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SUBACUTE ANTIDIABETIC PROPERTIES OF *AGERATUM CONYZOIDES* LEAVES IN DIABETIC RATS

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ABSTRACT: This study investigated the antidiabetic activity of the aqueous extract of the leaves of *Ageratum conyzoides.* Streptozotocin induced hyperglycemia male adult albino rats were used to study the antidiabetic effect of *Ageratum conyzoides* aqueous extract (at doses of 100, 200 and 300 mg/kg respectively). The diabetic rats were housed in metabolic cages, for the whole period of the experiment. Body weight was evaluated at the beginning of the experiment and on days 7, 14, and 21 afterward. Water intake, food intake and urine excretion volume were also estimated. At the end of the experiment the rats were sacrificed and serum insulin concentration, total serum proteins and hepatic glycogen content were estimated. Lipid profile (total cholesterol (TC), High density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and triglycerides (TG)) were also assessed. The results showed significant effect of stimulating body weight growth at the doses of 200, 300 mg/kg. The effect of the extract on the food, water intake and water excretion showed a significant decrease in the 200 and 300 mg/kg extract treated groups. In treated rats, both doses (200 and 300 mg/kg) induced a significant reduction in serum glucose and the Area Under the glucose Curve, with concomitant increases in serum insulin and protein levels. Furthermore, *A. conyzoides* treatment improve lipid profile by increase HDL and reducing triglycerides and LDL at 200 mg/kg. The present study clearly indicates that Ageratum conyzoidesaqueous extract leaves exhibited significant antidiabetic activity and supports its uses in traditional medicine.

INTRODUCTION: *Ageratum conyzoides* is an erect herbaceous annual plant. It was originally introduced as a garden plant, and now widely used in traditional medicine worldwide [1].

Since ancient times, the plant has been utilized for the treatment of various ailments, such as burns and wounds, infectious diseases, arthritis and fever[1].

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Specifically, it has been shown to have activity against larvae of the mosquito *Aedes aegypti* [2], anti-inflammatory properties with no apparent hepatotoxicity [3], radio-protective and gastroprotective effect [4, 5]. Isolated and structurally elucidated 5, 6, 7, 8, 3′, 4′, 5′-Heptamethoxy flavone from *Ageratum conyzoides*, showed low insecticidal activity against *D. hyalinata* and *R. dominica* [6]. In Africa *A. conyzoides* is used to treat fever, rheumatism, headache, colic, wounds caused by burns, dyspepsia, eye problems, uterine disorders and pneumonia, while it is used in traditional medicine for antiasthmatic, antispasmodic and haemostatic effects in East Africa [7].

Many bioactive compounds have been isolated from *A. conyzoides*, which include mono- and sesquiterpenes, chromene, chromone, benzofuran and coumarin, flavonoids, triterpene and sterols, alkaloids and some miscellaneous compounds [1]. According to previous ethno-botanical studies, *A. conyzoides* is used in the treatment of diabetes in Cameroon and Nigeria [8, 9].

Earlier pharmacological studies with crude leaves extract and butanol acetic acid fractions respectively, have demonstrated acute effect of *Ageratum conyzoides* on diabetic rats [10, 11]. The aim of this study was to evaluate the subacute antidiabetic effect of the aqueous extract of the leaves of *Ageratum conyzoides* on some antidiabetic parameters (blood glucose level, lipid profile, serum insulin, hepatic glycogen, total serum protein, water intake, urine excretion and food intake), and body weight.

MATERIALS AND METHODS:

Drugs and chemicals:

Glibenclamide (Glycomin®) was obtained from Strides Arcolat Ltd. Bangalore, India. Streptozotocin (Cat. No. S0130) was purchased from Sigma-Aldrich®, St Louis, MO.

Collection and preparation of plant material:

Mature plants of *Ageratum conyzoides* Linn. were harvested during the month of February 2009 in Yaoundé, Centre Region, Cameroon. Botanical identification was performed at the National Herbarium of Yaoundé, in comparison with the voucher specimen N°19050/SFR/Cam. The leaves were shade-dried and ground into powder.

Extraction:

A fine powder of the leaves (138 g) was boiled in distilled water (2.25 L) for 30 min. The decoction was taken and allowed to cool for 30 min at room temperature (24 ± 5°C), and filtered twice. The filtrate was dried in an oven (55°C) for 3 days to obtain the extract (Yield = 29% w/w).

Animals: Male albino *Wistar* rats (180 - 220 g) were maintained on standard laboratory diet and tap water *ad libitum* in the Animal House of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Cameroon. The study was carried out with the approval of the Institutional Animal Ethics Committee.

