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THERAPEUTIC PROPERTIES OF AQUEOUS LEAVE EXTRACT OF CHROMOLAENA ODORATA ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

Ifeanyi Anthony Egwuatu[1]*, Vivian Onyinye Atuadu[2], Onyinye Mary Ozioko[3], Chiadikobi Lawrence Ozoemena[4] and Thecla Isaac[5]

Department of Anatomy, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.

***Corresponding Author: Ifeanyi Anthony Egwuatu**

Department of Anatomy, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.

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ABSTRACT

Background: *Chromolaena Odorata* is used for its many medicinal properties, especially for external application as in wounds, skin infections, inflammation etc. The antidiabetic property of the aqueous leaf extract of Chromolaena odorata was evaluated in Wistar rats by inducing diabetes using a single intraperitoneal (I.P) injection of freshly prepared solution of alloxan monohydrate. **Materials and Methods:** 30 wistar rats weighing 150-200 kg were divided into 6 groups. Group A was negative control, Group B was positive control and was induced with 125 mg/kg of alloxan, Group C, D and E were induced with alloxan and then treated with *Chromolaena Odorata* extract at 100, 200, and 400 mg/bw. Group F was induced with alloxan and treated with 500 mg/kg of standard drug (Metformin). Animals with glucose level of 200 mg/dl and above were selected for the study. Treatment lasted for 21 days. After 21 days, blood samples were collected for insulin biochemical test. Blood glucose level was measured before induction, 7 days after alloxan induction, 14 days post induction/treatment and 21 days after treatment with Chromolaena odorata extract. At the end of the study, all of the animals of experiment were sacrificed for histopathological and biochemical examination. **Result:** Histopathological examination of the stained sections showed regeneration of β-cells of islets of pancreas of group C, D and E Chromolaena odorata-treated rats and the standard drug-treated rats when compared to untreated diabetic group of rats. **Conclusion:** In conclusion, this study shows that the Chromolaena odorata leaf extract has potential anti diabetic action (especially at low dosage) in Alloxan-induced diabetic wistar rat and the effect was found to be similar to the effect standard drugs (Metformin).

KEYWORDS: Chromolaena odorata; medicinal potential; antidiabetic; alloxan; metformin.

INTRODUCTION

Medicinal plants have been in use by man since ages in traditional medicine as a result of their therapeutic potential. The researches on medicinal plants have led to the discovery of new and novel drug candidates used against different diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs.[1] Therefore, this brings about the increasing recognition of herbal medicine as an alternative form of health care. The screening of medicinal plants for their active compounds has become significant and paramount as that may serve as talented sources of data bank for antibiotic and other ailment prototypes. The Herbal prescriptions and natural remedies are therefore a common practice in developing countries for the treatment of various diseases and this practice is oftentimes an alternative way to compensate for some perceived deficiencies in pharmacotherapy.[1]

Chromolaena odorata or Siam weed has a minimum 10-year life span. Chromolaena odorata is a scrambling perennial shrub which grows 2–3 m in height with straight, pithy, brittle stems that branch readily. The arrowhead-shaped leaves are 6–12 cm in length and 3–7 cm in width, with three veins in a pitchfork appearance. The leaves grow in opposite pairs along the stems and branches. There are 15–25 tubular florets per head, each 10 mm long and several colors such as white, purple, pink, or blue.[2] Its active phytochemical substances are as follows: flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin; terpenes and terpenoids, essential oils, alkaloids including pyrrolizidine, saponins and tannin, phenolic acids including ferulic acid, protocatechuic acid, phytoprostane compound including chromomoric acid.[2] Although much has been documented on the medicinal properties of this plant, this work, however, is designed to evaluate the possible therapeutic effect of the extract of this plant in order to

know the best extract to use in the treatment or amelioration of diabetes mellitus in folk medicine.

MATERIALS AND METHODS

Plant Material Collection

Fresh leaves of Chromolaena odorata (Siam weed) was harvested from their natural habitat at Enugu state university teaching hospital, Enugu state, Nigeria. The plant was authenticated by a taxonomist at the Department of Botany, University of Nigeria, Nsukka.

Plant Extraction and Phytochemical Analysis

The leaves were dried under shade for about 10 days after which they were ground to powder using an electric blender. 300 g of the powdered material was soaked in 1 litre of methanol and shaken vigorously. The sample was then filtered after 3 days using a Buckner funnel and Whatman No. 1 filtered paper. The extract was further concentrated using a rotary evaporator. The weight of the extract was 14.8 grams.

Phytochemical Analysis was done at Brain-Phosphorylation Scientific Solution Service. Ogui Road Enugu, Enugu State. Qualitative tests were carried out to determine the composition of some pharmacological active secondary metabolites.

