LETHAL DOSE EXAMINATION AND EFFECTS OF FLOWER, LEAVES, STEM AND ROOT EXTRACT OF *AGERATUM CONYZOIDES L* ON HEMATOLOGY AND SERUM ELECTROLYTES ON STREPTOZOTOCIN INDUCE DIABETIC RATS

ABSTRACT

Diabetes mellitus (DM) is a disease condition caused by decreased insulin production in the pancreas or as a result of the inability of insulin to act on target tissues that activates the absorption of blood glucose. This subsequently leads to an increased concentrations of glucose in the blood. Type 2 diabetes is the most recorded form of diabetes. It accounts for about 80% to 90% of all recorded forms the disease. The use of plant medicines is a very common practice from ancient time and is considered as much safer and less expensive therapeutic strategies for the management and treatment of various diseases including DM. The aim of this study is to investigate the antidiabetic effect of *Ageratum conyzoides* as claimed by herbal practitioners and to provide scientific evidences to back up the claim that the plant possess antidiabetic activity. Standard procedures were deployed in the aqueous extraction of the different parts (leaf, flower, stem, root and all parts) of the plant. Lethal dose of the plant was determined using Lorke's method. Subsequently, diabetes was induced into albino wistar rats using streptozotocin at 55mg/kg. 40 rats weighing 180g to 240g were divided into eight groups A to H, groups B to H were induced with diabetes. Groups A and B were labelled normal and diabetic respectively. C was treated standard drug (Metformin) at 1000mg/kg, groups D, E, F, G and H were with treated flower, leaf, stem, root and all parts extracts respectively at 2000mg/kg. Treatment in all groups was done for 28 days after which the rats were sacrificed and assayed for hematological and serum electrolytes. After DM was induced, rats were observed across groups for behavioral changes before, during and after treatment. LD_{50} of the aqueous extracts of *A. conyzoides* were found to be above 5000mg/kg. Significant differences were observed in the weights of the various groups at ($p<0.05$). Furthermore, Na^+, K^+ and Cl^- levels reduced significantly (<0.05), after treatment. Finally, WBC and its indices reduced significantly ($p<0.05$) while RBC and its indices increased

significantly ($p<0.05$) after treatment. These results shows that *A. conyzoides* possess antihyperglycemic and antilipidemic at 2000mg/kg.

Keywords: Ageratum conyzoides Linn; leaf, flower, stem and root extract; streptozotocin; diabetes mellitus; Glucose.

INTRODUCTION

Diabetes Mellitus (DM), is a disease of endocrine disorder in man and is considered one of the major health concerns globally today [1]. It is a disease of disordered metabolism of carbohydrate, protein and fat, caused by the complete or relative insufficiency of insulin secretion and/or insulin action [2]. It is characterized by a chronic hyperglycemic condition resulting from insufficient action of insulin. The main pathophysiological features of type 2 diabetes, which represents a great majority of diabetic cases, are impaired insulin secretion and increased insulin resistance. The impairment of pancreatic β-cell function notably shows progression over time.

It is a chronic disorder caused by decreased insulin production in the pancreas, or by the ineffectiveness of the insulin action on target tissues; this result in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, particularly the blood vessels and nerves [3]. The classical symptoms of diabetes include polyuria, glycosuria, weight loss, polydipsia, and polyphagia [4]. Derangements of carbohydrate metabolism in diabetes lead to chronic hyperglycemia in diabetes, which is associated with long-term damage, dysfunction and failure of various organs, especially the heart, eyes, blood vessels, kidneys, and nerves [5].

Plants have been of tremendous help to animals from time immemorial. Plants have been used by animals especially human beings as sole source of energy in the form of food and also medicine and beautification; the most important of these been the green plants [6].

In Nigeria and some parts of the world, history of traditional medicine show case thousands of plant species which have been used for many years in the practice of healing traditionally. In most part of Nigeria today, extracts from plants are still being used in their crude forms for the treatment of diseases. In most cases the therapeutic effects and other benefits derived are yet to be scientifically validated. Hence, there is a need for necessary scientific evaluation because of the rapid disappearances of forest habitats, and with time those in possession of this indigenous knowledge might die without transferring this knowledge and information to the next generation [7].