Induction of experimental diabetes:

Diabetes was induced in overnight fasted animals, by an intravenous injection of a freshly prepared streptozotocin (STZ) solution, at the dose of 52 mg/kg body weight in acidified saline solution (0.9%; pH 4.5), as previously described [12]. A set of control rats received only the acidified saline solution. After 72h, when the condition of diabetes was stabilized, the animals with blood glucose levels above 200 mg/dL were considered as diabetic animals and selected for the study.

Study of the effect of aqueous leaves extract of *Ageratum conyzoides* in diabetic rats:

Experimental design:

All rats were kept in metabolic cages (Nalgene) at the the animal house, with light/dark cycles of 12/12 hours at constant temperature and humidity of 20 °C and 65 % respectively, for 3 weeks before and during the experimental period. The diabetic animals were divided into five groups (n = 6) and *A. conyzoides* (100 mg/kg, 200 mg/kg or 300 mg/kg body weight respectively), glibenclamide (10 mg/ kg) and vehicle control were given orally. The vehicle control group (n = 6) received distilled water (1 mL/kg body weight). The treatments were administered orally, once per day, during 3 weeks.

Changes in body weight were assessed weekly in all groups of rats. Prior to the sacrifice on day 21, the animals were subjected to fasting for 16h but allowed free access to water. The fasting blood glucose levels were estimated on the initial day of the experiment, on day 7, on day 14 and at the end of the experiment (day 21).

At the end of the experiment (day 21), the animals were sacrificed by decapitation and the following biochemical parameters were evaluated in serum: insulin levels, lipid profiles ((Total cholesterol (TC), Triglycerides (TG), LDL - cholesterol (LDL-C) and HDL cholesterol (HDL-C)), total protein and liver glycogen levels.

Determination of body weight:

The body weight of diabetic rats was recorded once a week, from the beginning of the experiment, on

day 7, 14 and 21; these values were converted to the percentage of the initial weight.

Determination of the plasma glucose concentration:

Blood samples for glucose determination were obtained from the tip of the tail of rats at the beginning of the experiment before administration of drugs, at week 1, 2 and 3 (end of the experiment) respectively. Diabetic rats were used after 16 h of fasting. The glucose level was measured with a glucometer, Glucotrend®2 (An Accu-Chek system of the Roche Group Germany, Roche diagnostics GmbH D-68298 Mannheim, Germany) in all animals. The Area Under Curve was then calculated, and each value was compared to those of the vehicle control group.

Determination of water intake, urine excretion and food intake:

After 3 days of acclimatization, the rats were subsequently housed individually in metabolic cages with free access to tap water and food. Water and food intake were deducted from the initial quantities put in each cage. Urine was spontaneously voided after every 24 hours and collected in the metabolic cage. The urine collector was used to determine daily urinary volume excreted.

Determination of lipids profile:

Total cholesterol (TC), Triglycerides (TG), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) levels were measured following the commercial kit's instructions.

Serum insulin determination:

Serum was separated immediately after blood sampling by centrifugation at 4000 rpm at 4°C for 30 min. Serum insulin levels were estimated by a Radio Immuno Assay Kit (Millipore, St Charles, MO., Cat. No. RI-13K) according to the manufacturer's instructions.

Assay of glycogen content:

At the end of the experimental period (day 21), all diabetic rats were sacrificed and their livers removed. 100 mg of the liver tissue was homogenized in 5 volumes of an ice-cold 30% (w/v) KOH solution and dissolved in a boiling water-bath (100°C) for 30 min. The glycogen was precipitated with ethanol, washed, and re-solubilized in distilled water. Thereafter, hepatic glycogen content was determined by the anthrone-reagent method [13]. The amount of blue compound generated by the reaction was assayed with a spectrophotometer at 620 nm. The glycogen content was expressed as mg/100 g wet tissue.

Serum protein determination:

The protein content was estimated using a standard procedure [14], with slight modifications.

Statistical analysis:

The OGTT Area Under Curve was estimated with Graphpad software using the trapezoidal rule. The data were expressed as mean ± S.D. One-way analysis of variance (ANOVA) followed by Dunnet's post-test was used to identify significant differences between treated groups and that of vehicle control. Differences were considered to be significant at $p \leq 0.05$.

