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In vivo assessment of antidiabetic activity and safety of polyherbal teas from selected Cameroonian medicinal plants: *Persea americana, Ageratum conyzoides,* and *Mangifera indica*

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Abstract

Background: Diabetes mellitus (DM) is a metabolic disorder of the endocrine system which results in persistently high blood glucose levels. Available control measures do not completely cure the disease and most patients in the tropics cannot afford diabetes drugs regularly. Amongst other plants, *Mangifera indica* (MI), *Persea americana* (PA), and *Ageratum conyzoides* (AC) are used individually as traditional medicine by most people for the management of diabetes. This work was set to produce polyherbal teas (water infusion) from these plants and evaluate *in vivo* antidiabetic activity and safety of the teas.

Methods: Three (3) monoherbal teas (MI, PA, AC) and 4 polyherbal of which 3 composed of two plants each (MI+PA, MI+AC, PA+AC) and one including all the plants (MI+PA+AC) were formulated. The antidiabetic activity was assessed by evaluating the hypoglycaemic activity of the teas by carrying out the oral glucose tolerance test (OGTT) in male Wistar albino rats and the subacute antidiabetic assay in streptozotocin-induced diabetic rats. Safety was evaluated using the *in vivo* acute toxicity test in mouse model.

Results: The OGTT revealed a significant drop in post-prandial glucose peak in rats treated with 20mg/kg monoherbal tea from Mangifera indica leaf (MI), as compared to the negative control (distilled water). The polyherbal tea of all three plants (AC+MI+PA) at a dose of 20mg/kg was able to return the glucose levels to normal 30mins after the glucose peak. The tea AC+MI+PA also exhibited a higher antidiabetic activity as it produced a 60% glycemia recovery rate compared to the reference drug glibenclamide and monoherbal tea MI with 40% and 20% recovery rate respectively. Polyherbal tea and MI exhibited hepato-protective and nephron-protective activities. The *in vivo* acute toxicity test showed that the teas were not toxic as there was no record of mortality and adverse effects in physical appearance, behavior, change in body weight, water, and food intake.

Conclusion: Out of the seven teas produced, two showed promising antidiabetic potential, with both acute hypoglycemic effects and significant subacute efficacy. Further investigations are envisaged to pursue the development of improved alternative therapies from these, to address the enduring challenge of diabetes, particularly in resource-limited settings.

Keywords: Diabetes mellitus; antidiabetic; activity; polyherbal tea.

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Background

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system which occurs worldwide, and its incidence is rapidly increasing in most parts of the world [1]. It is caused by inherited and/or acquired deficiency in the production of insulin by beta cells of the pancreas, or by the ineffectiveness of the insulin produced. This dysfunction results in persistently high blood glucose alongside other physiological disorders. The heterogeneous etiology of diabetes also includes disturbances in carbohydrate, fat, and protein metabolism [2]. The etiological classification of diabetes has now been widely accepted, with type 1 and type 2 diabetes being the two main types of diabetes, and type 2 diabetes accounting for around 90% of all diabetes worldwide [3]. The number of diabetes patients is increasing worldwide, with 463 million patients between 20-79 years as of 2019. This incidence is expected to double in the years ahead according to the International Diabetes Federation (IDF) [3-4]. In Cameroon, over 615,000 adults suffer from diabetes within 20-79 years age group, and related deaths recorded to be 13,744 as of 2019 [3]. Some people with type 1 diabetes receive daily insulin injections to maintain their glucose level in the appropriate range. Type 2 diabetes is managed by the administration of anti-diabetic drugs (such as metformin and amylin analogs) and the adoption of a lifestyle that includes a healthy diet, regular physical activity, smoking cessation, and maintenance of healthy body weight [4]. However, these diabetic drugs have side effects (such as nausea and diarrhea), do not completely cure diabetes and cannot be afforded by most people regularly. Research has been carried out on some plants, amongst which the leaves of *Persea americana* (Lauraceae), *Ageratum conyzoides* (Asteraceae) and *Mangifera indica* (Anacardiaceae) have been found to possess anti-diabetic activities but remain under exploited as anti-diabetics. *P. americana* commonly known as Avocado tree is an evergreen medium to large tree, with a canopy ranging from low, dense, and symmetrical to upright and asymmetrical. *P. america* is a multipurpose food and medicinal plant widely used in Cameroonian fold medicine and in cosmetics to address several health problems: hair loss, fever, diabetes, wound healing, etc. [5-14]. Pharmacological potentials of different parts of *M. indica* trees have been extensively demonstrated, these include anticancer, anti-inflammatory, antidiabetic, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial and antihyperlipidemic effects [15]. *Ageratum conyzoides* is an annual herb with a long history of use in folk medicine in many parts of the world including Africa and Cameroon. Some widespread medicinal benefits apply to diseases such as arthrosis, headache, dyspnea, common wounds and burns, and diverse microbial infections [16]. This study aimed to formulate polyherbal teas from these plants with anti-diabetic activities using their leaves to see if the combination of these plants will produce a higher efficacy.