MATERIALS AND METHODS

PLANT MATERIALS

Ageratum conyzoides L. was obtained from Jos metropolis. The plant was identified and verified with a voucher number **(FHJ 246)** at the, Herbarium Department, Federal College of Forestry Jos, Plateau state.

EXPERIMENTAL ANIMALS

Adult male wister strain albino rats weighing from 180-200g were used to carry out the study. A minimum of twenty (40) adult albino rats were divided into 8 groups with 5 rats each. The rats were identified as head, back, head-back, tail, right hand, and left hand throughout diabetogen induction and plant aqueous treatment.

Lorke's method of LD50 Determination

This method has two phases which are phases 1 and 2 respectively.

Phase 1

This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behavior as well as if mortality will occur [8].

Phase 2

This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality [8].

Experimental Design

The animal groupings is as follows;

GROUP A- Normal control**,** GROUP B- Diabetic control, GROUP C – Diabetic + Metformin (1000mg/kg b.wt)**,** GROUP D- Diabetic + leaf extract (2000mg/kg b.wt)**,** GROUP E- Diabetic + flower extract (2000mg/kg b.wt)**,** GROUP F- Diabetic + stem extract (2000mg/kg b.wt)**,** GROUP G- Diabetic + root extract (2000mg/kg b.wt) and GROUP H- Diabetic + All parts extract (2000mg/kg b.wt)

Feeding and Randomization

After randomization into various groups and before the start of the experiment,the rats were acclimatized to the animal house condition (Kumal *et al.*, 2006; Miura *et al*., 2005; Nagappa *et al*., 2003) [9, 10, 3]. The rats were maintained on a standard rat feed consisting (70% Carbohydrate, 14.50% protein, 7.0% Fat, 7.20% fibre and 1.20% mineral) for 28 days.

Experimental Induction of Diabetes

Diabetes was induced by intraperitoneal injection of streptozotocin at (55mg/kg) in seven (7) groups namely Group B, C, D, E, F, G and H. The animals were left for 48 hours after which diabetes was confirmed from the fasting blood glucose using one touch glucometer. WHO [11] reported that blood glucose level reach 126mg/dl and accompanied with hyperglucosuric test 48 hours after streptozotocin injection. Prior to each study the animals will be made to fast for 14 hours but will have free access to water [12].

Preparation of Plant Extracts

The plant leaf was collected and removed from the stem and air dried at room temperature under shade. The dried plant leaf was pounded to powdery form using pestle and mortar. It was then sieved into a fine powder using mesh size of 180 micron. The powder was stored in an air tight container until required for use. The preparation of the plant extract was carried out using hot water.100g of the fine powder was boiled in one (1) Litre of distilled water for 15 minutes (to ensure maximum extractions of phytochemicals) using hot plate. The mixture was allowed to stand for 30 minutes before filtering using whatman filter paper No 1 to remove all extractable

matter. The filtrate was dried in the autoclave at a temperature of 50-60^{o}C for two weeks. The solid extract was kept in the refrigerator in an air tight container to be reconstituted in distilled water before use for treatment of diabetic rats.

ADMINISTRATION OF THE EXTRACT:

A. conyzoides L. Flower, leaf, stem, and root extract was administered through oral route at a dose of 2000 mg/kg body weight daily for 28 days. The lethal dose of different parts of the plant administered via oral route was found to be above 5000 mg/kg since no mortality was recording at 5000mg/kg.

Blood Collection

The blood was collected in both EDTA and plain sample bottle using the method of blood collection described by Parasuraman *et al.*, 2010[13] and was centrifuged in a sterile centrifuge tubes. Blood was collected after decapitation of rats. The EDTA collected blood was taking for haematological analysis, while the blood collected in the plain sterile sample bottle was allowed to clot for 40 minutes and spun at 3,500 rpm for 10 minutes. The serum was collected and transferred to bijou bottles and kept for analysis.

