Stereological Studies on Ameliorative Role of Ethanolic Extracts of VernoniaAmygdalinaand GongronemaLatifoliumAgainst Streptozocin- Induced Diabetic Splenic Tissue Damage in WistarRats.

Abstract

Background: Diabetes mellitus is a chronic metabolic dysfunction marked with prolonged excessive glucose level, with disruptions in the metabolism of starch, lipids, and amino acids due to excessive production and/or inadequate utilization of glucose.

Objective: To assess the protective impact of *Vernoniaamygdalina* (VA) and *Gongronemalatifolium* (GL) on splenic cytoarchitechure in STZ induced glycemic disorder rats using quantitative and histological methods.

Materials and Methods:A total of thirtyWistar rats were randomly divided into six groups of five rats each, weighing between 150-200g. The groups were marked as A, B, C, D, E and F. Group A [Normal control] received distilled water, B [Diabetic control] received 45mg/kg body weight of Streptozocin, C received 100mg/kg of the extract VA only; D received 100mg/kg of GL extract only. E received 5mg/kg of Metformin and F received combined 100mg/kg of VA and GLfor fourteen days. Twenty four hours after the last administration, the animals were sacrificed and the spleen was harvested, fixed, processed, stained with H&E and the spleen volume was estimate using Cavalieri methodand data were analyzed using ANOVA at $p<0.05$.

Results: The diabetic group treated with STZ onlyrevealed reduced white pulp and the marginal zone and an apparent decrease in the cellular density of the lymphocyte component of the pulp, with positive Perl's stain. The stereological analysis of the spleen showed a significant decrease of the splenic pulp volume density and a significant increase in the connective tissue volume density, which significantly improved after treatment with the combined extract of VA+GL-treated rats. The reduction of splenic pulp was mainly due to the decrease in the volume density of all structural components of the white pulp.

Conclusion:it can be suggested that combined ethanolic extracts of VA+GL when used in combination can be used in management of diabetes.

Keywords: Diabetes, Spleen, *VernoniaAmygdalina, Gongronemalatifolium,* STZ

Introduction

Diabetes is a chronic disorder associated with many debilitating multi-systemic complications. The spectrum of the diabetic pathology is complex and thus would require beyond single therapy for management of such complications. The more acceptable approach should embrace alternatives to orthodox care. Diabetes remains a public health concern with an estimated 463 million cases worldwide [8.8% of the adult population] [34].

"Diabetic patients have significant defects of antioxidant protection which may increase their vulnerability to oxidative damage and the development of diabetic complications"[23]. "Reactive Oxygen Species [ROS] are chemically reactive molecules containing oxygen, such as, H_2O_2, HOCl, and free radicals such as Superoxide anion, hydroxyl radicals"[3]. "Antioxidants are molecules involved in scavenging of free radicals. This defense mechanism involves both enzymatic and non-enzymatic strategies" [3]."In 2017, diabetes resulted in approximately 4.2 million deaths. It is the seventh leading cause of death globally"[8]. The pooled Prevalence

of 5.77% in Nigeria suggests that about 11.2 million people [1 out of every 17 adults] are living with the disease.
Recent research has also shown a connection between diabetes and dementia, hearing loss, and some forms of cancer[25]. Diabetes increases the risk of early death, and diabetes-related complications can lower quality of life [21].The High cost of anti-diabetic drugs, its side effects and numerous complications associated with poor prognosis arising from orthodox drugs has led manyto embrace plant-based therapy [27].As several studies revealed amelioration of Alzheimer's disease post-administration of *Telfairiaoccidentalis and Taliniumtriangulare* on rats induced with scopolamine[15,16,17,18,19],*Musa paradisiaca*stem juice limit the extent of status epilepticus, increased neuronal protein synthesis, reduced cytoarchitectural damage and astriogliosis as well as enhancing long term recognition memory in rats [40,41], star fruit ameliorates neurotoxicity in rats[4], other researchers also reported the antihyperglycemic and hypoglycemic action of *Vernoniaamygdalina* [VA] and *Gongronemalatifolium* [GL]in diabetic and non-diabetic rats [5]. The aqueous leaf extract has been shown to possess antihyperlipidemic and hypolipidemic effects on diabetic and non-diabetic rats [6]. According to [7], VA has continued to receive a lot of attention due to the numerous curative potentials that it has demonstrated; It is purported to possess antioxidant activity from radical scavengers[31]. Many people also believe in traditional ways of living, and this has influenced their health-seeking behavior [29],hence, the rationale to investigate the potentials of VA and GL on the cytoarchitecture of the spleen in streptozocin-induced diabetic Wistar rats in the present study.