RESULTS:

Effects of *Ageratum conyzoides* extract on body weight of STZ-induced diabetic rats:

Subacute administration of *Ageratum conyzoides* extract at doses of 200 mg/kg and 300 mg/kg body weight respectively increase significantly body weight of the diabetic rats at all weeks ($p \leq 0.01$). After the beginning of the experiment (the dose of 100 mg/kg of *Ageratum conyzoides extract* exhibits the same effect only at the third week (78.87 ± 2.30 to 88.01 ± 2.88; $p \leq 0.01$). Glibenclamide was more efficient during the three weeks of treatment (103.18 ± 3.30 to 135 ± 10.84; 83.48 ± 3.28 to 95.05 ± 3.50; 78.87 ± 2.30 to 135.12 ± 5.99; $p \leq 0.001$) (**Figure 1).**

Effects of *Ageratum conyzoides* extract on blood glucose of STZ-induced diabetic rats:

Daily treatment of STZ diabetic rats with *Ageratum conyzoides extract*, for 21 days result in a significant decrease of the Area Under Curve of the diabetic rats treated with the dose of 200 mg/kg and 300 mg/kg (8459.70 ± 259.10, to 6957.70 ± 487.40 and 5631.70 ± 283 respectively ($p \leq 0.001$)); when compared to that of vehicle control (8459.7 ± 259.10) ($p \leq 0.001$). Glibenclamide also lowered

significantly AUC-blood glucose ($p \leq 0.001$) (**Figures 2 & 3**).

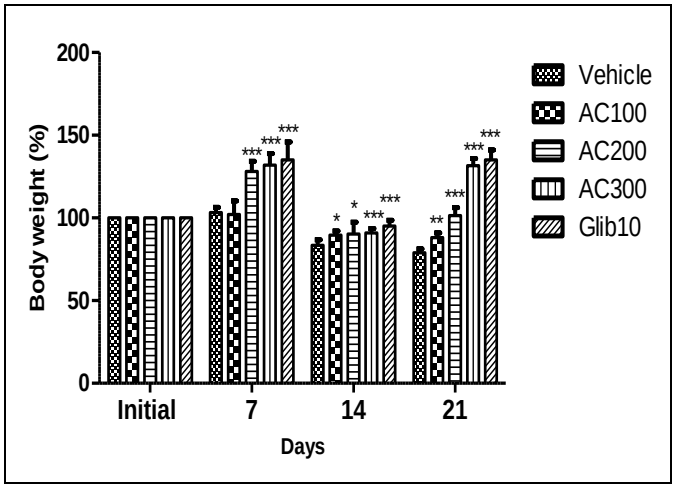

FIG.1: EFFECTS OF *AGERATUM CONYZOIDES* EXTRACT ON THE BODY WEIGHT OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). *$p \leq 0.05$ compared with the corresponding value for vehicle control rats; **$P \leq 0.01$ compared with the corresponding value for vehicle control rats; ***$p \leq 0.001$ compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg). AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

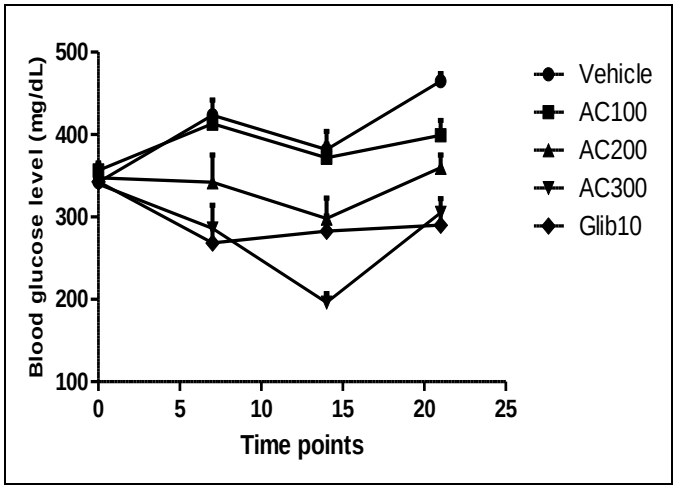

FIG.2: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON BLOOD GLUCOSE OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

Effect of *Ageratum conyzoides* extract on lipid profile of STZ-induced diabetic rats: The subacute treatment with *Ageratum conyzoides* extract, at all doses used, during the 21 days decreased total cholesterol, whenever significant at doses of 100 mg/kg of AC and 10 mg of glibenclamide ($p \leq 0.05$), although the dose 200 mg/kg increases HDL cholesterol (38.67 ± 2.16 to 46.33 ± 3.39) ($p \leq 0.001$) and decrease LDL Cholesterol (25.67 ± 2.50 to 15.83 ± 1.94) ($p \leq 0.001$). Serum triglycerides level of diabetics rats decrease significantly at the end of the treatment with 200 mg/kg, 300 mg/kg and glibenclamide (10 mg/kg) respectively (63.67 ± 3.14 to 55.17 ± 2.79 ($p \leq 0.01$), 46.67 ± 5.24 and 45.58 ± 5.02 ($p \leq 0.001$) **(Figure 4)**.