RESULTS

Table 1 Physical examination of experimental animals

GROUP	TREATMENT	NATURE OF FAECES	WATER INTAKE	CONDITION OF HAIR	CLUSTERING TOGETHER	WEAKNESS OR INACTIVENESS	DEATH
A	DC	Semi solid	Frequent	Hair lost	Frequent	Least active	No death
B	NC	Solid	Normal	No hair lost	Not frequent	More Active	No death
C	D + Metformin	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death
D	D + Flower	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death
E	D + Leaf	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death
F	D + Stem	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death
G	D + Root	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death
H	D + All Parts	Mild Solid	Not Frequent	Mild Hair lost	Not Frequent	Active	No death

Table 2: Effect of aqueous extract of *Agerantum conyzoides* on serum electrolytes concentration of streptozotocin induce diabetic rats.

GROUP	TREATMENT	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
A	DC	141.33±0.554	8.12±0.720	30.99±0.322
B	NC	128.73±0.379[a]	3.93±0.392[a]	22.39±0.843[a]
C	D + Metformin	130.70±0.588[ad]	6.98±0.862[ad]	24.68±0.554[ad]
D	D + Flower	138.93±1.770[ad]	6.64±0.163[ad]	30.033±0.351[ad]
E	D + Leaf	144.67±0.623[bd]	6.50±0.167[ad]	26.34±0.336[ac]
F	D + Stem	129.53±4.019[ac]	8.00±0.030[ad]	24.60±0.323[ac]
G	D + Root	130.27±2.301[ac]	5.61±0.360[ad]	25.51±1.038[ac]
H	D + All Parts	145.53±0.352[bc]	6.69±0.335[ad]	26.28±1.257[ac]
p-values	**-**	**<0.0001**	**<0.0001**	**<0.0001**

Values are expressed as mean ± SEM, n = 5.

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with diabetic control ($p < 0.05$)

[b]Values are significantly high when compared with diabetic control ($p < 0.05$)

[c]Values are significantly low when compared with normal control ($p < 0.05$)

[d]Values are significantly high when compared with normal control ($p < 0.05$)

Table 3: Effect of aqueous extract of *Agerantum conyzoides* on white blood cell and its parameters on streptozotocin induce diabetic rats.

TREATMENT	WBC	LYM	MID	GRA	L%	MI%	GR%
DC	18.25±1.783	11.44±0.775	1.88±0.181	7.47±0.627	53.06±2.715	10.40±0.374	37.90±2.178
NC	16.86±1.046[a]	3.77±0.086[a]	0.63±0.008[a]	2.21±0.046[a]	52.26±0.563[a]	6.80±0.108[a]	29.93±0.249[a]
D + Metformin	10.01±0.324[ac]	3.38±0.859[ac]	0.59±0.100[ac]	2.89±0.450[ad]	52.56±1.840[ad]	9.26±0.396[ad]	38.36±1.546[bc]
D + Flower	6.71±0.034[ac]	3.61±0.035[ac]	0.68±0.012[ad]	2.38±0.043[ad]	54.87±0.064[bd]	10.03±0.062[bd]	35.33±0.623[bd]
D + Leaf	5.13±0.034[ac]	2.76±0.025[ac]	0.53±0.013[ac]	1.85±0.024[ac]	53.90±0.081[bd]	10.13±0.062[bd]	36.30±0.108[bd]
D + Stem	17.65±2.421[ad]	6.44±0.047[ad]	0.84±0.006[ad]	3.33±0.062[ad]	59.26±0.165[bd]	8.36±0.143[ad]	32.70±0.318[ad]
D + Root	9.40±0.239[ac]	3.98±0.229[ad]	0.91±0.054[ad]	3.66±0.291[ad]	43.93±2.219[ac]	10.50±0.355[bd]	41.96±2.699[bd]
D + All Parts	6.60±0.040[ac]	3.54±0.022[ac]	0.66±0.021[ad]	7.00±0.024[ad]	43.33±0.623[ac]	5.46±0.143[ac]	52.83±0.124[bd]
p-values	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Values are expressed as mean ± SEM, n = 5.