Materials and methods
A total of 30 rats [150-200g] with ethical approval [FAREC/PA/[UC/049]were acquired from the animal house, College of Medical Sciences, University of Calabar. The rats were kept under standard conditions of temperature [27^{o}C-30^{o}C] and fed with rat chow purchased from Agro Feed Mill Nigeria Limited, Calabar and provided with distilled water for drinking. They acclimatized for a week prior to commencement of the experiment. The animals were housed in properly ventilated plastic cages.

Plant Extracts preparation
The fresh leaves of VA and GLwere purchased from Marian market, Calabar, Cross River State-Nigeria. These plants were identified by a botanist with reference number Bot/Herb/UCC/0188 and Bot/Herb/UCC/0718 respectively. The plants were cleaned and air dried, after which they were grounded into powered form. A measured amount of 120 grams of powdered leaves was extracted using 2.5 liters of absolute ethanol for 48hours with intermittent gyration of sample holder. After 48hours of soaking, the extract solution was first double filtered with chess cloth, then with filter paper [Whatman/filter paper]. The filtrate [extract] was thereafter concentrated under reduced pressure at 45^{o}c in rotary evaporator to 10% volume and then to complete dryness using regulated temperature water bath, yielding 22 grams [about 19.5g] of crude extract. The extract obtained was stored in a refrigerator until required.

Induction of Diabetes to the experimental rats.
Diabetes was induced in overnight fasted experimental animals by a single dose of intraperitoneal injection of freshly prepared Streptozocin [STZ] at 45mg/kg body weight reconstituted in 0.1M sodium citrate buffer at pH 4.5-5.0 as solvent using Lorke's method.

Diabetes was confirmed by use of glucometer to test for the fasting blood glucose concentration of experimental animals 48hrs after STZ administration, values above 180mg/dl were considered diabetic and found suitable for the study.

Determination of LD_{50}

$LD_{50}$of ethanol leaf extracts of VA and GL was established to be >2000mg/kg according to Lorke's method [1983]. The dosage was determined using 5% [100mg/kg] of 2000mg/kg body weight of ethanol extract of VA and GL leaves.

Plant extract and metformin administration

Group A, the Normal control rats were given only distil water and feed, Group B was the diabetic control rats, induced with diabetes [received 45mg/kg of STZ], Group C animals were induced with diabetes [received 45mg/kg of STZ] and were given*VernoniaAmygdalina*[100mg/kg]only. Group D were induced for diabetes [received 45mg/kg of STZ]and were given*Gongronemalatifolium*[100mg/kg]only. Group E were induced with diabetes [received 45mg/kg of STZ]and were given Metformin [100mg/kg] and Group F was induced with diabetes [received 45mg/kg of STZ] and were given both extracts of VernoniaAmygdalina [100mg/kg] and *Gongronemalatifolium*[100mg/kg]. Pre and post induction of STZ fasting blood sugar [FBS] and weight of animals were assessed. These procedures were carried out daily during the course of extracts administration.

Tissue processing procedure

Twenty four hours after the last administration, the experimental animals were sacrificed and the spleen was fixed with 10% formol saline. The tissues were dehydrated using ascending grades of alcohol followed by clearing with xylene, infiltrated, embedded with paraffin wax and sectioned [5µm] with rotary microtome. The ribbons of the sections were collected using clean slides and first straighten with 10% ethanol before being gently lowered into the surface of a warm water bath so as tostraighten wrinkles on sectioned tissues. The floated sections were mounted on an albumenized slides and it was dried in the oven at 60 degrees Celsius, for one hour before staining.

Hematoxylin and Eosin staining procedure [Cole, 1943]

Tissue sections were dewaxed in xylene for 5-10minutes,transferred through descending grades of alcohol [100%, 95% and 70%] 5mins respectively. Sections were rinsed in running tap for 10mins and stained in hematoxylin for 10 minutes. Theywere rinsed in water and Blueing in tap water for 5 minutes. Sections were differentiated in 1% acid alcohol so as to help nucleus absorbs the stain`. They were counter stained in 1% Eosin for 30 seconds. Various sections were blued under a running tap water for 2-3mins.Finally, sections were passed through ascending grades of alcohol, cleared in xylene and mounted in distyrene, plasticizer and xylene [DPX].