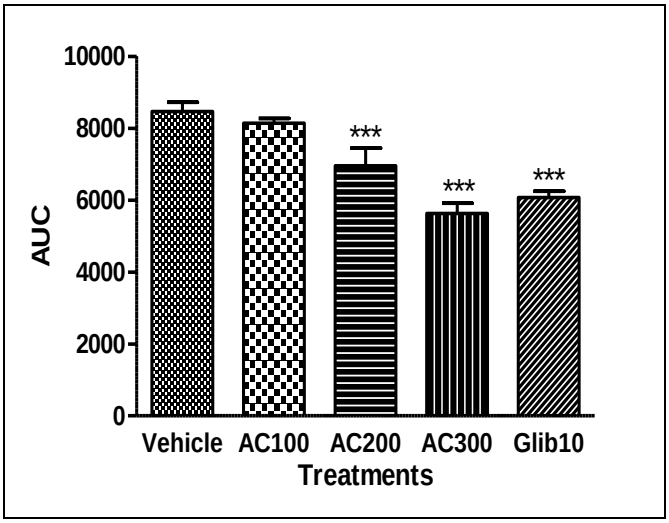

FIG. 3: AREA UNDER CURVE OF THE EFFECT OF *AGERATUM CONYZOIDES* ON BLOOD GLUCOSE OF OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). ***$P \leq 0.001$ compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

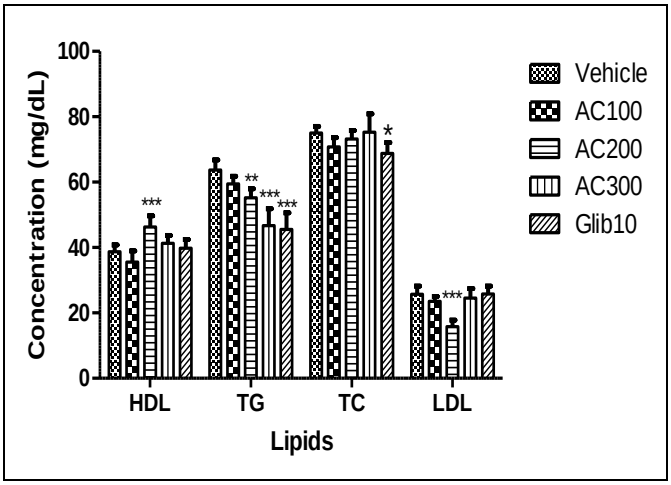

FIG. 4: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON LIPID PROFILE OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). *p ≤ 0.05 compared with the corresponding value for vehicle control rats; **p ≤ 0.01 compared with the corresponding value for vehicle control rats; ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. HDL: HDL cholesterol; TG: triglycerides; TC: Total Cholesterol; LDL: LDL Cholesterol. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

Effect of *Ageratum conyzoides* subacute treatment (3 weeks) on the food intake, water intake and urine excretion in streptozotocin-diabetic rats:

Amongst the doses used, only the doses of 200 mg/kg and 300 mg/kg decreased food intake (207.98 ± 17.62 to 129.10 ± 39.06 and to 120.32 ± 38.52) (p ≤ 0.05)) respectively, water intake (589.53 ± 20.25 to 342.16 ± 16.63, and to 509.03 ± 9.23 respectively) (p ≤ 0.001) and urine excretion (158.65 ± 12.64 to 101.96 ± 10.25) (p ≤ 0.001), and to 134.13 ± 6.95) (p ≤ 0.05). Glibenclamide showed the best effect in lowering food intake (207.98 ± 17.62 to 100.34 ± 30.02) (p ≤ 0.001), lowered water intake (589.53 ± 20.25 to 372.17 ± 14.33) (p ≤ 0.001) and decrease urine excretion (158.65 ± 12.64 to 85 ± 5.15) at p ≤ 0.001 (**Figures 5, 6 and 7).**

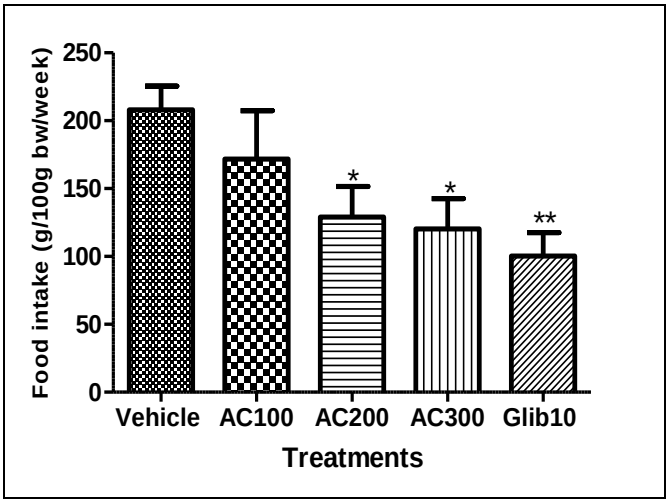

FIG. 5: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON RELATIVE FOOD INTAKE OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). *p ≤ 0.05 compared with the corresponding value for vehicle control rats; **p ≤ 0.01 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