Induction of experimental diabetes

- Each group consists of five rats. Diabetes mellitus was induced according to Sovia et al., (2017) (in group B, C, D, E and F test rats) by single intraperitoneal injection of 125 mg/kg/bw of alloxan freshly dissolved in 0.9% saline. Control rats (group A) were injected with only 0.9% saline intraperitoneally. The test animals in group B to F became diabetic within 72 hours after alloxan administration. The diabetic state was confirmed by measuring blood glucose concentration 72 hours after alloxan injection using accu-chek glucometer.
- Diabetes was allowed to develop and stabilize in these alloxan treated rats over a period of 3-5 days. All animals in all groups were kept and maintained under laboratory conditions and were allowed free access to food (standard pellet diet) and water. Before commencement of the experiments, both the control and alloxan treated diabetic test rats were subjected to 8 hours fasting, but still allowed free access to water throughout. At the end of the 8 hours fasting period, blood glucose level of the control and alloxan-treated rats were determined and recorded. Alloxan-treated rats that fasted and still had blood glucose concentration of 200 mg/dl and above were considered to be diabetic, and used for this study. A blood sample was obtained from the tail vain of the animals and their fasting glucose determined in mg/dl using a digital glucometer (Accu-check). C.odorata leaf extract 100, 200, and 400 mg/kg body weight was administered orally to the diabetic rats in groups C, D and E respectively. The administration of C. odorata was commenced as from the 7th day post alloxan injection for 21 days. Also the standard drug (metformin) was administered the same day as the C.odorata extract.
- Weight, blood glucose, and behavioral changes in the animals were observed and documented.

Ethical Clearance

Ethical clearance for this study was obtained from the Health Research Ethical committee. University of Nigeria, Enugu State.

Experimental Animal

The animals used in this study were male albino rats weighing between 120 and 150 grams. The rat was randomly divided into 6 experimental groups of 5 rats each (A-F): A (control), B (Alloxan- treated), C (Alloxan and 100mg/kg body weight C.odorata- treated), D (Alloxan and 200mg/kg body weight C.odorata-treated), and E (Alloxan and 400mg/kg body weight C.odorata- treated). They were kept in the Experimental Animal house of the Department of Anatomy, University of Nigeria Enugu campus throughout the period of this study. They were housed in rats' cages and were fed with standard rat pellets. They were given access to clean water at all times. All experimental procedures were in conformity with the University of Nigeria Ethics committee on Research in Animals as well as internationally-accepted principles for Laboratory animal upkeep and use.

Table 1: Experimental Design and Administration.

GROUPS	NO OF RATS PER CAGE	INDUCING AGENT/ EXTRACT	DOSAGE	DURATION
Group A (negative control group)	5	Normal saline	0.9%	21 days
Group B(positive control)	5	Alloxan	125mg/kg	21 days
Group C	5	Alloxan + C.odorata extract(low dose)	125mg/kg +100mg/kg	21 days
Group D	5	Alloxan+ C.odorata extract(medium dose)	125mg/kg+200mg/kg	21 days
Group E	5	Alloxan + C.odorata extract (high dose)	125mg/kg+400 0mg/kg	21 days
Group F	5	Alloxan + metformin (standard drug)	125mg/kg+500mg/kg	21 days

Sacrifice of Experimental Animal Blood Collection

After 21 days, the animals were kept overnight fast and sacrificed under light chloroform anesthesia. Blood was drawn from the ventricles, centrifuged and the serum was used immediately for various biochemical estimations. Pancreas was excised immediately, washed with ice cold saline stored in 10% formalin and 0.9% saline, for histopathological studies.[3]

Biochemical Analysis

The whole blood sample was used for the estimation of glucose (Accu-check glucometer). The plasma sample was used for the estimation of insulin.

Estimation of glucose: A drop of the whole blood sample was used for measuring glucose using Accu-check glucometer, with gluco strips.

Histological Study

The histological analyses of the tissues were done according to the method of Drury.[4] The stained tissues were micrographed and interpreted by a pathologist at the University of Nigeria, Nsukka.

Statistical Analysis

Results of biochemical analysis were analyzed using SPSS (version 21), expressed as Mean ±Standard Error of Mean (SEM). Statistical differences in mean between groups were analyzed using one way ANOVA (Analysis of variance). P-value less than 0.05 were considered as statistically significant.

