.	150 *F. sur*	7.40±0.25*	460.61
4.	300 *F. sur*	11.54±0.59*	774.24
5.	150 *J. tanjorensis*	7.02±0.25*	431.82
6.	300 *J. tanjorensis*	7.70±0.79*	483.33
7.	150 *M. pruriens*	7.0±0.73*	430.30
8.	300 *M. pruriens*	8.90±0.31*	574.24
9.	150 *C. odorata*	4.14±0.24*	213.64
10.	300 *C. odorata*	1.11±0.42	-15.90

**P< 0.05 versus diabetic control*

Table 3. Effects of plants extracts on clotting time in alloxan induced diabetic rats

Group	Treatment (mg/kg)	Clotting time (Min.)	% Increase in clotting time
1.	Normal control	2.0 ±0.18	
2.	Diabetic control	1.29±0.09	
3.	150 *F. sur*	2.41±0.13*	86.82
4.	300 *F. sur*	2.32±0.14*	79.84
5.	150 *J. tanjorensis*	2.11±0.18*	63.57
6.	300 *J. tanjorensis*	1.98±0.24*	53.49
7.	150 *M. pruriens*	1.91±0.19*	48.06
8.	300 *M. pruriens*	2.42±0.21*	87.60
9.	150 *C. odorata*	1.85±0.21*	43.41
10.	300 *C. odorata*	1.37±0.04	6.20

**P<0.05 versus diabetic control*

4. DISCUSSION

The results of this indicate increase in platelets counts which may have accounted for the lowered bleeding and clotting times observed in the diabetic rats and tends to agree with existing literature reports. It is reported that platelets hyperactivity in patients with hyperglycaemia resulting from a dysregulated signaling pathways that lead to an increased tendency to activate and aggregate response to a given stimulus [14]. Platelets activation therefore triggers thrombus formation, microcapillary embolization and facilitates the development of other cardiovascular diseases. Akingbami et al. [15] added that diabetes mellitus is characterized by enhanced platelets activation and coagulation proteins and reduced fibrinolytic activity which usually precede the development of cardiovascular complications. The increased platelets counts in the diabetic rats may have been responsible for the observed decrease in bleeding and clotting times and may increase the risk of intravascular blood clotting and associated diseases [16,3].

The significant ($p<0.05$) reduction in platelets counts and corresponding elevations in bleeding and clotting times in all diabetic rats treated with leaf extracts of *Ficus sur*, *Jatropha tanjorensis, and Mucuna pruriens* suggest that the extracts contain strong principles with anti-hemostatic and fibrinolytic properties in addition to their hypoglycaemic properties reported by [5,6,7]. The extracts may have achieved these effects by stimulating decrease in formation and activation of blood platelets, a process which usually does not favor the blood clotting and shown by increased bleeding and clotting times. *C. odorata* ethanol leaf extract, reported to have hypoglycaemic activity which could be of value in the management of diabetes mellitus [6,17], its use in diabetes requires further evaluation as the extract was observed not to significantly affect bleeding and clotting times in the treated diabetic rats and has been reported to have haemostatic and fibrinolytic activity in experimental rats [18]. This may be the reason for the use of *C. odorata* leaf extract to promote wound healing and to stop bleeding [11].

The results obtained agree with Houghton and Skari [19], on the use of *Mucuna pruriens* extract

to prolong bleeding and clotting times and justifies the use of *Ficus sur, Jatropha tanjorensis* and *Mucuna pruriens* in bleeding disorders associated with diabetes mellitus.

5. CONCLUSION

The results of this study have shown that ethanol leaf extracts of *Ficus sur, Jatropha tanjorensis and Mucuna pruriens,* can be used to remedy the abnormal platelets, bleeding and clotting times values observed in diabetic rats and justify the use of these agents in diabetes management as their effects may be useful in the management of thrombosis and other blood coagulation problems associated with the condition, but leaves the use of *Chromolaena odorata* for the same purpose to be further evaluated.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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