Methods

Chemicals and reagents

Streptozotocin, glibenclamide (Glib), glucose, chloroform, and other laboratory consumables were purchased from Sigma-Aldrich (Germany). The different kits for biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (γ-GT), creatinine, uric acid, and glucose, were bought from ChronoLab® (Barcelona, Spain).

Plant materials and identifications

Matured fresh leaves of PA, AC, and MI were harvested on the University of Buea campus, Buea, the capital of the Southwest Region of Cameroon. The plant identification was confirmed by the Limbe Botanic Garden where voucher specimens were submitted. Upon collection, leaves were air-dried in a shade for two weeks after identification and ground into powder using a blender.

Preparation of teas and dose determination

Following the traditional mode of preparation of remedies from the selected plants, an amount of 10g of dried leaves from each plant which is corresponding to two teaspoons was used to prepare monoherbal teas by infusion in 200 ml of hot water (at 100^{o}C) for 5 mins. Equal amounts each (5g of the powdered leaves of each plant for the combination of two plants, and 3.33g of the powdered leaves of each plant for the combination of all three plants) of dried leaves of the plants were added to form combinations which were used to prepare polyherbal teas by infusing each combination in 200 ml of hot water for 5mins. To determine the various doses (mg extract/kg body weight), the three monoherbal teas and four polyherbal teas (200ml) prepared were put one at a time in an oven and evaporated to dryness at a temperature of 40^{o}C for 72 hours. The dry matter of each tea obtained after evaporation was weighed to reflect the amount represented in 200mL. Considering the volume administered to animals based on their body weight, the doses in mg/Kg body weight were then derived as 20mg/kg for the teas MI, PA, AC+MI+PA, and MI+PA, while AC, PA+AC, and MI+AC had a dose of 25mg/kg.

Experimental animals

Male Wistar albino rats (*Rattus norvegicus*) were obtained from the breeding house of the Medical Research and Applied Biochemistry Laboratory (MRABL), University of Buea. Male animals were preferred for both acute and subacute antidiabetic testing due to the susceptibility of males to diabetes, as compared to females, but also the high hormonal (estrogen) variability in females which also affect energy metabolism and diabetes outcome [17]. The male and female Balb/c mice used for the acute toxicity assay were obtained from the breeding house of the Biotechnology Unit, University of Buea. Animals were handled according to the ethical guidelines of the Institutional Animal Care and Use Committee of the University of Buea (Authorization Number: *UB-IACUC N^{o} 012/2020*).

Oral glucose tolerance test (OGTT) in non-diabetic rats

The hypoglycemic and antihyperglycemic activities of the teas were determined by carrying out the oral glucose tolerance test (OGTT). Blood glucose levels will rise after a meal, because of absorption of glucose from the meal or oral glucose load. This postprandial hyperglycemia is reversed by regulatory mechanisms of the organism generally within 2-4 hours [18]. The main objective of the OGTT was to investigate the ability of the different teas to stimulate regulations/reduction of blood sugar levels, in non-diabetic subjects. A total of 45 male Wistar albino rats were divided into 9 groups of 5 animals each.

The animals were fasted overnight prior to the commencement of the test (day 0). The tail of each animal was gently massaged to increase blood flow and pricked using a lancet at the tip of the tail to collect a drop of blood. The blood sample collected migrated by capillary action when applied on a glucose strip already inserted in a glucometer (CodeFree®, SD Biosensor, South Korea). The fasting blood sugar (FBS) level was measured, and the time was recorded as $t_{-30mins}$. Immediately after FBS measurement, animals were orally administered corresponding products (tea extract for the test groups, glibenclamide for the positive control, and distilled water for the negative control group). After 30 mins, fasting blood sugar levels of the animals were again measured (time recorded as t_{0mins}) and all the animals were then given an oral dose of glucose (2 g/kg body weight). The fasting blood sugar levels of the animals were then further determined after 30 mins, 60 mins, 90 mins, 120 mins, and 240 mins (referred to as $t_{+30\ min}$, $t_{+60\ min}$, $t_{+90\ min}$, $t_{+120\ min}$ and $t_{+240\ min}$).

Subacute antidiabetic activity evaluation of the teas in diabetic experimental rats