Stereology

The absolute volume of the spleen was estimated using the Cavalieri estimator of volume according to the method of West *et al.* [1999]. To achieve this, spleen of each Wistar rat per group was isolated, processed and sectioned serially using a microtome, as shown by [22] to provide a number of sections after pilot study on how many slices could be derived and how many slices will give the lowest coefficient of error, each section had a section of the spleen.

Tissue sections of the spleen were selected using a systematic uniform random sampling method. The sections derived were stained using H and E stains. A transparent counting grid was placed randomly over the cut surface of every spleen slice. The number of points hitting the spleen was counted. The volume was then estimated using the Cavalieri's principle [22] as follows:

V total [mm^3]: = $T \times {}^{a}/_{p} \times \sum pi$

where ''**: =**'' indicates that the result is the estimated value rather than the true value, "V" is the total volume of spleen, "T" = 100. Slice thickness =5 µm x100 = 500 µm.Convert 500 µm to mm, 500/1000 = 0.5 mmis the average slice thickness, "a/p" ¼ is the area per point associated with each point inthe counting grid [4mm^2], and "Σp" is the total number of points hitting the spleen.

Data Analysis

Results obtained at the end of the experiment were analyzed using the statistical software, statistical package for social science [IBM SPSS *version* 23.0] and Microsoft Office Excel 2019 were used for charts. Results were expressed as mean ± S.E.M. One-wayanalysis of variance [ANOVA] was used to compare mean difference between groups followed by least significant difference [LSD]*post hoc test*. Paired *t*-test was employed for the comparisons of means as appropriate. Values were considered significant when $p<0.05$.

Results

H&E stained Sections from the control group showed normal histology of the spleen with normal white pulp [WP] and numerous white blood cells, red pulp [RP], the marginal zone and the central vessels [BV] following H&E staining [Plate 1].Group B revealed shrunken white pulp [WP] and enlarged red pulp [RP] withreduced germinal center using, the spleen also showed numerous lymphocytes in the shrunken white pulp area indicating the presence of inflammation and immune response and distortion of the splenic cytoarchitecture, [Plate 2]**.** Group C revealed signs of regenerating white pulp [WP] compared to the diabetic group, with lymphocytes infiltrating into the red pulp [RP] area [Plate 3]. Group D showed prominent white pulp (WP) with numerous white blood cells and reduced red pulp [RP][Plate 4]. Group E showed normal appearing white pulp [WP] with white blood cells and reduced red pulp [RP][Plate 5].Group F revealed signs of regenerating normal white pulp, red pulp, increased lymphocytes in the white pulp with normal central vessel [Plate 6].

Stereological estimation of Spleen Volume of diabetic control group B [970±250.98][CE = 0.035] was significantly [$p<0.05$] lower when compared to normal control group A [2398.7±352.70] [CE = 0.018] [Figure 1].Spleen volume of all treated groups: group C [1630±321.23] [CE = 0.028], group D [2538±284.32] [CE = 0.015], group E [3458±515.54] [CE = 0.004] and group F [2548±871.34] were significantly [$p<0.05$] higher when compared to the volume of the diabetic control group B [970±250.98] [CE = 0.023][Figure 1]