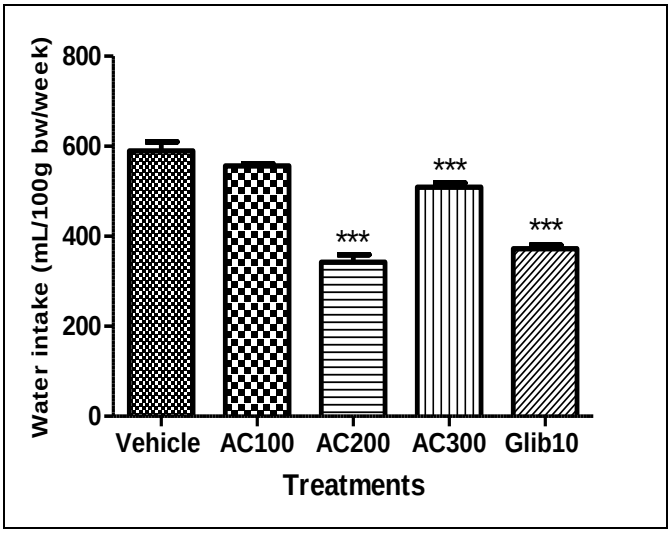

FIG. 6: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON RELATIVE WATER INTAKE OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

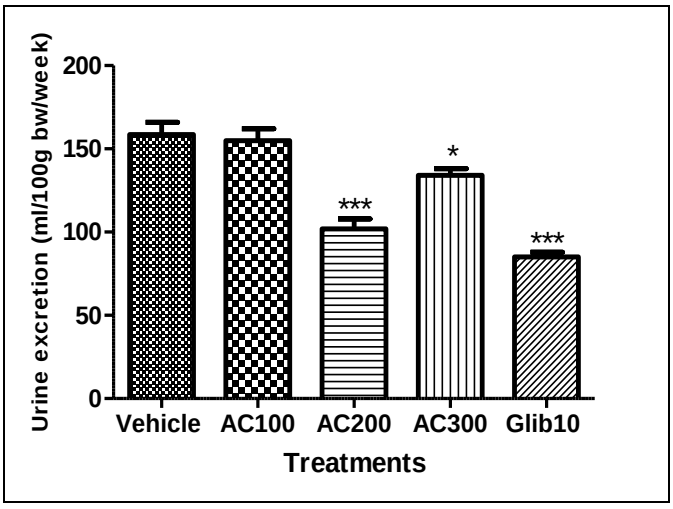

FIG.7: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON RELATIVE URINE EXCRETION OF STZ-INDUCED DIABETIC RATS

Data are expressed as means ± S.D (n = 6). *p ≤ 0.05 compared with the corresponding value for vehicle control rats; ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

Effect of *Ageratum conyzoides* extract on insulin level of STZ-induced diabetic rats at the end of the three weeks of treatment:

A significant increase of serum insulin of diabetic rats after 3 weeks of treatment with the extract, at

200, 300mg/kg and with glibenclamide (10 mg/kg), when compared to diabetic control (**Figure 8**). This increase was by a percentage of 23.64, 29.94 and 79.86% respectively, and dose related.

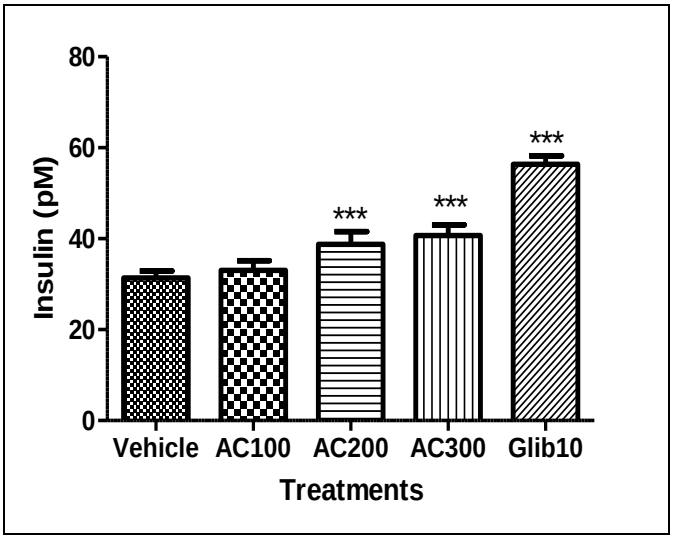

FIG.8: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON INSULIN LEVEL OF STZ-INDUCED DIABETIC RATS AT THE END OF THE THREE WEEKS OF TREATMENT