The antidiabetic activities of selected teas with significant hypoglycaemic and antihyperglycaemic activities after the OGTT (monoherbal tea from *M. indica* and polyherbal tea from a combination of all three plants) were evaluated for sub-acute antidiabetic activities. A total of 20 diabetic rats were divided into 4 groups of 5 animals each, having statistically similar average fasting blood sugar levels on day 0. The groups were assigned different treatments as follows: Group I, positive control (Glibenclamide 3 mg/kg/day); Group II, negative control (distilled water); Group III, *M. indica* tea and Group IV, a combination of *P. americana, A. conyzoides and M. indica* tea. The different groups received food and water *ad libitum* and the different treatments were administered daily, for 20 consecutive days.
The antidiabetic activity was determined based on both clinical markers (change in food and water intake, urine elimination, and change in body weight) and the main biochemical indicator (fasting blood sugar levels). The volume of urine eliminated, food, and water consumed were measured daily. Body weight of the animals in the various groups was measured on days 0, 7, 14, and 20. The animals were also monitored for any behavioral changes. Fasting blood sugar level was measured on day 0 (before drug administration), day 6, day 10, day 14, and day 20 (end of the treatment period) to track changes in glucose levels. Also, the effect of the different treatments was assessed on the functions of the liver (ALT, AST, ALP, and γ-GT activities) and kidney (creatinine and urea levels) which are affected by diabetes. At the end of the treatment period, the rats were sacrificed, and blood samples were collected through cardiac puncture into tubes. The pancreas, liver, and kidney were equally dissected and weighed.

In vivo acute toxicity assessment of the teas

The acute toxicity of the 2 selected teas with more promising antidiabetic effects was assessed using both adult male and female Balb/C mice, according to Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals as previously implemented [18]. The animals of each sex were randomly divided into 3 groups of 5 each, making a total of 6 groups which were kept in their respective cages for 5-days acclimatization. For each sex, animals in the test groups received either the polyherbal tea (AC+MI+PA) or monoherbal tea (MI) at a dose of 2000 mg/kg, while the other animals in the control group were administered the vehicle (distilled water). The teas and vehicle were administered orally using a gavage needle. It should be noted that the animals were fasted 4 hours prior to administration and 2 hours after administration with teas or vehicles, while water was made available throughout the experiment. The mice were observed for 4 hours following administration of teas/vehicle and periodically every day for 14 days, for detection of lethality and any change in physical appearance, as well as behavioral alterations including activeness or lethargy, tremors, and convulsions. Body weight, food, and water consumed were measured on days 7 and 14.

Results

Hypoglycemic effect of teas in OGTT

The acute hypoglycemic effect of the teas was evaluated in rats using OGTT and the results are presented in Table 1 and Figure 1. Monoherbal teas from MI, PA, and AC produced lower post-prandial peaks. However, only MI generated a significantly lower peak ($p= 0.07$) compared to the negative control. Monoherbal tea MI, polyherbal tea AC+MI+PA and the reference drug Glib returned the glucose levels back to normal just 30 mins after the glucose peak, i.e., 60 mins following oral glucose load, and sustained this effect throughout the experimental time (240mins). The positive control group, PA, AC, and MI produced higher post-prandial glucose peak suppression compared to the negative control group. The positive control group and the teas MI and AC+MI+PA produced significantly lower FBS levels, compared to the negative control group.

Subacute antidiabetic activity evaluation of the teas in diabetic experimental rats

The findings on the sub-acute antidiabetic effects of the selected teas with antihyperglycemic activities (MI, MI+AC+PA) are presented based on evolution in body weight, food, and water intake, urine elimination, FBS, lipid profile as well as the biomarkers of liver and kidney functions.

Effect of the different treatments on clinical markers

From this study, it was observed that the quantity of food consumed decreased in all the groups throughout the experiment. The positive control group (Glib) and the test groups (MI and AC+MI+PA) caused a significant decrease ($p<0.05$) in food intake compared to the negative control group. Similarly, there was a decrease in water consumption in all the groups throughout the experiment. The test groups showed the highest decrease in the volume of water consumed compared to the negative control group (distilled water). The volume of urine eliminated throughout the experiment decreased in all the groups. The test groups excreted significantly lower ($p<0.05$) volumes of urine compared to the negative control group.

Effects of the teas on FBS in STZ-induced diabetic rats

From Figure 2, the mean FBS levels decreased in all the groups from day 0 to day 10 but there was no significant difference between the test groups, positive and negative control groups. From day 10 to day 14, there was an increase in FBS levels in all the groups. However, mean FBS levels of animals receiving MI and AC+MI+PA were significantly lower compared to that of the negative control group ($p<0.05$) and were not significantly different

from the positive control group (Glib). On day 20, no significant difference in FBS levels was recorded between the test groups and the control groups.

Recovery rates from STZ-induced diabetes

There was no death record in groups which received the monoherbal tea, MI and polyherbal tea, AC+MI+PA whereas 60% and 20% of the animals died in the negative control and positive control groups respectively. AC+MI+PA caused 60% of its animals to recover compared to the positive control group which had 40% recovery and MI with 20% recovery rate (Figure 3).

Effects of the different treatments on the liver and kidney functions

Figure 4 shows a significant increase in the relative weight of the liver was observed in the group MI compared to the normoglycemic group. However, the relative weight of the liver of the group MI was not significantly different from AC+MI+PA, negative and positive control groups. No significant difference was observed between AC+MI+PA, normoglycemic group, negative and positive control groups.

