Asian Journal of Research in Biochemistry

Volume 15, Issue 1, Page 1-8, 2025; Article no.AJRB.129175
ISSN: 2582-0516

Effects of the Combination of Aqueous Extracts of *Vernonia amygdalina* and *Tamarindus indica* on Digestive Enzymes and Hematological Profile of Streptozotocin-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrb/2025/v15i1345

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/129175

Original Research Article

Received: 28/10/2024
Accepted: 30/12/2024
Published: 01/01/2025

ABSTRACT

Aims: Many strategies have been put in place to stop diabetes and its related complications: the use of plants has proved to be less costly and with few side-effects. Among these plants *Vernonia amygdalina* and *Tamarindus indica* are used because of their richness in bioactive compounds, notably phenols and dietary fiber. The aim of this work was first to evaluate the effect of combining

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Cite as: *Amel, Tekou Florian, Kemtsop Michel Pegui, Kuate Dieudonné, and Dzoyem Jean Paul. 2025. "Effects of the Combination of Aqueous Extracts of Vernonia Amygdalina and Tamarindus Indica on Digestive Enzymes and Hematological Profile of Streptozotocin-Induced Diabetic Rats". Asian Journal of Research in Biochemistry 15 (1):1-8. https://doi.org/10.9734/ajrb/2025/v15i1345.*

aqueous extracts of *Vernonia amygdalina* leaves and *Tamarindus indica pulp* on two energy metabolism enzymes (alphaamylase and pancreatic lipase), and secondly to assess the effect of combining these extracts on the hematological profile of streptozotocin-induced diabetic *wistar* rats.
Methodology: *Vernonia amygdalina* leaves and *Tamarindus indica* pulp was subject to aqueous extraction, the two extracts obtained were mixed *in vitro* in the presence of the two enzymes and their inhibitory power evaluated; streptozotocin was used to induce diabetes in *Wistar* rats which were then treated with our extracts and the combination of these; their blood was collected and hematological analysis carried out.
Results: Our findings revealed that at 100 mg/l, the mixture showed a 23.56±4.6% inhibition of alphaamylase. The percentage inhibition of pancreatic lipase varied depending on the extract concentration from 8.81±1.90 at 62.5µg/ml to 31.95±1.90 at 1000µg/ml, and the mixture also improved hematological parameters in diabetic rats.
Conclusions: combining these two extracts makes it possible to increase the inhibitory power with respect to these enzymes and improves the hematological profile, this could therefore be beneficial for the non drug management of diabetes.

Keywords: Type 2 diabetes mellitus; Vernonia amydalina; Tamrindus indica; α-amylase; pancreatic lipase.

1. INTRODUCTION

Everywhere in the world today, we can observe an increase in the prevalence of non-communicable diseases including high blood pressure, obesity and diabetes. The World Health Organization defines diabetes as a disorder that occurs when a person's body is unable to produce a hormone called insulin, or when the body does not properly use the insulin it produces (World Health Organization, 2016). This disorder results in abnormally high blood sugar levels, and the clinical diagnosis is based on fasting blood sugar levels above 1.26 g/l on two occasions. Diabetes also leads to anemia and immunosuppression, manifested by disturbances in the hematological profile of patients (Pretorius et al., 2015). Lack of insulin production leads to type 1diabetes, while bad utilization of the insulin produced by the body is responsible for type 2 diabetes, the most common type with over 80% of diagnosed cases. Type 2 diabetes appears as a complication of obesity, to which it is closely linked. From 108 million in 1980, the number of people suffering from diabetes has risen to 422 million in 2014, and the International Diabetes Federation predicts over 629 million diabetics worldwide by 2045 if the current rate of growth continues, representing an overall increase of 54% (International Diabetes Federation, 2019). These alarming figures make diabetes a real problem. The first weapons used to combat diabetes were essentially synthetic drugs. Although the modes of action are different, these drugs have helped in common to induce a reduction in glycemia and improve patients' physiological parameters. These drugs include insulin secretors, biguanides, α-glucosidase inhibitors, thiazolidine-diones and insulin therapy; however, diabetic patients face other problems such as flatulence, acidosis, diarrhea, prompting researchers to look to other sources of molecules with anti-diabetic activity that would be less costly, easily accessible and have few or no side effects. Plants and functional foods would be a good alternative, acting in several different ways. For example, work by Nivesh et al. (2020) revealed that the aqueous extract of *Tamarindus indica* pulp exerts its anti-diabetic potential by acting as an inhibitor of pancreatic α-amylase and lipase; in 2020, Waldemar et al. (2020) showed that ethanolic extracts of *Vernoniaamygdalina* were able to protect the pancreas. Hoang et al. (2019) reported that saponins extracted from the leaves of *Vernonia amygdalina* were able to act as α-aamylase and alpha glucosidase inhibitors. Ouédraogo et al. (2020) showed that *Tamarindus indica* was able to act as an alphaamylase inhibitor. However, it is important to note that few scientists have been the effect the combination of these two plants on lipids and carbohydrate metabolism. The present work was therefore undertaken with the aim of evaluating the inhibitory potential of the combination of aqueous extracts of *Vernoniaamygdalina* and *Tamarindusindica* for anti-diabetic activity.