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with diabetic control ($p < 0.05$)

[b]Values are significantly high when compared with diabetic control ($p < 0.05$)

[c]Values are significantly low when compared with normal control ($p < 0.05$)

[d]Values are significantly high when compared with normal control ($p < 0.05$)

Table 4: Effect of aqueous extract of *Agerantum conyzoides* on red blood cell and its parameters on streptozotocin induce diabetic rats.

GROUP	TREATMENT	RBC	HB	HCT	MCV	MAT	MCHC
A	DC	7.59±0.267	134.00±1.780	47.54±1.223	54.73±0.188	15.46±0.201	284.67±0.849
B	NC	9.78±0.238[b]	145.67±0.623[b]	51.38±0.358[b]	59.33±1.247[b]	17.83±0.704[b]	292.00±1.080[b]
C	D + Metformin	8.87±0.449[bc]	142.00±3.240[bc]	48.46±1.255[bc]	57.33±0.623[bc]	16.70±0.040[bc]	293.00±1.633[bd]
D	D + Flower	9.05±0.054[bc]	136.67.0.849[bc]	45.69±0.250[bc]	51.33±0.623[bc]	15.30±0.147[bc]	295.33±1.247[bd]
E	D + Leaf	8.93±0.046[bc]	144.33±0.623[bc]	47.87±0.072[bc]	53.33±0.623[bc]	16.00±0.408[bc]	301.33±0.623[bd]
F	D + Stem	8.79±0.071[bc]	143.9±0.062[bc]	49.40±0.178[bc]	57.47±0.209[bc]	16.50±0.040[bc]	291.67±0.623[bc]
G	D + Root	9.02±0.271[bc]	151.00±0.408[bd]	49.39±0.588[bc]	53.66±0.849[bc]	16.16±0.352[bc]	297.00±3.082[bd]
H	D + All Parts	9.45±0.143[bc]	145.33±0.623[bc]	46.50±0.198[bc]	55.33±0.849[bc]	16.63±0.478[bc]	282.00±11.669[bc]
p-values	**-**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**0.0019**	**0.0854**

Values are expressed as mean ± SEM, n = 5.

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with diabetic control ($p < 0.05$)

[b]Values are significantly high when compared with diabetic control ($p < 0.05$)

[c]Values are significantly low when compared with normal control ($p < 0.05$)

[d]Values are significantly high when compared with normal control ($p < 0.05$)

Table 5: Effect of aqueous extract of *Agerantum conyzoides* on red blood cell and its parameters on streptozotocin induce diabetic rats.

GROUP	TREATMENT	ROW	MPV	PCT	PDW	PLT
A	DC	15.33±0.239	6.32±0.107	0.20±0.0023	17.76±0.347	560.00±4.082
B	NC	17.56±0.572[b]	8.36±0.347[b]	0.95±0.006[b]	41.23±0.408[b]	1880.00±14.855[b]
C	D + Metformin	17.60±0.285[bd]	7.66±0.312[bc]	0.53±0.004[bc]	33.53±0.810[bc]	713.67±28.567[bc]
D	D + Flower	16.33±0.102[bc]	7.93±0.143[bc]	0.43±0.020[bc]	33.70±0.267[bc]	528.67±2.461[bc]
E	D + Leaf	16.70±0.081[bc]	8.73±0.062[bd]	0.87±0.012[bc]	36.93±0.107[bc]	1020.70±1.027[bc]
F	D + Stem	16.40±0.040[bc]	7.56±0.023[bc]	0.67±0.004[bc]	33.26±0.084[bc]	863.00±0.408[bc]
G	D + Root	16.26±0.286[bc]	7.73±0.347[bc]	0.61±0.114[bc]	35.05±0.716[bc]	764.33±180.55[bc]
H	D + All Parts	16.83±0.390[bc]	7.30±0.122[bc]	0.84±0.010[bc]	36.76±0.102[bc]	1186.30±2.248[bc]
p-values	**-**	**0.0004**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**

Values are expressed as mean ± SEM, n = 5.