Serum ALT activity in the negative control group (Distilled water) and MI were significantly higher than that of the normoglycemic group (non-diabetic rats). However, ALT activity in the group of diabetic rats treated with MI was significantly lower than that of the negative control group whereas it was not significantly different from the positive control group (Glib). The group AC+MI+PA caused a significant reduction in serum ALT activity compared to the negative and positive control groups, whereas it was not significantly different from the normoglycemic group. The positive control group produced significantly lower ALT activity compared to the negative control group.

A significant increase in AST activity was observed in the negative and positive control diabetic rats compared to the normoglycemic group. No significant difference in AST activity was observed between the test groups; MI and AC+MI+PA, and the normoglycemic group. The monoherbal tea, MI, produced a significantly lower AST activity compared to the negative control group. There was no significant difference in AST activity in the group which received AC+MI+PA and the positive control group compared to the negative control group.

There was no significant difference in γ-GT activity between the diabetic groups and the normoglycemic group. The γ-GT activity of the positive control, MI, and AC+MI+PA groups was not significantly different from γ-GT activity of the negative control group. From Figure 5, the relative weight of the kidneys in the groups; negative control, MI, and AC+MI+PA were significantly different compared to the normoglycemic group. The positive control group was not significantly different from the negative control, MI, AC+MI+PA and normoglycemic group. There was no significant difference between the test groups; MI and AC+MI+PA compared to the negative control group. No significant difference in serum creatinine and urea levels was observed in the test groups; MI and AC+MI+PA, the controls; positive and negative control groups compared to the normoglycemic group. There was no significant difference in serum creatinine and urea levels in the groups MI, AC+MI+PA, and Glib compared to the negative control group.

Acute toxicity in mice

Effect of teas on bodyweight in mice

Findings from the acute toxicity study revealed that there was a general increase in body weight in males for both the control group and the test groups (MI, AC+MI+PA) from day 0 to day 7, where the increase was highest in the group which received AC+MI+PA. Body weight decreased in all the groups from day 7 to day 14, especially in the control group. However, there was no significant difference between the test groups and the control group on days 7 and 14 with p value ≥ 0.05. In females, a similar trend was observed from day 0 to day 7, as there was a general increase in body weight both in the test groups (MI and AC+MI+PA) and the control group. From day 7 to day 14, there was an increase in body weight in the control group and in the test group which received AC+MI+PA while the body weight remained constant in animals that received MI. However, there was no significant difference in body weight between the control group and the test groups with p-values of 0.342 and 0.366 on days 7 and 14, respectively.

Effect of teas on food consumption in mice

There was a general increase in food intake in all the groups in males. The test groups (MI and AC+MI+PA) showed a significantly increased amount of food consumed compared to the control group. Generally, there was an increase in food consumption in all the groups from day 7 to 14 in females. The test groups (MI and AC+MI+PA) were not significantly different from the control group.

Effects of teas on water consumption in mice

The volume of water intake increased in all the groups in males. However, no significant difference was observed between the test groups; MI and AC+MI+PA, and the control group, p ≥ 0.05. Water intake generally increased in females across the groups, with the increase more pronounced in the group which received MI. However, no significant difference was observed between the control group and the test groups.

Physical appearance and mortality

No loss of fur was observed in any of the groups and none of the groups had a change in fur and eye color. No abnormal secretions from the eyes, nose, and genitals were observed. Movements and reactivity to stimuli were assessed by agility. Animals in control and test groups moved with ease and all the animals involved survived through the experiment.

Discussion

Diabetes mellitus has remained an enduring public health challenge for centuries. To date, despite considerable efforts worldwide, curative treatment is yet to be officially established. The present work aimed to formulate polyherbal teas from the leaves of these plants and assess the antidiabetic effectiveness using Oral Glucose Tolerance Test (OGTT) and Streptozotocin-induced diabetes in male Wistar Albino rats (*Rattus norvegicus*). The study was conducted in three phases: (i) Preliminary screening of the three monoherbal and four polyherbal teas (water infusion) from leaves of the three selected plants; (ii) Subacute antidiabetic determination of the two most promising teas (MI and AC+MI+PA) in streptozotocin-induced diabetic male Wistar albino rats; (iii)