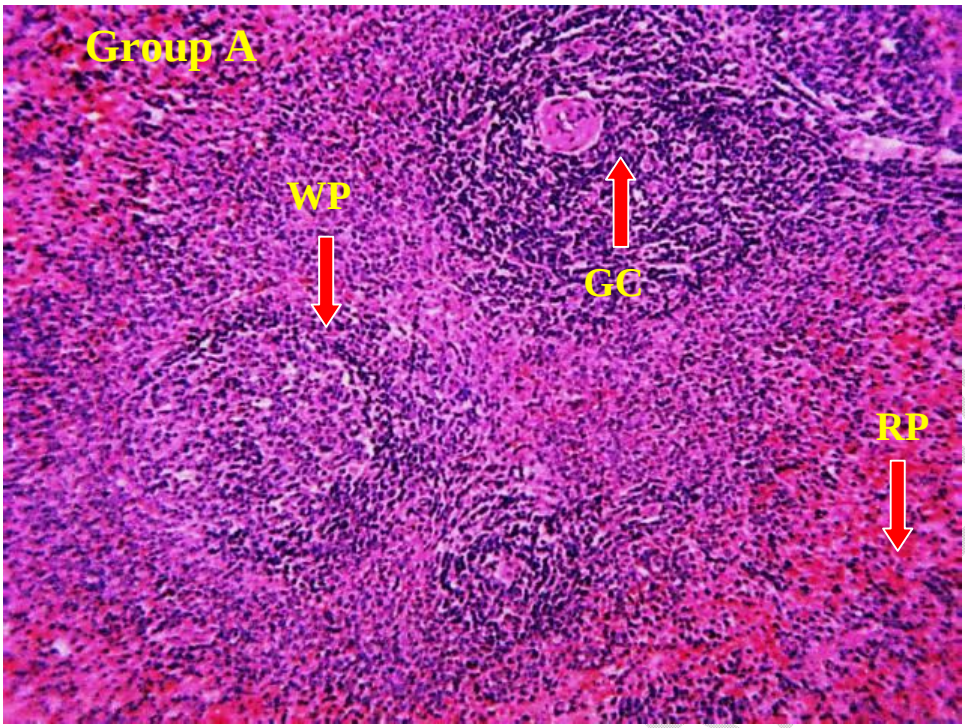

Plate I: Photomicrograph of spleen of Wistar rats in normal control group treated with H_20 showing normal white pulp and red pulps with germinal center, H&E stain, [mag x 100]

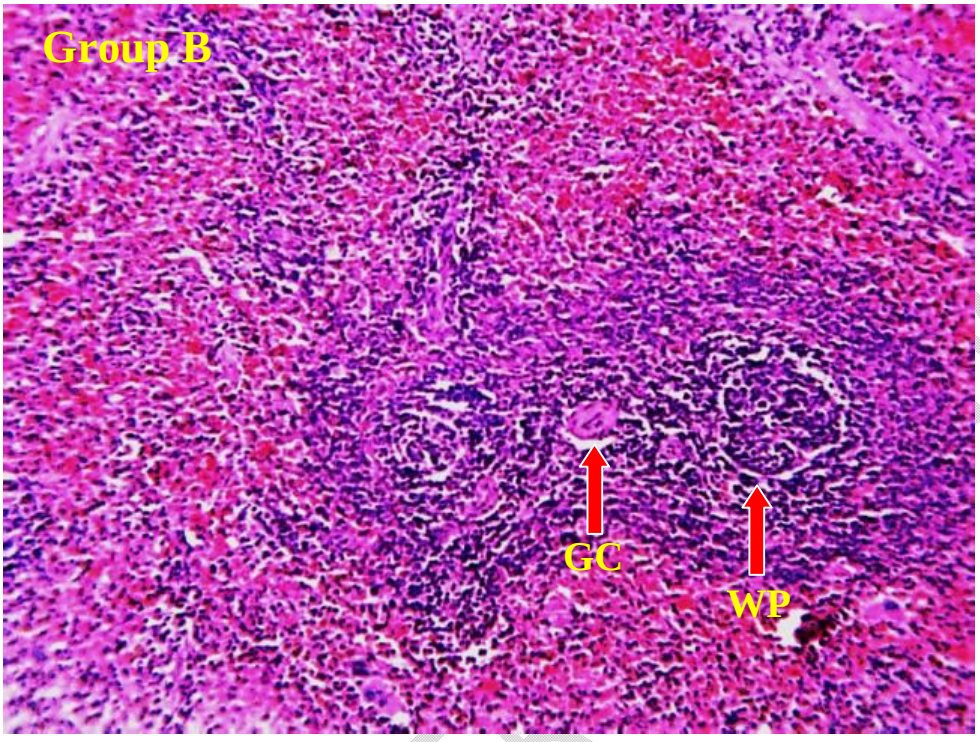

Plate 2: Photomicrograph of spleen of Wistar rats in Diabetic control group treated with 45 mg/kg streptozocin only showing shrunken and distorted white pulp and reduced germinal center with blood vessel, H&E stain, [mag x100]