2. MATERIALS AND METHODS

2.1 Chemicals

Alpha amylase, pancreatic lipase and, streptozotocin were purchased from Sigma-

Aldrich, Germany. Many other chemicals and reagents such as olive oil, phosphate buffer, cacuim chloride, sodium hydroxide were used in this study. The extracts and reagents were prepared on the day of the experiments. Streptozotocin as a disease inducer was given to the rats intraperitoneally (ip) while the extracts and the extract mixture were administrated by oral gavage.

2.2 Plant Materials

Vernonia amygdalina leaves were collected in the West region of Cameroon and *Tamarindus indica* fruit samples were collected in the North Cameroon region. These samples were identified in the national herbarium and sent to the Research Unit of Biochemistry, Medicinal Plants, Food Sciences and Nutrition (RUBPMAN laboratory of the Biochemistry)

2.3 Extraction

Aqueous extract of *Vernoniaamygdalina* leaves and *Tamarindus indica* pulps were obtained according to the method described by Razali et al*.* (2012) by maceration of powders (10g) of samples into 100 ml of water for 24 hours with gentle stirring, after which the mixtures were filtered using a Whatman N°1 filter paper. The resulting filtrates were dried at 45 ˚C in an air oven to obtain the aqueous extract. The extracts were weighed to calculate the yield, then a mixture of extracts was made using 1/3 extract of*Vernoniaamygdalina* leaves and 2/3 *Tamarindus indica*; mixture and extracts werestored in the freezer at -4°C for later use.

Yield (%) = Mass of extract×100 / Mass of powder

2.4 Laboratory Animals

Four-week-old *Wistar* rats were obtained from the Department Animal Centre and allowed to be accustomed to the new environment for 1 week. They were maintained in accordance with the guidelines of the OECDand were randomly distributed into height groups of seven animals each (including two controls). The animals were individually housed under controlled temperature (25 °C) and lighting (12:12-hours light-dark cycle) and had free access to water and diet. The test groups and the positive control were fed a high-fat high sucrose diet (17.6% fat and 7% sucrose-enriched) while the negative control received a basal diet. The high-fat-high-sucrose diet-induced obesity was carried out for twelve (12) weeks and diagnosed by a body mass index greater than 0.68 (Novelli et al., 2007). These obese animals were administered with a single dose of 45 mg/kg of streptozotocin. Three days after the injection of streptozotocin, animals having a fasting blood glucose concentration higher than 200 g/l were considered diabetic (Chang, 2000) and used for the procedure that latest for twenty-one days.

2.5 Alphaamylase Inhibition

The *in vitro* α-amylase inhibitory activity of extracts and mixture of extracts was carried out using the method described by Bernfeld (1955). In brief, 0.02–4 µL of the different extracts reacted with 200 µL of α-amylase enzyme, and 100 µL of 2mM of phosphate buffer (pH, 6.9). After 20-min preincubation, 100 µL of 1% starch solution was added. The same was performed for the controls. After incubation of 5min, 500 µL of dinitrosalicylic acid reagent was added to both the control and test. They were kept in boiling water bath for 5min, the absorbance was recorded at 540nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the following formula: % Inhibition = [(Control - Test)/Control]×100. A reference inhibitor (Acarbose) was use as control.