Data are expressed as means ± S.D (n = 6). ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

Effect of *Ageratum conyzoides* extract on glycogen level of STZ-induced diabetic rats at the end of the three weeks of treatment:

The hepatic glycogen content was significantly elevated respectively at 200 and 300 mg/kg of *Ageratum conyzoides* extract when compared to vehicle control (42.61 ± 2.95 to 48.03 ± 3.54) ($p \leq 0.05$) and (42.61 ± 2.95 to 50.39 ±2.85) ($p \leq 0.01$). Glibenclamide was more active to enhance hepatic glycogen content when compared to vehicle control (42.61 ± 2.95 to 56.37 ± 3.32) ($p \leq 0.001$) (**Figure 9**).

Effects of *Ageratum conyzoides* extract on serum total protein in STZ- diabetic rats at the end of the three weeks of treatment:

Amongst the doses of *Ageratum conyzoides* extract used, only the doses of 200 and 300 mg/kg increased the level of serum total protein (59.50 ± 5.01 to 75.50 ± 3.45 and 59.50 ± 5.01 to 72 ± 7.32) ($p \leq 0.01$ and $p \leq 0.001$ respecticely). Glibenclamide was more effective and increased the serum total protein by 59.67 % from 59.50 ± 5.01 to 95 ± 5.55 ($p \leq 0.001$) (**Figure 10**).

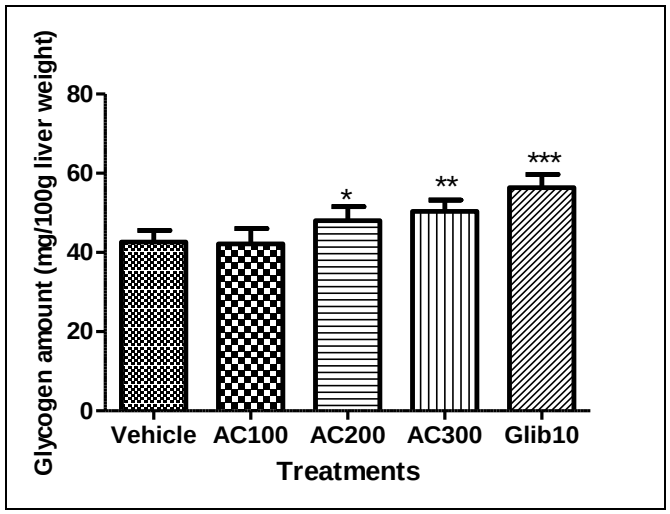

FIG.9: EFFECT OF *AGERATUM CONYZOIDES* EXTRACT ON GLYCOGEN LEVEL OF STZ-INDUCED DIABETIC RATS AT THE END OF THE THREE WEEKS OF TREATMENT

Data are expressed as means ± S.D (n = 6). *p ≤ 0.05 compared with the corresponding value for vehicle control rats; **p ≤ 0.01 compared with the corresponding value for vehicle control rats; ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg)

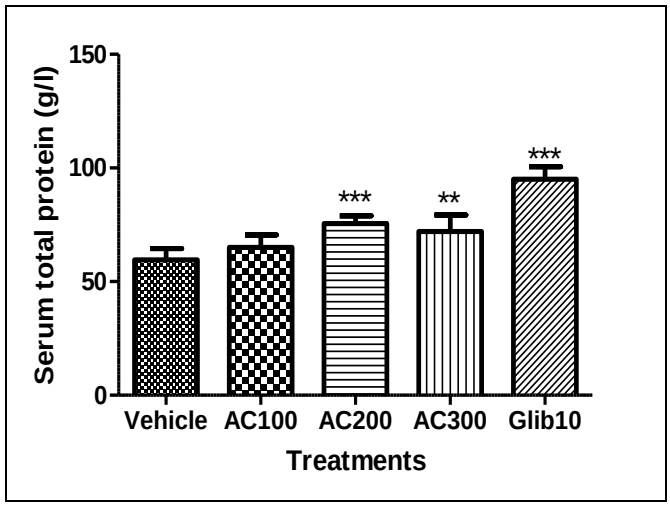

FIG.10: EFFECTS OF *AGERATUM CONYZOIDES* EXTRACT ON SERUM TOTAL PROTEIN LEVEL OF STZ-INDUCED DIABETIC RATS AT THE END OF THE THREE WEEKS OF TREATMENT

Data are expressed as means ± S.D (n = 6). **p ≤ 0.01 compared with the corresponding value for vehicle control rats; ***p ≤ 0.001 compared with the corresponding value for vehicle control rats. AC100: *Ageratum conyzoides* (100 mg/kg); AC200: *Ageratum conyzoides* (200 mg/kg); AC300: *Ageratum conyzoides* (300 mg/kg); Glib10: Glibenclamide (10 mg/kg).