RESULT

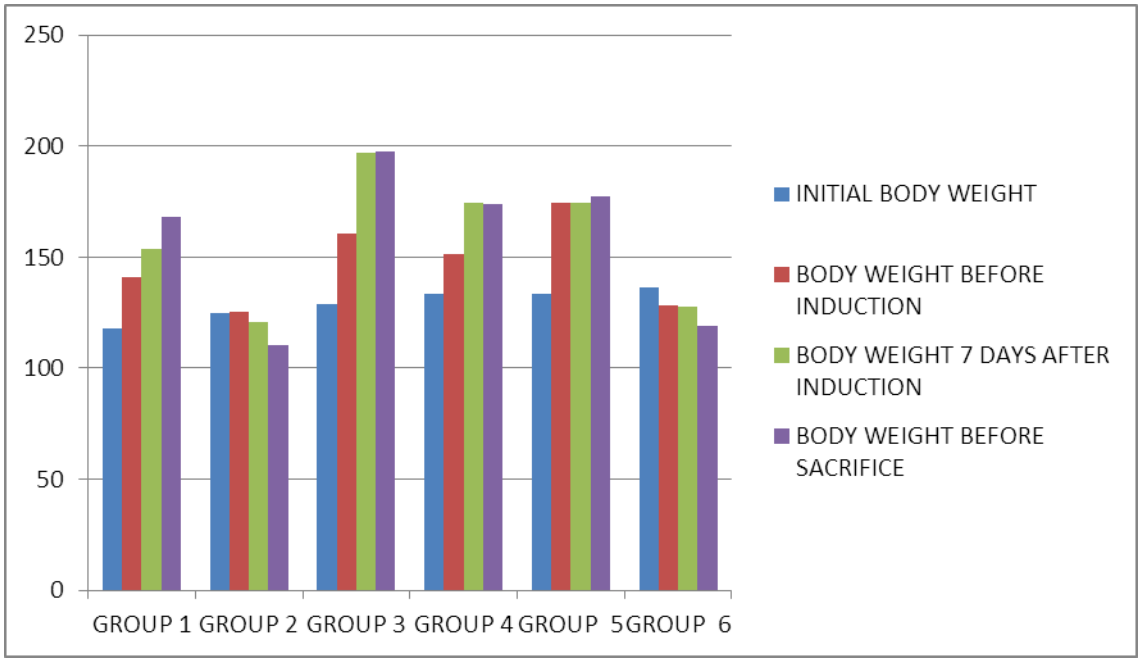

Figure 1: Results of the effect of the plant extract on the Body Weight (G) in the Different Groups of Experimental Animals before and after treatment.

Fig 1: showing the initial body weights, before induction of diabetes, seven days after induction and at the end of the treatment: values were expressed as mean ± SD.

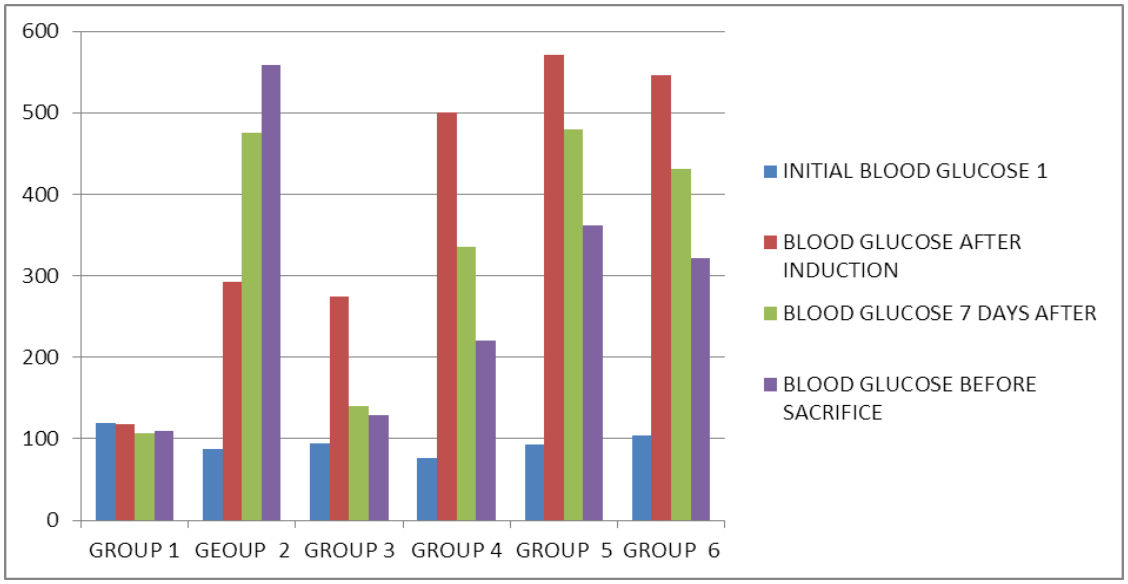

Figure 2: Results of the effect of the plant extract on Glucose concentration before and after treatment with the extract.