2.6. Pancreatic lipase Inhibition

The modified method of Beisson et al*.* (2000) was used to assess lipase inhibitory activity. This technique was adopted to measure the ability of the extracts tested to reduce the production of free fatty acids in a reaction medium containing an emulsified substrate and pancreatic lipase. Released fatty acids were titrated by adding 0.1 N sodium hydroxide to the reaction medium. A decrease in the volume of sodium hydroxide compared with the control corresponds to the percentage of enzyme inhibition by the extract.

To the test tubes containing 100 µl of extract solutions at concentrations of 1000, 500 and 250 µg/ml, prepared in distilled water, we added 1 ml of 10% olive oil emulsion (10 ml olive oil in 90 ml of 10% gum Arabic) Rathelot et al*.,* (1975) (100g gum arabic in one liter of boiled water, then filtered) and 500 µl of pancreatic lipase solution (2 IU/ml) prepared in phosphate buffer (0.2 M, pH 8) containing 1 mM $CaCl_2$. The whole was incubated for 30 minutes at 40°C in a water bath, then 20 ml of distilled water and 3 drops of

phenolphthalein were added. The mixture was titrated with 0.1 N sodium hydroxide solution. Pink coloration indicated turning, which persisted for 5 min. The percentage of lipase inhibition by the extracts was determined by the following formula:

$$\%I = \frac{Vc - Ve}{Vc} X\ 100$$

Where:

-Vc = volume of NaOH in the control tube without extract,
-Ve = volume of NaOH in the test tube containing extract.

2.7 Treatment of Rats with Extracts and Mixture of Extract

The rats were randomly distributed into eight groups of 7 rats each as follows: Group I and Group II received saline and served as the normal and positive control. Group III received glibenclamide (80 mg/kg) and served as a model group. Group IV received aqueous extract of *Vernoniaamygdalina* (500 mg/kg), groups V received aqueous extract of *T.indica* (500 mg/kg), group VI received combination of *V. amygdalina* and *T. Indica* (250 mg/kg each), group VII received association of *V. amygdalina* and *T. indica*(125 mg/kg each) and group VIII received both association of *V. amygdalina* and Tamarindus indica (250 mg/kg each) and glybenclamide (80 mg/kg).

2.8 Animal Sacrifice, Blood Collection and Hematological Tests

Twenty-four hours after the last administration, the animals were sacrificed under light anesthesia using ketamine. Dissecting instruments were washed with 5% nitric acid and rinsed several times with distilled water before use. Blood samples collected in EDTA tubes were used to perform a haemogram on an impedance haematology machine (QBC Autoread Plus, UK). The counting principle is based on impedance variation and flow cytometry to determine the size and type of blood cells. Flow cytometry measures on a suspension of particles (cells, bacteria, parasites, beads) the individual characteristics of each particle such as size, shape and, complexity of any component or function that can be detected by a fluorescent compound. Cells in suspension pass one by one past one or more laser beams, and detectors pick up signals emitted by each cell. Each time a cell passes through the aperture, the electrical resistance increases. This increase is translated into electrical pulses, the height of which is directly proportional to the cell volume. Cells emit signals, either naturally or after treatment, which are analyzed by the computer linked to the cytometer, enabling the leukocyte formula to be established, giving the percentages of the different types of leukocytes.

The number of red blood cells was determined by the total number of pulses recorded. The hematocrit level was then deduced from the formula:

> Hematocrit = red blood cell × mean corpuscular volume /10.

Hemoglobin was determined spectrophotometrically (525 to 550 nm) after lysis of the red blood cells.