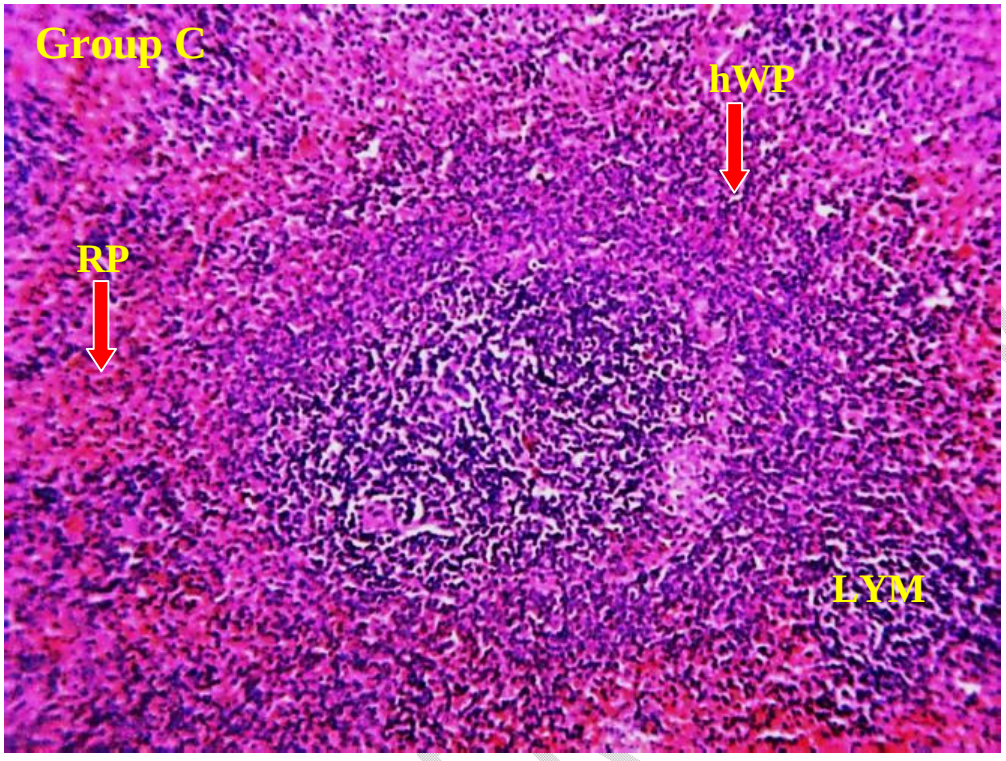

Plate 3: Photomicrograph of spleen of Wistar rats in group C treated with 45 mg/kg streptozocin and 100 mg/kg of VA only showing regenerating white pulp [rWP] with lymphocytes [LYM] and reduced red pulp [RP] area, H&E stain, [mag x 100]

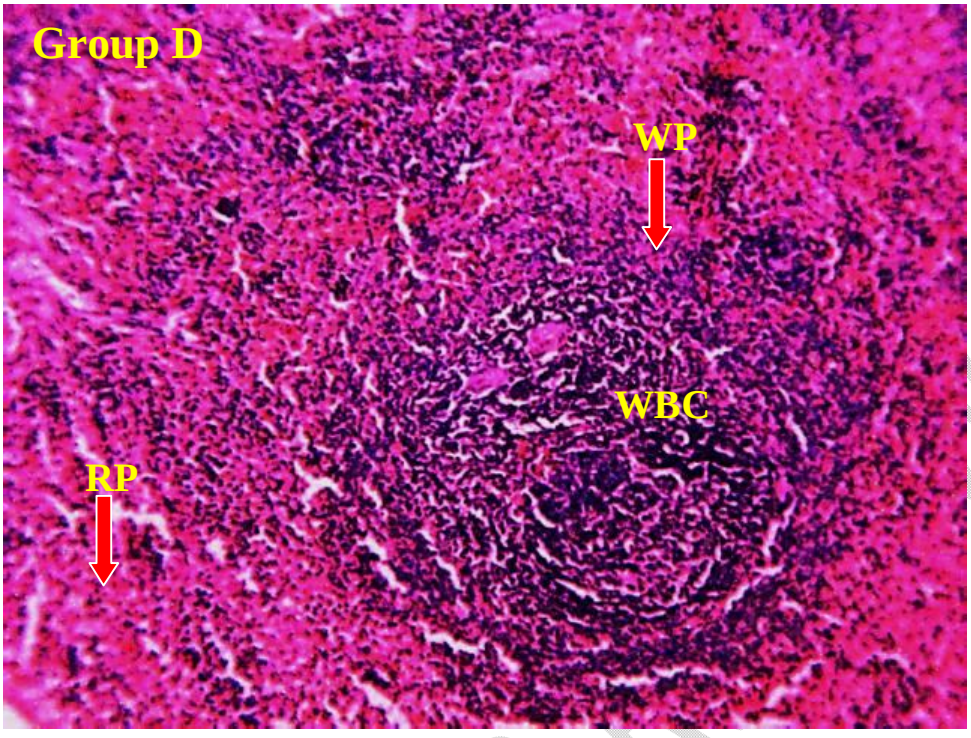

Plate 4: Photomicrograph of spleen of Wistar rats in group D inducedwith 45 mg/kg streptozocin and treated with 100 mg/kg GL only showing regenerating white pulp [WP] filled with increased white blood cells [WBC] and reduced red pulp [RP] area, H&E stain, [mag x100]