Fig 2: showing the initial blood glucose, after induction of diabetes, seven days after induction and at the end of the treatment: values were expressed as mean ± SD

Table 2: Result of the effects of the plant extract on the Mean ± Standard Deviation of the Insulin and Organ Weight in normal and diabetic rats.

GROUP	INSULIN LEVEL	ORGAN WEIGHT
1	3.17±0.01	0.75±0.07
2	3.09±0.08	0.50±0.00[a]
3	3.35±0.42	0.60±0.42
4	3.07±0.28	0.65±0.21
5	3.25±0.23	0.90±0.14[b]
6	2.59±0.37[a]	1.10±0.14[a]

[a]$P < 0.05$ (statistically significant compared with control),
[b]$P < 0.05$ (statistically significant compared with diabetic rats).

Effect of Aqueous Leave Extract of *Chromolaena Odorata* (Siam Weed) on the Histo architecture of Alloxan-treated Diabetic Wistar Rats

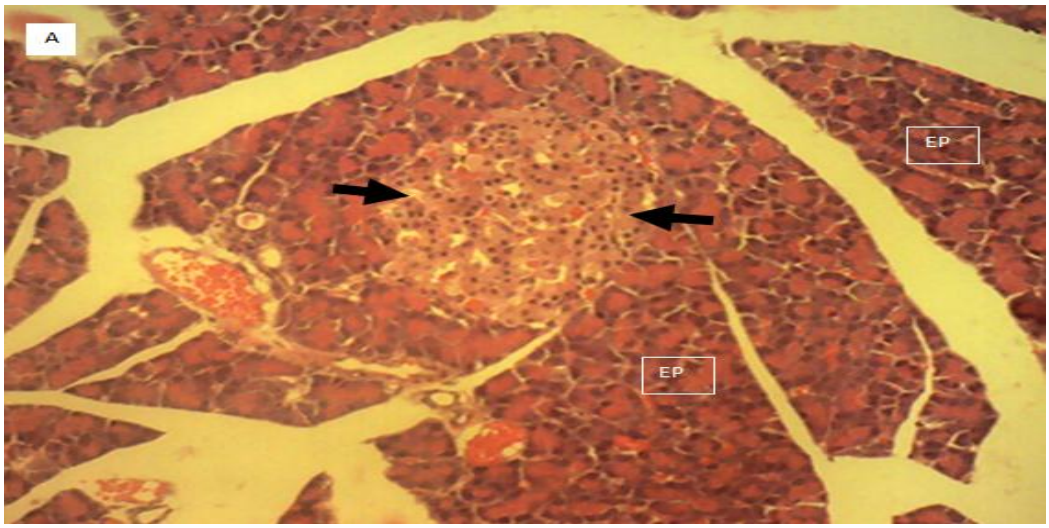

Plate 1: photomicrograph Sections of the pancreas after treatment with 0.9% of normal saline, the sections of the pancreas presented in Group A showed the histo-architecture of the endocrine and exocrine pancreas. The pancreatic islets (arrow), made up of normal islet cells, was obviously multiple and mostly occupying 30 -50% of the pancreatic lobules. Exocrine pancreas (EP). H&E x160; x400.