2.9 Statistical Analysis

Data were expressed as the mean ± standard error of the mean. After the analysis of variance, the comparison of means between different groups was carried out by the Waller-Duncan test using SPSS version 26.0 software. P values =0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Extraction Yields

After obtaining the various extracts, the extraction yields evaluated were 9.98% for *Vernoniaamygdalina* and 17.26% for *Tamarindusindica*.

3.2 α-Amylase Inhibitory Activitivity

The following Fig. 1 shows the ability of extracts and mixture of extracts to inhibit α-amylase at 100mg/ l. It appears that the two extracts were able to act as α-amylase inhibitors but the percentage of inhibition is lower than that of the reference drug used (Acarbose). However, we can observe that the combination of the two extracts gives a percentage of inhibition higher than any of the two extracts.

The combination of aqueous extracts of *Vernonia amygdalina* and *Tamarindus indica* inhibits α-amylase and this can be attributed to the bioactive compounds present in our two

materials, notably phenols and dietary fibres. Indeed, work by Quan et al. (2019) reveals that the position of the hydroxyl and methoxy groups of the phenolic compounds contained in these extracts could be at the origin of the antioxidant and alpha amylase inhibitory activities.

3.3 Pancreatic Lipase Inhibitory Activity

The ability of our different samples to act as an inhibitor of pancreatic lipase is reported on the following Fig. 2. It appears that the percentage of inhibition increase with the concentration of extracts and mixture; it also appears that the combination of the two extracts canincrease the inhibitory ability of extracts. The main function of pancreatic lipase is to catalyse hydrolysis reactions (Pandey et al., 1999) the pancreatic lipase inhibitory activity observed here can be attributed to several classes of compounds, including catechins, coumarins, quercetins and tannins, which act as pancreatic lipase inhibitors (Dunaif & Schneeman, 1981) it can also be attributed to dietary fiber which have the capacity to limit lipolysis thus increasing the fecal excretion of lipids (Kundu et al., 2014) extracts of *Vernoniaamygdalina* and *Tamarindusindica*are known to be rich dietary fiber and many phenolic compounds (Sadiq et al., 2016) Phenols and fibers therefore act various ways, including limiting glycation of red blood cells and reducing apoptosis of leukocytes (Kundu et al., 2014). These results corroborate those obtained by Auger et al. (2019) who demonstrate that phenols from extracts of nine Niger plants were capable of inhibiting pancreatic lipase.

3.4 Effect of Plant Extracts and the Mixture of Extracts on Hematologic Parameters

Table 1 shows the number of white blood cells, the percentage of lymphocytes and, the number of granulocytes in the blood of animals treated with extracts and a mixture of extracts. It appears that the combination of extracts can increase the number of white blood cells and granulocytes.

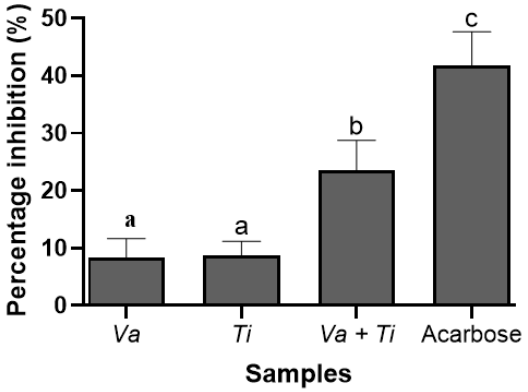

Fig. 1. Effect of extracts and mixture of extracts in vitro on α-amylase activity in comparison with acarbose.Va: *Vernoniaamygdalina*, Ti: *Tamarindusindica*, Va+Ti: Mixture. Samples with different letters are significantly different at p=0.05

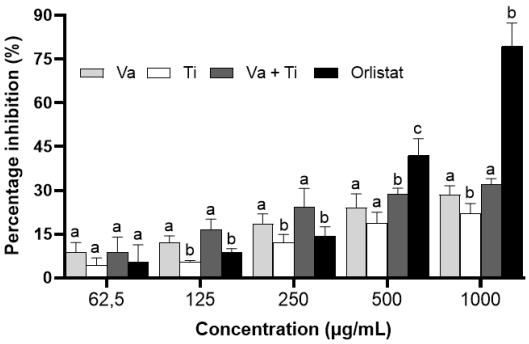

Fig. 2. Effect of extracts and mixture of extracts *in vitro* pancreatic lipase activity,in comparison with Orlistat. Va: *Vernoniaamygdalina*, Ti: *Tamarindusindica*, Va+Ti: Mixture. Samples with different letters are significantly different at p=0.05