Safety evaluation (acute toxicity) of the teas with promising hypoglycemic activity in male and female Balb/c mice. The main feature of diabetes mellitus is persistent hyperglycemia even during fasting and intolerance to glucose or carbohydrate intake. Preliminary hypoglycemic and antihyperglycemic activities of the teas formulated were assessed by carrying out the OGTT. The aim of this step was to evaluate the ability of the different teas to stimulate glucose uptake and storage. The results obtained revealed a significant reduction in the post prandial glucose peak in rats receiving monoherbal tea (MI) and the reference drug Glib as compared to the negative control which received distilled water. This result following a slight drop in glycemia 30mins after administration of the tea or reference drug (Glib) may highlight the substantial hypoglycemic effect or antihyperglycemic properties of the MI tea. On the contrary, the peaks were rather more pronounced in groups receiving MI and AC (MI+AC), PA and AC (PA+AC), and MI and PA (MI+PA) combination teas. Combining the active MI with AC or PA compromises MI hypoglycemic effect. This could reflect antagonism between constituents of these teas. As such, combinations of MI with PA or AC should be strongly discouraged based on the present findings. Just 30 mins after the glucose peak, the initial glycemia was already re-established in animals receiving Glib, MI, and AC+MI+PA, unlike the other groups. These findings corroborate previous studies by Khan et *al.* [19] who demonstrated that aqueous extracts of the leaves of MI possess hypoglycemic activity comparable to chloropropamide. These authors hypothesized that MI might act by reducing glucose absorption in the intestine. However, this hypothesis requires confirmatory studies. Other parts of MI have equally been exploited for hypoglycemic properties, including seed kernels for which the antidiabetic activity of both the aqueous and methanolic extracts was reported [20]. The fact that present results confirm previous findings, might be a good justification for the common use of MI tea for diabetes management in several communities in Cameroon. In summary, this preliminary hypoglycemic screening using the OGTT assay reveal promising hypoglycemic activity in the monoherbal MI and polyherbal AC+MI+PA teas which were retained for further subacute antidiabetic investigations.

Subacute antidiabetic activity of the two most promising teas, MI, and AC+MI+PA in streptozotocin-induced diabetic rat model was determined. The aim of this step was to determine the ability of these teas to return and maintain glucose levels within the normal range.

The hypoglycemic and antihyperglycemic effects of MI and AC+MI+PA were later confirmed by a significant recovery from STZ-induced diabetes in rats as reflected in the food and water intake, body weight, and urine elimination. Because polyuria and polydipsia are some of the major characteristics of diabetes mellitus, a decrease in water intake and urine elimination can be interpreted as a result of recovery from the disease in animals which were treated with MI and AC+MI+PA.

Based on FBS levels, results obtained show a significant reduction in the mean FBS levels of rats receiving AC+MI+PA (polyherbal tea), MI (monoherbal tea), and the reference drug (Glib) compared to the negative control which received distilled water. The negative control group lost a total of three animals. Two of the animals with highest FBS levels (487 and 476 mg/dL) died on day 16 leaving two animals with FBS levels (169 and 297 mg/dL). This explains the drop in FBS levels in the negative control group on day 20, though the two rats left in the group had slightly higher FBS levels than the treated groups. These results were complimented by the fact that over 60% of the animals in the negative control group died with 0% recovery. There was no significant difference between the test groups (MI and AC+MI+PA) and the positive control group. These results show that MI and AC+MI+PA teas can demonstrate similar recovery from STZ-induced diabetes in animals like the reference drug (Glib). However, percentage recovery (60%) was highest with the polyherbal tea (AC+MI+PA) compared to the reference drug (Glib) and monoherbal tea (MI) with 40% and 20% recovery respectively. Several studies have reported the hypoglycemic effects of MI in rat models [21-22]. It is suggested that the decrease in fasting blood sugar might be explained by the presence of phytochemicals such as flavonoids, tannins, steroids, terpenoids, and phenols. Also, Reddeman *et al.* [23] reported that hydro-alcoholic mixture of *Mangifera indica* leaves exhibited a decrease in postprandial blood glucose following seven days of therapy in alloxan-induced diabetic mice. The study suggested that the leaf extract of the plant possesses antidiabetic activity possibly due to the presence of mangiferin and other phytochemicals such as phenolic and flavonoid compounds. Combining MI, PA, and AC was shown to improve the activity, which might be an indication of the synergistic effects of constituents of the three plants. In fact, *Persea americana* also contains phytochemical components such as flavonoids, tannins, saponins, phenolics, and alkaloids [14].

The enzyme ALT is produced specifically in the liver. It catalyzes the reversible transfer of an amino acid group from alanine to α-ketoglutarate to form glutamate and pyruvate. The group which received MI had significantly lower ALT and AST, but similar γ-GT levels compared to the negative control group. This implies that MI prevented liver damage as ALT did not leak out of the liver. These results are confirmed by those reported previously [24], according to which MI aqueous extract (400 mg/kg) had no toxic effect on the liver as AST, ALP, and ALT were seen in lower concentrations. The polyherbal tea caused a significant reduction in serum ALT levels compared to the negative and positive control groups. However, similar ALT, AST, and γ-GT levels were observed between the polyherbal tea and the normoglycemic group, suggesting the absence of any hepatotoxicity induced by the tea.

The serum levels of creatinine and urea in groups that received MI and AC+MI+PA were similar to those of the negative control group. This implies that the teas did not impair kidney function. Results are confirmed by those reported by Amrita *et al.* [25], and Lucka *et al.* [26] which showed that the methanol extract of MI reduced the level of nephrotic markers in a significant manner.