DISCUSSION:

Previous acute studies have showed a short-term hypoglycaemic effect of oral administration of *Ageratum conyzoides* leaves extract in diabetic rats [10, 11]. The present study aimed at investigating the subacute antidiabetic effect of *A. conyzoides* aqueous extract in diabetic male rats. Oral administration of *A. conyzoides* aqueous extract caused important differences in body weight, serum levels of blood glucose, insulin and lipid profile as well as in the amount of liver glycogen and total serum protein.

The following of the blood glucose level over time in this experiment showed that *Ageratum conyzoides* leaves extract reduced plasma glucose concentration in STZ-diabetic rats. The present study also revealed a significant increase of serum insulin of diabetic rats after 3 weeks of treatment with the extract, at 200, 300 mg/kg and with glibenclamide, when compared to diabetic control. These results are in the same line with the observations of other authors [15], who reported that the mode of reduction in blood glucose (insulinomimetic or insulin secretagogues activity) could be due to some active phytoconstituents having insulin mimetics activity.

Since streptozotocin selectively destroys β-cells of the pancreas, we would expect the extract to exert no effect on blood glucose level in STZ diabetic rats, if the mode of action is mediated through insulin production. However the present results showed significantly elevated levels of insulin in diabetic rats treated with the extract, at the doses of 200, 300 mg/kg and glibenclamide; This suggest that the extract increased insulin production.

Ageratum conyzoides extract were significantly effective in lowering AUC-glucose curves of STZ-diabetic albino rats, and this relates with a significant increase of glycogen level at 200 and 300 mg/kg of the extract and 10 mg/kg of glibenclamide. This correlated also well with the observations of other authors [16], who demonstrated that glibenclamide is able to maintain prolonged increase in serum insulin. It binds to receptors on the surface of pancreatic β-cells; as a result, the cell membrane creates an influx of calcium ions and a subsequent release of insulin [17].

The present investigation also shows that daily administration of *A. conyzoides* extract at the doses of 200 mg/kg and 300 mg/kg body weight, as well as the tested dose level of 10 mg/kg of glibenclamide induced an increase in liver glycogen concentration; a process likely to have a positive impact on glucose homeostasis in diabetic rats. This effect is in accordance with the findings of many investigators [18, 19, 20], who attributed the increase in liver glycogen of diabetic treated rats with different plants extracts and glibenclamide to the increased insulin response, which in turn promotes conversion of inactive form of glycogen synthetase to the active form and enhances conversion of blood glucose into glycogen.

Those results taken together suggest that both pancreatic and extrapancreatic mechanism might be involved in its antidiabetic/antihyperglycemic action. However, the extrapancreatic actions could consist of (i) a stimulation of peripheral glucose utilization; (ii) an enhancement of glycolytic and glycogenic processes and/or (iii) a glycemia reduction through the inhibition of glucose intake [21].

The antidiabetic activity might be due to two major explanations [22]. First, by preventing the death of β-cells and/or second, it may permit recovery of partially destroyed β-cells. In our study, *Ageratum conyzoides* extract might have acted also if having the ability to release the insulin by the stimulation of a regeneration process and revitalization of the remaining ß-cells, like gymnemic acid molecules dihydroxy gymnemic triacetate from *Gymnema sylvestre*, as postulated before [23].

The hypoglycemic effect of *A. conyzoides* extract and glibenclamide was pronounced noticeably at the treatment extends. Many authors have previously reported hypoglycemic effect of different plants extracts [24, 25]. The hypoglycemic effect may also be due to the presence of insulin-like substance found in various plants [26]. Those results can mainly be attributed to the phytochemical constituents of the extract which possess antidiabetic activities. This hypoglycemic effect may be due to the presence of glycosides,

alkaloids, saponins, tannins, resins and triterpenes reported before [1]. Such components were showed to be responsible of the hypoglycemic action.

Our results revealed that treatment of STZ-diabetic rats with *A. conyzoides* extract at efficiency doses, or glibenclamide produced great improvement of the altered serum lipid variables. The ability of *A. conyzoides* extract in reducing serum lipids variable could be explained on the basis of insulin releasing capacity. Others results reported that the rate of lipogenesis is normalized by Cinnamon extract, due to insulinogenic effect on the lipid metabolism or it could be due to the achievement of normoglycemia where there was no further degradation of already accumulated lipid for otherwise glucose starved cells [27, 28].