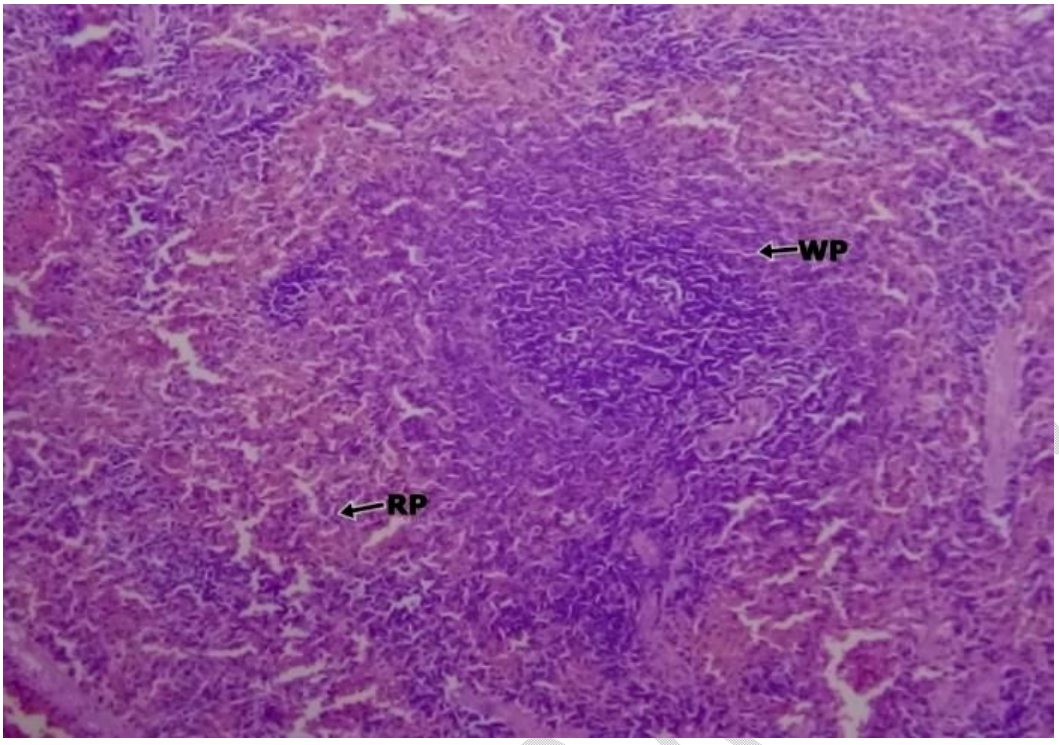

Plate 5: Photomicrograph of Group E [Metformin solution 5mg/kg] showing regenerating splenic white pulp with white blood cells infiltrating the reduced red pulp [RP], H&E stain, [mag x 100]