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with diabetic control ($p < 0.05$)

[b]Values are significantly high when compared with diabetic control ($p < 0.05$)

[c]Values are significantly low when compared with normal control ($p < 0.05$)

[d]Values are significantly high when compared with normal control ($p < 0.05$)

DISCUSSION

Continuous observation of experimental animals after induction of streptozotocin gave some eminent symptoms and confirmed the emergence of diabetes in the experimental rats. Table 1 shows the results of close observation and physical examination of experimental rats. During administration of plant extracts, the nature of faece, rate of water intake and loss of hair were closely monitored.

The nature of faeces in all groups induced with diabetes became semi solid in nature with the exception of normal control whose faeces was solid in nature. Consequently, as treatment progresses, there were noticeable change in the fecal material to almost normal state, indicative of the fact that there is gradual reversal to the normal state after treatment. This observation agreed with the findings by Satoshi and Hitoshi, that Diabetes and some of its complications can affect the digestive system, leading to persistent diarrhea. The symptoms of diabetic diarrhea is similar to other forms of diarrhea but it is different from other forms of diarrhea, although it can be hard to distinguish from other types. It can occur during the day or night, and it can impact a person's quality of life and social interaction [14]. According to a study by Miguel et al., the pathogenesis of chronic diarrhea in persons suffering from DM is unclear. However, there are several mechanisms which could possibly be the reasons for this phenomena. They are; anorectal dysfunction, abnormalities in intestinal motility and secretion, bacterial overgrowth in the small bowel, bile acid catharsis, exocrine pancreatic insufficiency, and celiac disease [15]. Furthermore, Ogbonnaya and Arem in a review conducted in 1990 found that there are several pathogenic mechanisms responsible for diabetic diarrhea among which autonomic neuropathy, bacterial overgrowth, and pancreatic exocrine insufficiency have been implicated. Pancreatic exocrine insufficiency have being the most important underlying aberrations.

Excessive thirst (polydipsia) can be caused by high blood sugar (hyperglycemia). It's often one of the first noticeable symptoms of type 2 diabetes. Lal, explains that incessant water intake and frequent urination are typical signs of diabetes mellitus. This occurrences is as a result of an increase in glucose (sugar) in the blood. This will eventually lead to frequent urination. He added that if a patient insulin is ineffective, or absent, this will in turn result in the inability of the kidneys to filter glucose back into the blood. This will further trigger the demand for water consumption which is needed to dilute glucose concentration in the kidney, causing excessive water inflow in the kidney, triggering frequent urination [16].

In a study carried out in 2019 by Patricia *et al.*, [17] to determine the relationship between hair loss and T2DM among African American women. The study observed that T2DM increases the risk of severe central scalp hair loss in AA women. Thereby supporting the hypothesis that contributes to a large extent to hair loss among patients. Furthermore, they suggest that physicians should caution women with type 2 diabetes that their risk of severe central hair loss may be increased and should recommend early screening. In the same vein, medical practitioners should examine patients for central hair loss as early signs of T2DM. Also, Miranda et al. in a 2016 study asserted that hair follicle, which is also an organ is vulnerable to high plasma sugar damage. They mentioned that this phenomena is seen in the relationship between high blood sugar level and androgenetic alopecia, and the hair loss of T2DM patients. Therefore, a close observation of hair follicles status can serve as an entry point to the diagnosis of hyperglycemia organ damage effect.

Uncontrolled or untreated diabetes can result in a person's blood sugar levels becoming too high. Persistently high blood sugar levels can lead to damage in various tissues, organs, and blood vessels within the body. Damage to blood vessels can restrict blood flow, resulting in certain cells getting less oxygen and nutrients than they need. This deficiency can negatively impact the normal growth cycle of hair follicles, which can lead to hair loss.