Furthermore, the improvement with *A. conyzoides* administration is similar to another study, reporting marked decrease in lipid variables after treatment with *M. indica* and its polyphenol compound mangiferin which may be ascribed to lipid lowering activity of mangiferin or due to its influence on various lipid regulation systems [29]. This last hypothesis can be correlated in our study with the phytochemical polyphenol content of aqueous extract of *A. conyzoides*.

Lipids play a vital role in the pathogenesis of diabetes mellitus. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. In our study, we have noticed elevated levels of total cholesterol and triglycerides in non-treated diabetic rats. The levels of increased serum lipids in diabetes represent risk factor for coronary heart disease [30]. Under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triglycerides [31]. Insulin increases uptake of fatty acids into adipose tissue and increases triglyceride synthesis. Moreover, insulin inhibits lipolysis.

In case of insulin deficiency, lipolysis is not inhibited and we have increased lipolysis which finally leads to hyperlipidemia. In insulin-deficient diabetes, the concentration of serum free fatty acids is elevated as a result of free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification-triglyceride lipolysis cycle is displaced in favor of lipolysis [31]. HDL-Cholesterol is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissues into the liver and thereby acts as a protective factor against coronary heart disease. The level of HDL-Cholesterol, which increased after *A. conyzoides* administration, might be due to the increase in the activity of lecithin cholesterol acyl transferase, which may contribute to the regulation of blood lipids. Administration of *A. conyzoides* lowers triglycerides and to a lesser extent LDL Cholesterol level, increases the serum HDL-Cholesterol in diabetic rats. These results are in agreement with those of previous investigators [32].

Our results showed that the growth rate increase of body weight was dose-dependent. Only the doses of 200 and 300 mg/kg were able to maintain a significant growth of diabetic rats. This growth rate can be related also to the food intake amount of the rats. The increased in body weight of animals treated with the extract after 7 days is correlated with its stimulant effect on food intake.

The large volume in urine outputs by the animals administrated with 100 mg/kg explained the corresponding increase in water intake observed in the rats, and hence the diuretic activity of the plant extract exhibited by the rats treated with 100 mg/kg dosage. It has been documented that metabolic behaviours that regulate water and food intake are controlled by neural and physiological mechanisms [33]. The significant decrease of water intake volume in the treated groups suggests that the plant may have some effects on the neural and physiological mechanisms; though it is known that water intake and urine outputs are biologically dependent on many factors [34].

The increased urine output due at 100 mg/kg dosage may be associated with an impairment of water excretion as a result of kidney abnormality or the inappropriate secretion of antidiuretic hormone [35]. *A. conyzoides* was effective in preventing polyuria and polydepsia conditions during diabetes. The beneficial effect of *A. conyzoides* extract could also be attributed to its high fibre content [36].

It is well known that dietary fibres facilitate slow absorption of glucose along the passage through

gastrointestinal tract [37, 38]. The role of fermentation product of dietary fibres in the form of short chain fatty acids such as acetate, propionate and butyrate is also to be considered in the amelioration of diabetic status [39]. Some earlier studies already showed beneficial effects of butyric acid during diabetes [40].

A gradual return to normal levels of total serum protein in diabetic rats treated with the *A. conyzoides* extracts was dose-dependent: On the last day of the study, *Ageratum conyzoides* at doses of 200, 300 mg/kg and glibenclamide (10 mg/kg) increased total serum protein concentration by 26.89, 21.01 and 59.67 % respectively.

In diabetes mellitus, deranged glucagon-mediated regulation of cyclic AMP formation in insulin deficiency leads to accelerated proteolysis [41]. In addition, secondary hypoalbumenia commonly observed in diabetic patients is generally attributed to the nephrotoxicity and/ or may be due to increased protein catabolism [42].

The progressive restoration of total protein in the serum of STZ diabetic rats treated by *Ageratum conyzoides* extracts and glibenclamide may be due to inhibition of proteolytic activity as a result of increasing insulin secretion and proper utilization of blood glucose. Similar effects have also been reported by some workers on total protein and albumin concentrations [43]. In the same way, good correlation between protein synthesis and insulin level has been earlier recorded [19].

CONCLUSIONS: Our finding showed that oral administration of *A. conyzoides* produces significant antihyperglycemic effect, lowers both triglyceride levels and, at the same time, increases HDL-cholesterol in STZ-induced diabetic rats. This investigation reveals the potential of *A. conyzoides* for the use as a natural oral agent, with both antihyperglycemic and hypolipidemic effects. These observations taken together suggest that *A. conyzoides* leaves extract has a potential in the management of diabetes mellitus.

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