Results from the *in vivo* acute toxicity assessment shows that the test groups (polyherbal and monoherbal teas) were not toxic as there was no record of mortality in the control nor in the test groups (MI and AC+MI+PA) both in the male and female Balb/c mice. Also, the teas (MI and AC+MI+PA) did not induce any sign of toxicity in terms of change in body weight, water, and food intake, as well as altered behavior. These results are in line with those obtained by Sudha *et al.* [24] as they established that aqueous and ethanol extracts of *Mangifera indica* produced no toxic effect after a 90-day repeated dose oral toxicity in rats. The no observable adverse effect dose was determined to be at least 2000mg/kg bwt/day, which is about 100fold of the therapeutic doses tested. This result might indicate the suitability of the teas MI and AC+MI+PA for use as safe therapies against type 2 diabetes mellitus.

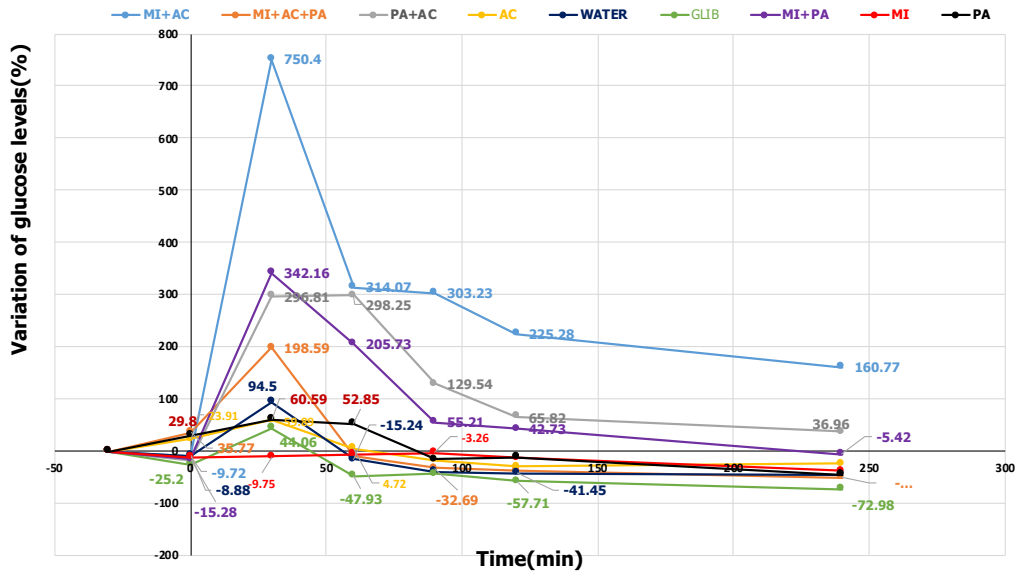

Figure 1. Variations of FBS in different treatments, polyherbal teas and negative control (A) and monoherbal teas, reference drug and negative control (B) during oral glucose tolerance test in healthy male rats**.**

Negative control (water): Distilled water; Positive control (Glib): Glibenclamide; MI+AC (25mg/kg): Extract combination of *M. indica* leaves and *A. conyzoides* leaves; PA+AC (25mg/kg): Extract combination of *P. americana* leaves and *A. conyzoides* leaves*;* MI+PA (20mg/kg): Extract combination of *M. indica* leaves *and P. americana* leaves*; MI* (20mg/kg): Extract of *M. indica* leaves*; PA* (20mg/kg)*:* Extract of *P. americana* leaves; AC+MI+PA (20mg/kg): Extract combination of *A. conyzoides* leaves, *M. indica* leaves and *P. americana* leaves; AC (25mg/kg): Extract of *A. conyzoides* leaves*.*