The toxicity assessment of *A. conyzoides L.* was performed using Lorkes method as explained by Chinedu *et al.*, [18]. Table 1 shows the acute toxicity result for flower, leaf, stem, and root of *A. conyzoides L.* It indicates that the acute toxicity or LD_{50} of *A. conyzoides*

L is above 5000mg/kg, considering the fact that no mortality was recorded in both the low doses of 10mg/kg, 100mg/kg, 1000mg/kg and the high doses of 1600mg/kg, 2800mg/kg and 5000mg/kg tested.

Table 2 shows the levels of serum electrolytes. The levels of sodium, potassium and chloride of diabetic control and normal control are statistically significant ($p<0.05$) when compared to treatment groups. The root extract shows more electrolyte lowering effect compare to other parts of the plants in Na^+ and K^-, while the stem showed more lowering effect compare to other parts of the plant in chloride. Research shows that the association between glycemia and serum electrolytes is multi factorial and it is related to a number of other factors, which includes age and associated conditions. Increased urination in diabetic condition could lead to loss of electrolytes, water and could result to a physiological imbalance which may distort sodium and potassium levels in the body.

According to the findings by Sarguru *et al.,* [19] DM patients are more prone to mild hyperkalemia, when compared to the healthy patients which are the controls. Other studies have shown that the exogenous insulin can induce mild hyperkalemia because it promotes the potassium influx into the skeletal muscles and hepatic cells which increases the activity of Na^+ and K^+ ATPase pump [19]. Hyperkalemia is also linked with an impaired insulin secretion and decreased peripheral glucose utilization which could lead to carbohydrate intolerance and hyperglycemia [20].

Elevated serum Cl^- levels were also observed in diabetic patients and this may be as a result of diabetic ketoacidosis. Ketoacidosis are known to cause reduction in blood pH which further disturbs acid-base balance and this distortion often leads to the elevation of serum chloride. This condition is often observed in diabetic conditions [19].

Research have shown that administration of medicinal compounds or drugs have the tendency to alter the normal functionin of haematological parameters [21]. These alterations can be positive or negative [22]. Assessment of haematological parameters are useful in determining the extent of deleterious effect on blood constituents of organism [23, 24].

Table 3 shows the results of white blood cell haematological parmeters. WBC, lymphocyte and other WBC parameters increased significantly ($p<0.05$) for diabetic control compare to those of normal control and diabetic treated groups.

Table 4 and 5 shows the results of red blood cell and its related parameters. Significant difference was observed in diabetic control, normal control and diabetic treated groups ($p<0.05$). RBC and its related parameters were seen to show significant reduction in diabetic control compare to normal control group and diabetic treated groups. These results could be as a result of anaemia or other blood complications that occur as a result of hyperglycemia.

Oyedemi *et al.*, [25] reported the occurance of anaemia in diabetes mellitus. This he said is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia. The oxidation of these proteins and hyperglycaemia in this disease condition leads to an increase in the production of lipid peroxides that eventually results to haemolysis of RBC [26]. The decrease in MCH and MCHC values, observed after administration of STZ, is an indication of abnormal hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolarity [27].

Studies have shown that hematological alterations in streptozotocin-induced diabetes mellitus distort the normal functioning of a diabetic rat. Akpan and Ekaidem reported in 2015 [28] that hematological alterations often occur in diabetes mellitus as a result of oxidative stress induced by diabetes mellitus. BW of streptozotocin in rats. The results of this investigation showed that the diabetic control had significantly higher level of WBC count than the normal control. Red blood cell (RBC), Hemoglobin (HGB) and Packed cell volume (PCV) were all significantly reduced in comparison to normal control as well as red blood cell indices including MCV, MCH and MCHC [29].

Biological or chemical induced alterations in tissue arrangement could result into metabolic disorders and this distortion could be reversed by the active principles contained in herbal remedies [30].

Koneri *et al*., found out that saponin isolated from *Momordica cymbalaria* Fenzl caused considerable quantitative increase and rejuvenation of β-cells (75%) of streptozotocin-induced diabetic rats, in which oleanane-type triterpenoid saponin was discovered to be the active hyperglycemic compound contained in the plant extract.