Table 1. White blood cells, lymphocyte and granulocytes in the blood of animals after treatment

	WBC*10^9(/L)	LYM (%)	GRAN*10^9(/L)
Ti	3.1±0.30^b	74.5± 1.10^b	2.16±0.20^b
Va	2.43±0.15^a	86.4±3.01^d	1.6±0.26^a
Ti+Va 500	4.56 ±0.35^c	81.5±1.24^d	2.3±0.26^b
Ti+Va 250	2.16± 0.49^a	78.63±1.40^c	1.63±0.25^a
Ti+Va+Gly	3.5±0.10^b	83.83±4.7^d	2.2±0.36^b
Glib	5.2±0.26^d	77.63 ± 2.47^c	2.13± 0.32^b
C(+)	2.53±0.25^a	69.34±2.72^a	1.33 ± 0.15^a
C(-)	7.06 ± 0.47^e	82.7 ±4.94^d	3.96 ±0.70^c

WBC: White blood cells, LYM: lymphocytes, GRAN: granulocytes, Va: Vernoniaamygdalina, Ti: Tamarindusindica, Va+Ti: Mixture. Glib: Glibenclamide, C(+): positive control, C(-): negative control, Values in the same colum, with different letters are significantly different at p=0.05.

Table 2. Red blood cells, hemoglobin and hematocrit in the blood of animals after treatment

	RBC*10^{12}(/L)	HGB (g/dl)	HCT (%)
Ti	8.1± 0.17	14.73±0.37	46.6±0.10
Va	8.27±0.21	15.26 ±0.30	50.2± 0.72
Ti+Va 500	7.25±0.12	13.5± 0.95	43.33±2.49
Ti+Va 250	8.08 ±0.50	14.2± 0.81	45.2 ± 3.87
Ti+Va+Gli	8.083± 0.11	14.7±0.36	47.36± 0.80
Gli	7.8±0.23	14.4 ± 0.36	46.7± 2.74
C(+)	27.36 ±0.30	13.36±0.49	42.8± 0.28
C(-)	8.46 ±0.24	15.36 ±0.55	47.46±1.11

White bloodcells, HGB : Hemoglobin, HCT : Hematocrin ; Va: Vernonia amygdalina, Ti: Tamarindusindica, Va+Ti: Mixture, Glib: Glibenclamide, C(+) : positive control C(-) : negative control.

The number of red blood cells, hemoglobin and, the hematocrinhaved also been evaluated; the results are grouped in the following Table 2. It can be observe that the number of red blood cells and hemoglobin of animals treated with extracts and the mixture of extracts were nearly the same as those of animals treated with the reference drug (Glibenclamide). The mixture of extracts improved hematological parameters in treated animals, reflecting the ability of the compounds in our mixture to reduce immunological complications associated with diabetes.These results corroborate those of Woumbo et al. (2022) who report that the phenols in a nutraceutical formulated from extracts of three plants improve the hematological profile of streptozotocin-induced diabetic rats (OECD, n.d.).

4. CONCLUSION

The aim of the present work was to evaluate the effect of the combination of aqueous extract of *Vernonia amygdalina* and *Tamarindus indica* on two keys enzymes of energetic metabolism (namely alphaamylase and panreatic lipase) and also their effect on the hematologic profile of streptozotocin- induced diabetic rats. It was found that the mixture of these extracts is able to act as good inhibitors of these enzyms; the mixture is also able to improve the hematologic profile of streptozotocin- induced diabetic rats with better activities than extracts taken separately. Further detailed studies with purified compounds may provide evidence to effectively control diabetes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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Details of the AI usage are given below:

1. GRAMALY

ACKNOWLEDGEMENTS

The authors are thankful to the University of Dschang which provided laboratory facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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