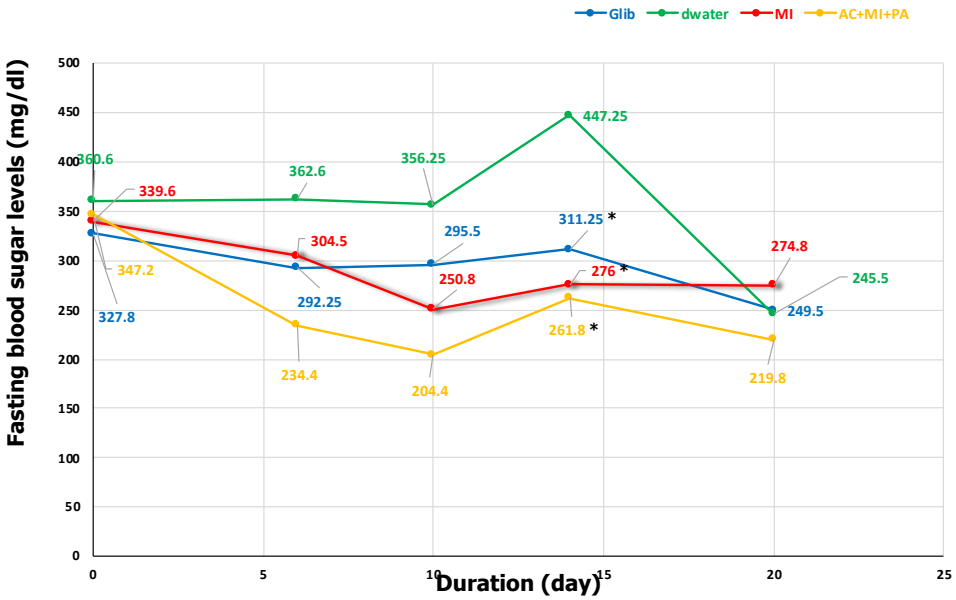

Figure 2: Variation in mean fasting blood sugar levels in diabetic rats

AC+MI+PA (20mg/kg): Extract combination of *A. conyzoides* leaves, *M. indica* leaves and *P. americana* leaves; *MI* (20mg/kg): Extract of *M. indica* leaves; Glib(3mg/kg): Glibenclamide; dwater: Distilled water. ***Note*:** The two animals with the highest glycaemia died between day 14 and day 20 in the negative control group, so only two animals were left in the group.

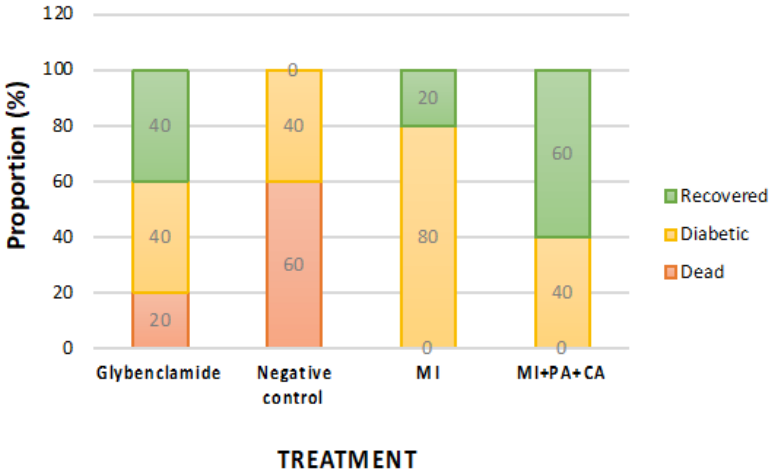

Figure 3. Recovery rate of diabetic rats based on survival.
AC+MI+PA (20mg/kg): Extract combination of *A. conyzoides* leaves, *M. indica* leaves and *P. americana* leaves; *MI* (20mg/kg): Extract of *M. indica* leaves; Glib(3mg/kg): Glibenclamide; dwater: Distilled water.

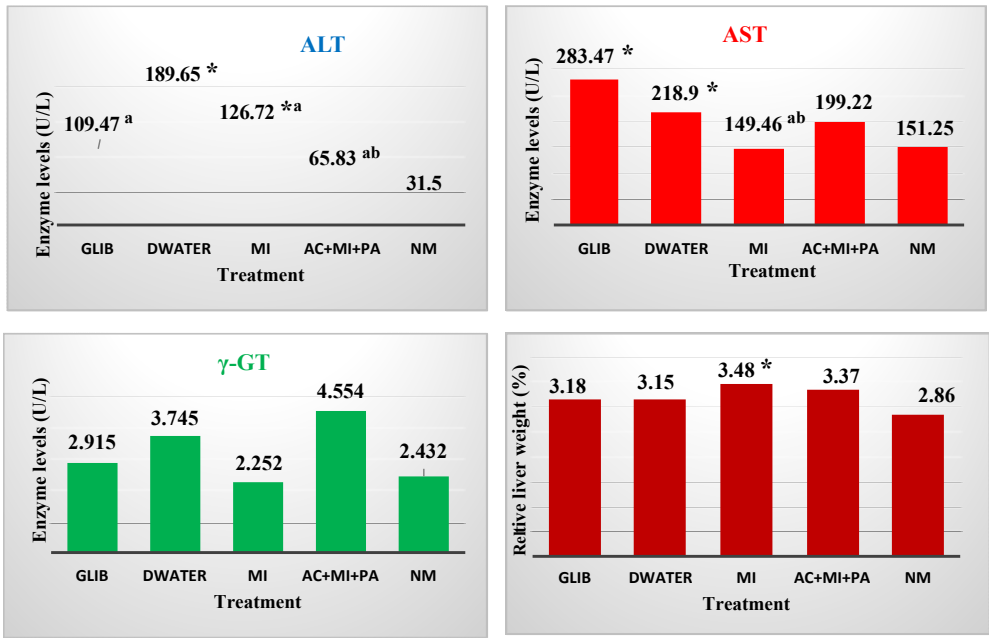

Figure 4. Effects of selected teas on liver function
(A) AST levels (B) ALT levels (C) γ-GT levels (D) Relative liver weight; AC+MI+PA (20mg/kg): Extract combination of A. conyzoides leaves, M. indica leaves and P. americana leaves; MI (20mg/kg): Extract of M. indica leaves; Glib: Glibenclamide (3mg/kg); dwater: Distilled water (Negative control); Normoglycaemic: Non-diabetic rats. *Significantly higher than the normoglycaemic group, p≤0.05. [a] significantly lower than the negative control group, p≤0.05. [b] significantly lower than the positive control group, p≤0.05.