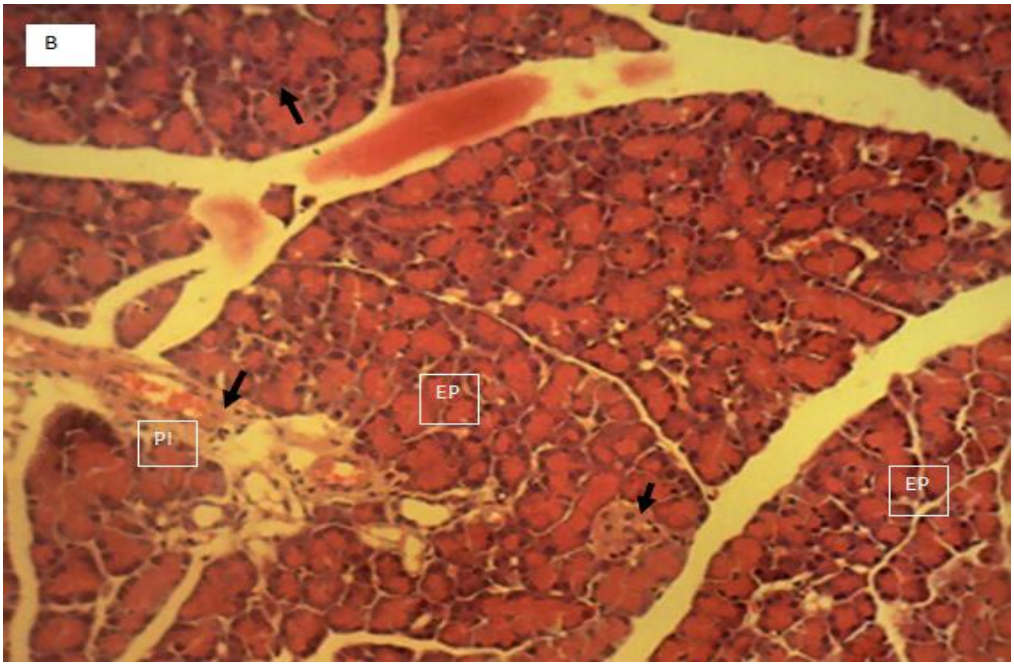

Plate 2: photomicrograph Sections of the pancreas after treatment with alloxan 125mg/kg only, Sections of the pancreas presented in Group B showed a marked depletion in the number of pancreatic islets (PI) embedded in the exocrine pancreas (EP). The pancreatic islets appear small to relatively inconspicuous (arrow) and show varying degrees of islet cell depletion due to degeneration and necrosis with nuclear pyknosis (arrow head) and karyolysis. H&E x400; x160.

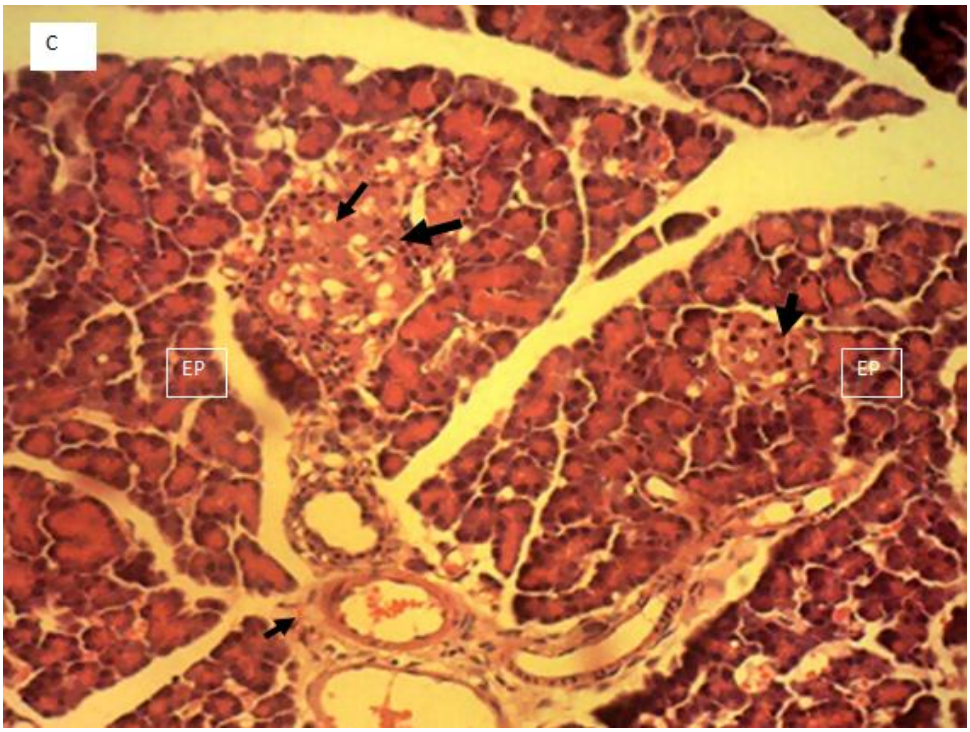

Plate 3: photomicrograph Sections of the pancreas after 125mg/kg and *C.Odorata* extract 100mg/kg for fourteen days, Sections of the pancreas presented in Group C showed a marked some regeneration of depleted pancreatic islets embedded in the exocrine pancreas (EP). Pancreatic islet (arrow); Exocrine pancreas(EP) H&E x160.