Also, Abou El-Soud *et al*., [31] discovered and reported that alkaloid extract of fenugreek dried seeds (*Trigonella foenum-graecum* L.) has glycemic control, reverse renal and hepatic tissues damage in streptozotocin induced diabetes and its ability to reverse renal and

hepatic tissue damages are not unconnected with the outcomes of phytochemical interactions among the composite herbal extracts, which may either display synergy or antagonism [32].

CONCLUSION

In conclusion, the result obtained clearly shows that flower, Leaf, Stem and Root Extract of *Ageratum conyzoides L* does not possess any toxic effect on albino wistar rats and was seen to possess antidiabetic effects. This is evident in its ameliorative effect on serum electrolytes and hematology.

ETHICAL APPROVAL

The animals were fed with standard feed throughout the period of the research. All experiments on animals were in accordance with the guidelines of both the University of Jos ethical committee and the international guidelines for handling of laboratory animals

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the

authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Rohilla, A. & Ali, S. (2012). Alloxan–induced diabetes, Mechanisms and effect. *Int.J.Res*
2. Ivorra, M .D., Paya, M. & Villar, A(1989). A review of natural products and plants as potential anti - diabetic drugs. *J.Ethnopharmacol*, 27:243-275.
3. Nagappa, A. N., Thakurdesai, P. A., Raob, N. V. & Singh, J. (2003). Antidiabetic activity of *Terminalia catappa* Linn fruits. *J. Ethnopharmacol*; 45-50.
4. Cooke, D. W. & Plotnick, L. (2008) Type 1 diabetes mellitus in pediatrics, *Pediatr Rev*; 29(11):374–84.
5. Assim, A. & Khalid, A. B. A. (2006). Assessment of care for type 2 diabetic patients at the primary care clinics of a referral hospital. *Journal of Family and Community Medicine*; 13(1):13-18.
6. Luka, C. D., Istifanus, G., George, M. & Jesse, N. S. (2018). Effect of Aqueous Extract of *Dodonaea viscosa* Linn on Some Biochemical Parameters in Alloxan induced Diabetic Rats. *Current Journal of Applied Science and Technology* 27(3): 1-7.
7. Idu, M. & Ndukwu, B.C (2006).Studies of Plants Used in Ethno medicine in Ethiope council area of Delta State.Nigeria.pp 40.
8. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275–87.

9. Kumar, G. P. S., Arulselvan, P., Kumar, D. S. and Subramanian, S. P. (2006). Antidiabetic activity of fruits of Terminalia chebula on streptozotocin induced diabetic rats. *Journal of health science*, 52: 283-291
10. Miura, T., Koike, T. and Ishida, T. (2005). Antidiabetic activity of green tea (Thea sinensis L.) in genetically Type 2 diabetic mice. *Journal of health science*, 51: 708- 710.
11. WHO (2005). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia; Report of a WHO/IDF Consultation.
12. Ragavan and S. KrishnaKumari (2006). Antidiabetic effect of T. arjuna bark extract in Alloxan induced diabetic rats. *Indian Journal of Clinical Biochemistry*. 21:123-128.
13. Parasuraman, S., Raveendran, R. & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother*; 1: 87–93.
14. Satoshi Murao and Hitoshi Hosokawa (2010). Serotonin 5-HT3 Receptor Antagonist for Treatment of Severe Diabetic Diarrhea. *Online letters* DOI: 10.2337/dc09-2131
15. Miguel A. Valdovinos. Michael Camilleri and Bruce R. Zimmerman (1993). Chronic Diarrhea in Diabetes Mellitus: Mechanisms and an Approach to Diagnosis and Treatment. *Mayo Clinic Proceedings.* VOLUME 68, ISSUE 7, P691-702.
16. Lal, B. S (2016). DIABETES: CAUSES, SYMPTOMS AND TREATMENTS. *Chapter 5*. Pg 55-67.
17. Patricia F. Coogan, Traci N. Bethea, Yvette C. Cozier, Kimberly A. Bertrand, Julie R. Palmer, Lynn Rosenberg and Yolanda Lenzy (2019). Association of type 2 diabetes with central-scalp hair loss in a large cohort study of African American women. *International Journal of Women's Dermatology* (5) 261–266.
18. Enegide Chinedu, David Arome, Fidelis Solomon Ameh (2013). A New Method for Determining Acute Toxicity in Animal Models. *Toxicology International* / Vol-20 / Issue-3.
19. Sarguru Datchinamoorthi, Vanaja R and Balaji Rajagopalan (2016). Evaluation of Serum Electrolytes in Type II Diabetes Mellitus. Int. J. Pharm. Sci. Rev. Res., 40(1), September – October 2016; Article No. 45, Pages: 251-253.
20. Lindner, G. & Funk, G. C. (2013). Hypernatremia in critically ill patients. *J Crit Care*, 28, 2013, 216.e11-216.e20.
21. Adeneye, A. A. (2008). Haematopoetic effect of methanol seed extract of C*itrus paradise* Macfad (grape fruit) in Wistar rats. *Biomed Res*; 19:23-6.
22. Ajagbonna, O. P., Onifade, K. I. & Suleiman, U. (1999). Haematological and biochemical changes in rats given extract of *Calotropis procera*. *Sokoto J Vet Sci*; 1:36-42.
23. Muhammad, N. O., Oloyede, O. B., Owoyele, B. V. & Olajide, J. E. (2004). Deleterious effect of defatted *Terminalia catappa* seed meal-based diet on haematological and urinary parameters of albino rats. *NISEB J*; 4(2):51-7