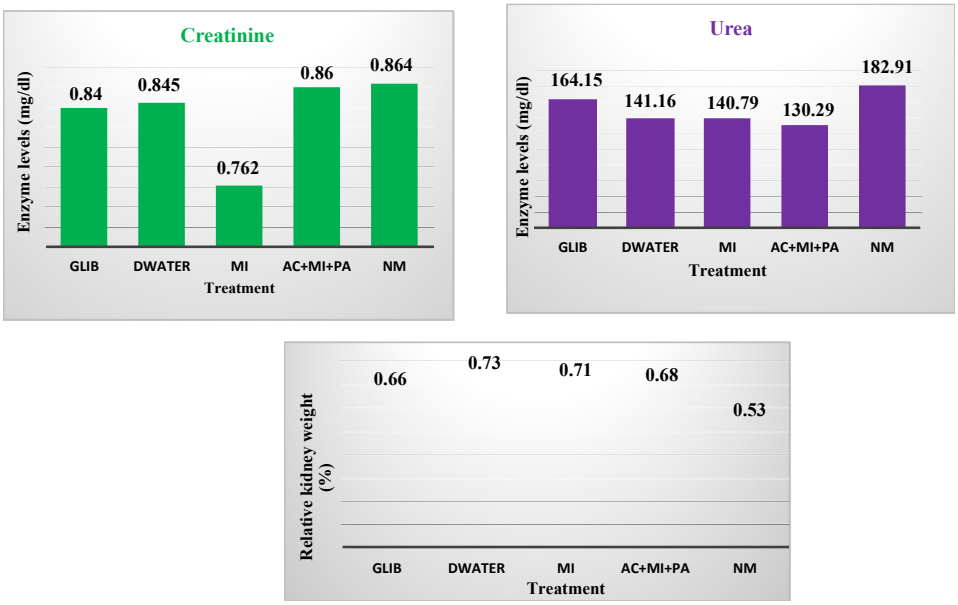

Figure 5. Effects of the selected teas on kidney function.
(A) Creatinine concentration (mg/dl) (B) Urea concentration (mg/dl) (C)Relative kidney weight (%); AC+MI+PA (20mg/kg): Extract combination of *A. conyzoides* leaves, *M. indica* leaves and *P. americana* leaves; *MI* (20mg/kg): Extract of *M. indica* leaves; Glib(3mg/kg): Glibenclamide; dwater: Distilled water; Normoglycaemic: Non-diabetic rats. *Significantly different p≤0.05.

Table 1. Percentage post prandial glucose peak suppression and restoration time in different treatment

Parameter	Dwater (negative control)	**Treatment**							
		MI	AC+MI+PA	Glib positive control)	MI+PA	MI+AC	PA+AC	PA	AC
Percentage peak suppression (%)	0	-110.32*	110.15	-53.38*	262.07	694.07	214.08	-35.88*	-36.62*
Glucose restoration time (mins)	60	+30[a]	+60[a]	+60[a]	240	X	X	90	90

AC+MI+PA (20mg/kg): Extract combination of *A. conyzoides* leaves, *M. indica* leaves and *P. americana* leaves; *MI* (20mg/kg): Extract of *M. indica* leaves; Glib(3mg/kg): Glibenclamide; dwater: Distilled water; Normoglycaemic: Non-diabetic rats. *Significantly difference from the normoglycaemic group, p≤0.05. [a]The group is significantly different from the negative control group, p≤0.05.

Conclusion

The polyherbal tea (AC+MI+PA) and monoherbal tea (MI) showed significant antidiabetic efficacy, at the dose of 20 mg/kg/day, with the polyherbal exhibiting the highest recovery rate (60%) in the STZ-rat model and no dead, above glibenclamide. Both AC+MI+PA and MI formulations improved the liver and kidney functions, as well as the lipid profile in diabetic animals. In addition, these two formulations show no significant acute toxicity in mice. Further investigations on the mechanism of action of MI and AC+MI+PA, as well as the preparation of improved traditional medicines from these plants are envisaged.

Abbreviations

AC: *Argeratum conyzoides* leaf extract (20mg/kg)
AC+MI+PA: Extract combination of *A. conyzoides* leaf, *M. indica* leaf and *P. americana* leaf (20mg/kg)
MI: Extract of *Mangifera indica* leaf extract (20mg/kg)
PA: *Persea americana* leaf extract (20mg/kg)
Glib: Glibenclamide (3mg/kg)
dwater: Distilled water (Negative control)
DM: Diabetes Mellitus

Authors' Contribution

DZ conceived the work, oversaw the laboratory work, and edited the manuscript; GS and EEM carried out the bench work and took part in drafting the manuscript; FPTM, DDFS, AUA; CTM, J-CGA and JFT participated in the supervision of bench and edited the manuscript. All the authors approved the last version of the work and its submission to the journal.

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Conflict of interest

The authors declare no conflict of interest.

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