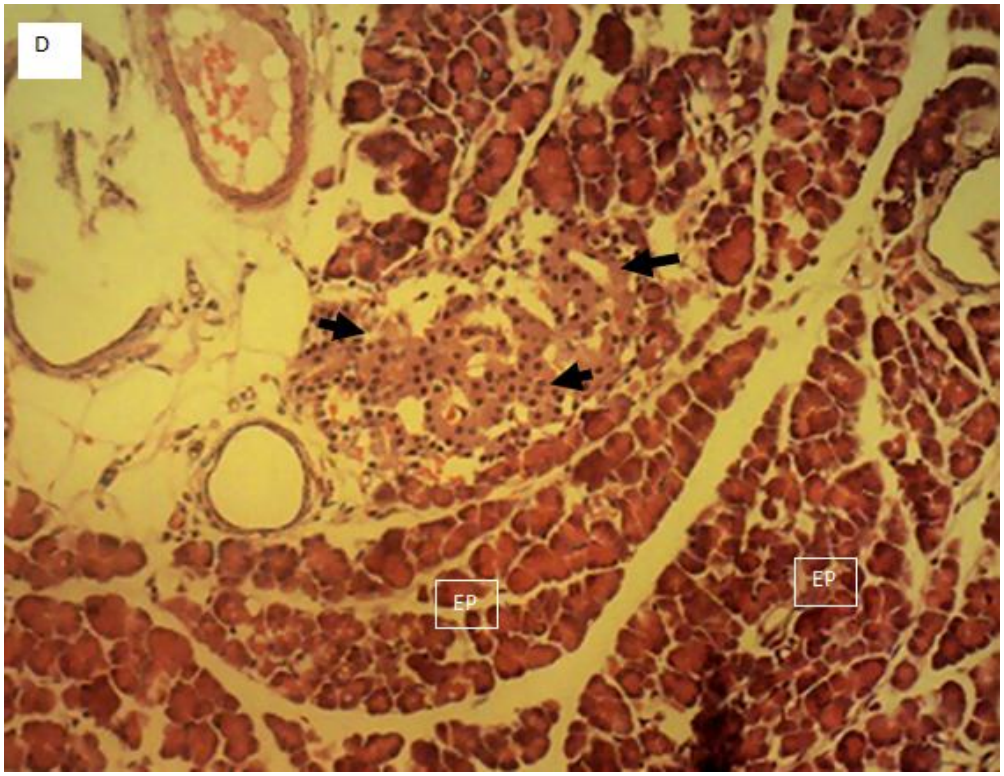

Plate 4: photomicrograph Sections of the pancreas after treatment with alloxan 125mg/kg and *C.Odorata* extract 200mg/kg for fourteen days, Section of the pancreas presented in Group D showed a decrease in the number of pancreatic islets, however a few large islets was observed. These islets showed cord-like arrangement of the islet cells (arrow). H&E x160.

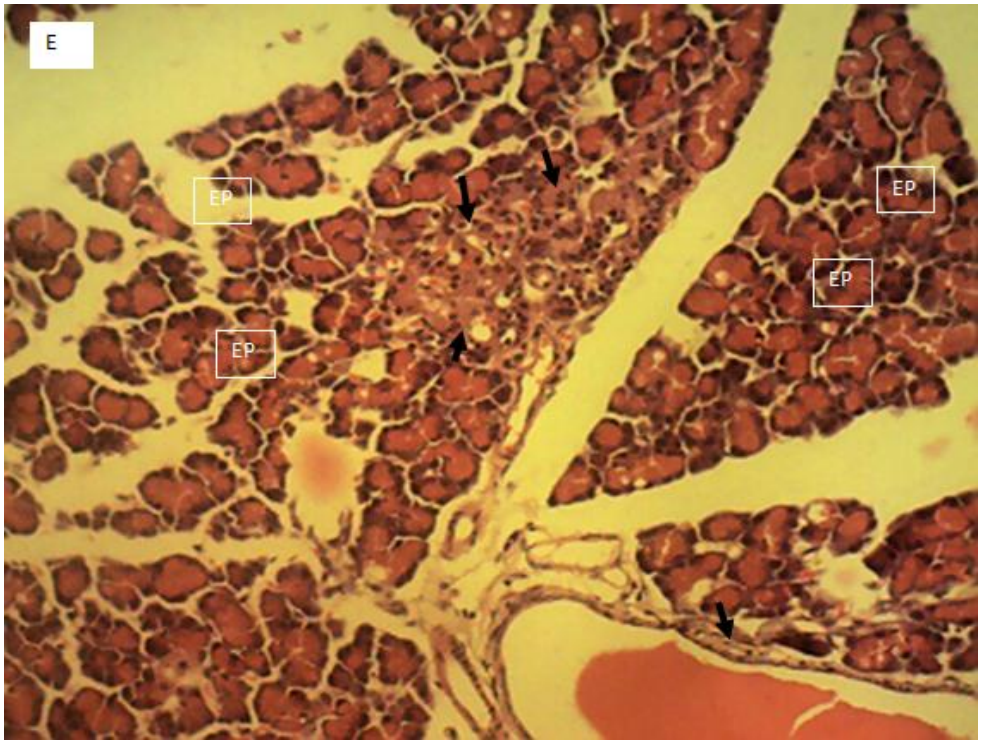

Plate 5: photomicrograph sections of the pancreas after treatment with alloxan 125 mg/kg and *C.Odorata* extract 400 mg/kg for fourteen days, Section of the pancreas presented in Group E showed a number of pancreatic islets, These islets showed cord-like arrangement of the islet cells (arrow). Exocrine pancreas (EP). H&E x100.