24. Ashafa, O. T., Yakubu, M. T., Grierson, D. S. & Afolayan, A. J. (2009). Toxicological evaluation of the aqueous extract of *Felicia muricata* Thunb. leaves in Wistar rats. *Afr J Biotechnol*; 8:949-54.
25. Oyedemi, S. O., Yakubu, M. T. & Afolayan, A. J. (2011). Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. *J Med Plant Res*; 5:119-25.
26. Arun, G. S. & Ramesh, K. G. (2002). Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocindiabetic rats. *Indian J Pharmacol*; 34:156-64.
27. Stookey, J. D., Burg, M., Sellmeyer, D. E., Greenleaf, J. E., Arieff, A. & Van Hove, L. (2007). A proposed method for assessing plasma hypertonicity in vivo. *Eur J Clin Nutr*; 61:143-6.
28. Akpan, H. D. & Ekaidem, I. (2015). Modulation of Immunological and Hematological Disturbances of Diabetes Mellitus by Diets Containing Combined Leaves of *Vernonia amygdalina* and *Gongronema latifolium*. *British Journal of Applied Science & Technology*; 6(5):534-44.
29.
30. Zafar, M., Naqvi, S. N-u-H., Ahmed, M. & Khani, Z. A. K. (2009). Altered kidney morphology and enzymes in streptozotocininduced diabetic rats. *Int J Morphol*; 27(3):783-90.
31. Ahmed, D., Kumar, V., Verma, A., Gupta, P. S., Kumar, H. & Dhingra, V. (2014). Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of *Albizzia Lebbeck* Benth. stem bark (ALEx) on streptozotocin induced diabetic rats. *BMC Complement Altern Med*; 14:243.
32. Koneri, R. B., Samaddar, S. & Ramaiah, C. T. (2014). Antidiabetic activity of a triterpenoid saponin isolated from *Momordica cymbalaria* Fenzl. *Indian J Exp Biol*; 52:46-52.
33. Abou El-Soud, N. H., Khalil, M. Y., Hussein, J.S., Oraby, F. S. & Farrag, A. R. (2007). Anti-diabetic effects of Fenugreek alkaloid extract in streptozotocin induced hyperglycemic rats. *J Appl Sci Res*; 3:1073-83.
34. Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A. & Sabharwal, M. (2014). Herbal antioxidant in clinical practice: A review. *Asian Pac J Trop Biomed*; 4:78-84.