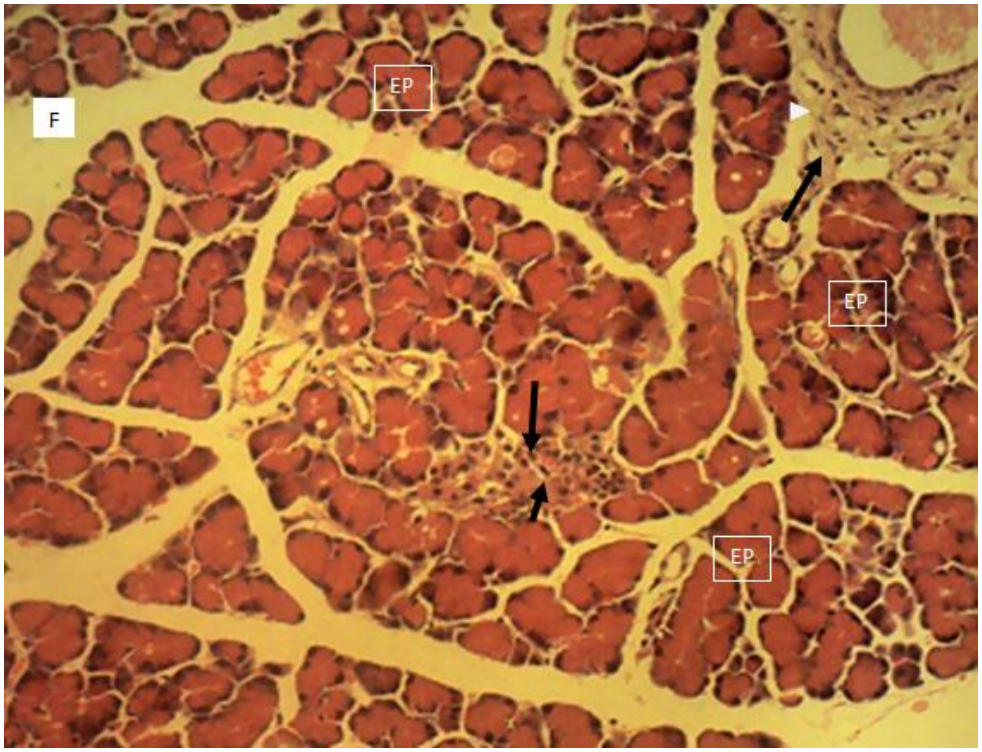

Plate 6: photomicrograph sections of the pancreas after treatment with alloxan 125 mg/kg and Metformin 500 mg/kg for fourteen days, sections of the pancreas presented in Group F showed regenerated pancreatic cells showing in vacuoles within their cytoplasm. Pancreatic islet (arrow); exocrine pancreas (EP). H&E x400; x160.

DISCUSSION

The basis for the application of *Chromolaena Odorata* was found to be their profound antioxidant actions due to high concentration of phenolic compounds.[5] The aim of this study was to evaluate the effect of Chromolaena odorata leaf extract on the pancreas of alloxan-induced diabetes in Wistar rats**.** Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism.

Diabetes mellitus was induced by a single intraperitoneal (I. P) injection of freshly prepared solution of alloxan monohydrate where β cells of Islets of Langerhans of the pancreas are destroyed resulting in a decrease in endogenous insulin secretion leading to decrease utilization of glucose by body tissues.[6], It results in elevation of blood glucose level.[7]

In diabetes mellitus, anaemia has always been observed particularly the hypochromic type, due to fall in the iron content of the body resulting from oxidation stress associated with the condition.[8][9][10] The anti-anaemic activity of the extract in this can be attributed to the high iron content of its chlorophyll, as seen in other green, leafy vegetables.[9]

The results of body weight indicated that the final body weight was significantly increased in the normal control rats when compared to initial body weight, whereas in the diabetic control rats there was a significant decrease in the body weight (Figure 1). The decrease in body weight observed in diabetic rats is reason out as the result of degradation of proteins (muscle wasting). Structural proteins are known to contribute to the body weight.[11]

In the absence of glucose and lipid sources, proteins are the next main source of energy in body. So it is clear that the decrease in body weight in diabetic rats were mainly because of degradation of structural proteins.

Diabetic rats treated with *C. Odorata* restored the body weight, this may be explained that the extracts improved the insulin secretion which reduces the hyperglycemia by peripheral utilization of glucose by the cells which ultimately improved the body weight.

Table 2 and figure 2 shows the concentration of blood glucose, plasma insulin in control and experimental groups. Administration of alloxan to diabetic control group significantly increased the blood glucose level. Alloxan produce diabetes mellitus with a single dose of administration through selective necrosis of pancreatic βcells of islets of langerhans that initiate insulin deficiency.

Oral administration of aqueous extract of C.odorata at (100, 200, 400mg/kg) significantly reduced ($p<0.05$) the blood glucose and increased the insulin level in diabetic rats, but not to the level of control rats. Since, C.odorata brought down the blood glucose level and increase the insulin level, the mechanism behind the reduction of blood glucose may be of its increasing ability in releasing the insulin from the β-cells.

Numerous studies have demonstrated that a variety of plant have been reported to contain substances like glycosides alkaloids, terpenoids, flavonoids and tannin etc which have been proved to be antidiabetic by different mechanism of action.[12]

Significant increase in glucose and significant reduction in insulin ($p<0.05$) were found in alloxan treated diabetic rats. Oral administration of the standard antidiabetic drug, metformin (500mg/kg) significantly reduced ($p<0.05$) the blood glucose level in diabetic rats and also the insulin levels.

Also the weights of the organs in Table 2 shows significant decrease in organ weights compared to the organ weight of rats treated with C.odorata and standard drug metformin.

Results of histological studies showed that the section of the pancreas presented in the normal control rats unveiled the histo-architecture of the endocrine and exocrine pancreas. It is made up of normal islet cells, obviously multiple and mostly occupying 30 -50% of the pancreatic lobules. Exocrine pancreas (EP). H&E x160; x400, while the positive control rats treated with alloxan monohydrate only showed a marked depletion in the number of pancreatic islets (PI) embedded in the exocrine pancreas (EP). The pancreatic islets appear small to relatively inconspicuous and show varying degrees of islet cell depletion due to degeneration and necrosis with nuclear pyknosis (as shown by an arrow head) and karyolysis and Alloxan has been reported to establish a redox cycle with the formation of superoxide radicals, which submit dismutation to hydrogen peroxide (H_2O_2) and a highly reactive hydroxyl radicals which are formed by Fenton reaction. Moreover, the action of reactive oxygen species (ROS) with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of pancreatic β-cells and thus hyperglycemia is occurred.[13] Inhibition of glucokinase enzyme is involved in the toxic action of alloxan on pancreatic β-cells.[14]

The experimental rats that received low, medium and high doses of *C. Odorata* extract and alloxan resulted in the dose dependent mitigation of the effect of alloxan. This was evident in the treated rats which showed reduced cell death in their number of pancreatic islets. These islets showed cord-like arrangement of the islet cells.

The *C. Odorata* extract may have functioned to restore the integrity and functions of the damaged pancreatic tissues. The specific mechanism of this tissue repair may not have been completely understood. Nonetheless, it could have been as a result of the large implication of oxidative stress[15][16] in damage to the pancreas, it seems reasonable to suggest that the antioxidant[17][18][19][20][21] and radical scavenging[22][23] effects of this plant may have played a key role in the protection of the pancreatic tissues from oxidants including that generated by alloxan. Alloxan destroys insulin-producing pancreatic ß-cells through the formation of reactive oxygen species that cause tissue damage.[24]

The rats which were Alloxan-induced and treated with 500mg/kg body weight metformin showed regenerated pancreatic cells showing in vacuoles within their cytoplasm. The effect of metformin after oral administration between normal and diabetic rats differed, when compared with negative control (group A) there is a mass reduction of the number of islet cells as the cells observed in groups treated with Metformin were regenerating from the damages done to the pancreatic cell when administered with Alloxan while that of the negative control has multiple number of islet cells showing a clear histo-architecture of the endocrine and exocrine pancreas. The positive control (alloxan induced only) when compared has the pancreatic islets appearing small to relatively inconspicuous and shows varying degrees of islet cell depletion due to degeneration and necrosis with nuclear pyknosis and karyolysis.

Histopathological data obtained from the current study were also consistent with Hadi[25] who reported that the size and number of pancreatic islets were decreased in diabetic rats in comparison to normal rats, while treated rats with mixture of plants extracts showed regeneration of the islets cells, according to Cigliola.[26]

CONCLUSION

In conclusion, this study indicates that *Chromolaena odorata* leaves contain substances with anti-diabetic properties and could be a safe and potent agent to be considered in the treatment of diabetes mellitus.

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