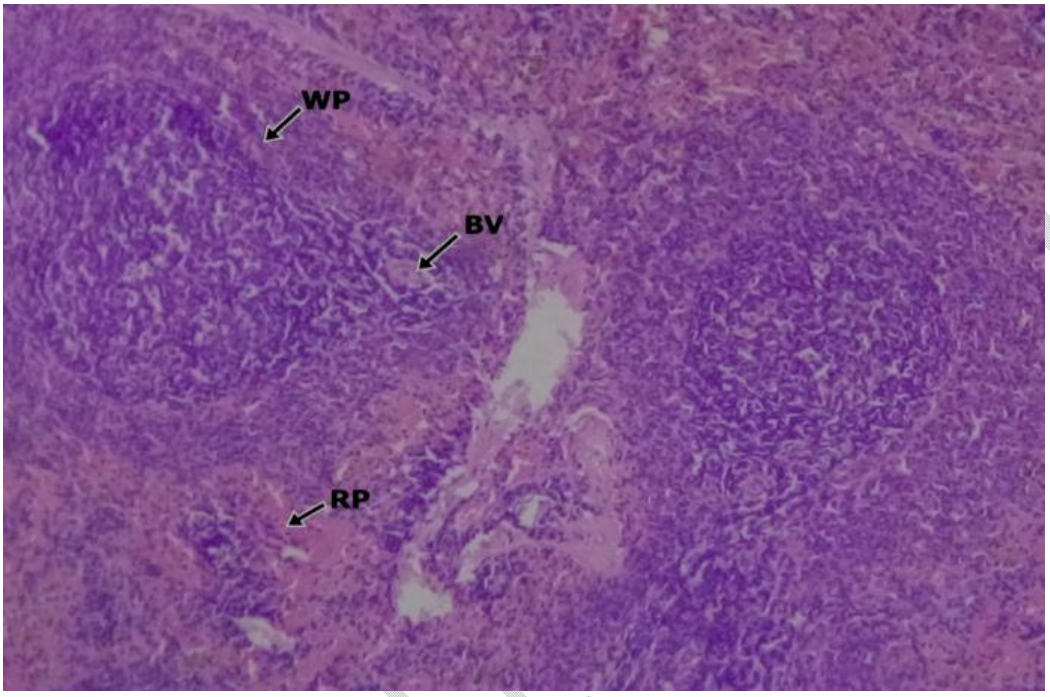

Plate 6: Photomicrograph of the spleen of Wistar rats in Group F treated with 45 mg/kg streptozocin and combined extracts of 200 mg/kg VA + GL showing regenerating white pulp, with reduced red pulp with presence of numerous normal lymphocytes [LYM] in the white pulp and central vessel. H&E stain [mag x100)]

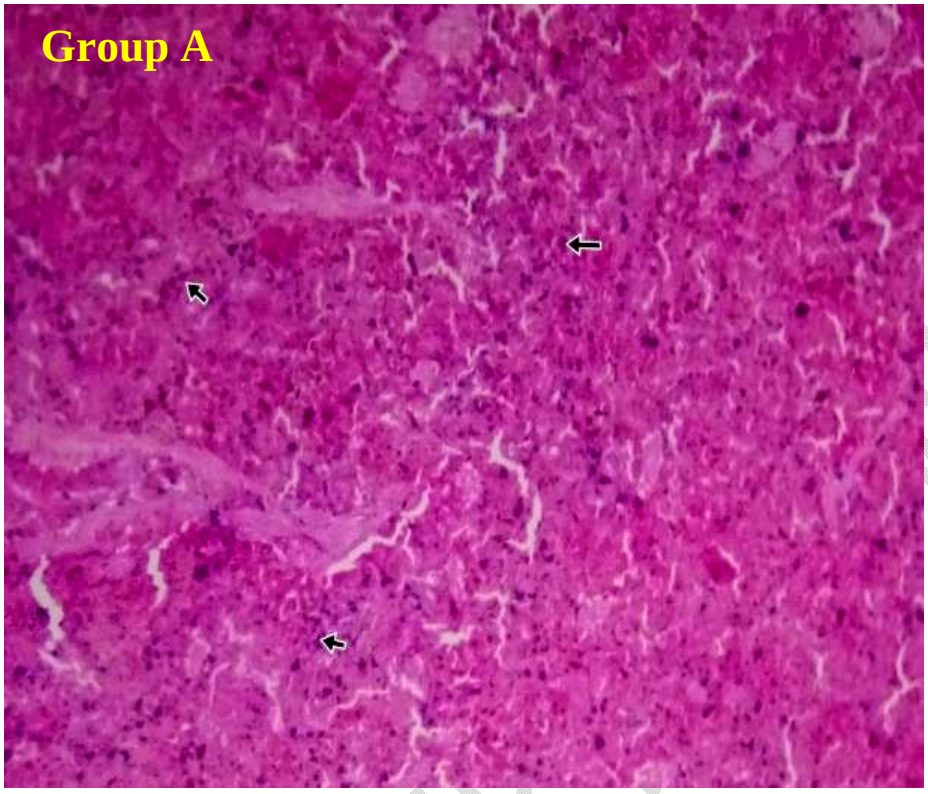

PLATE 7: Photomicrograph of spleen of Wistar rats in normal control group treated with distilled water showing weakly positive Perl's stain with sparse hemosiderin deposits. Perl's Prussian stain, [mag x100]

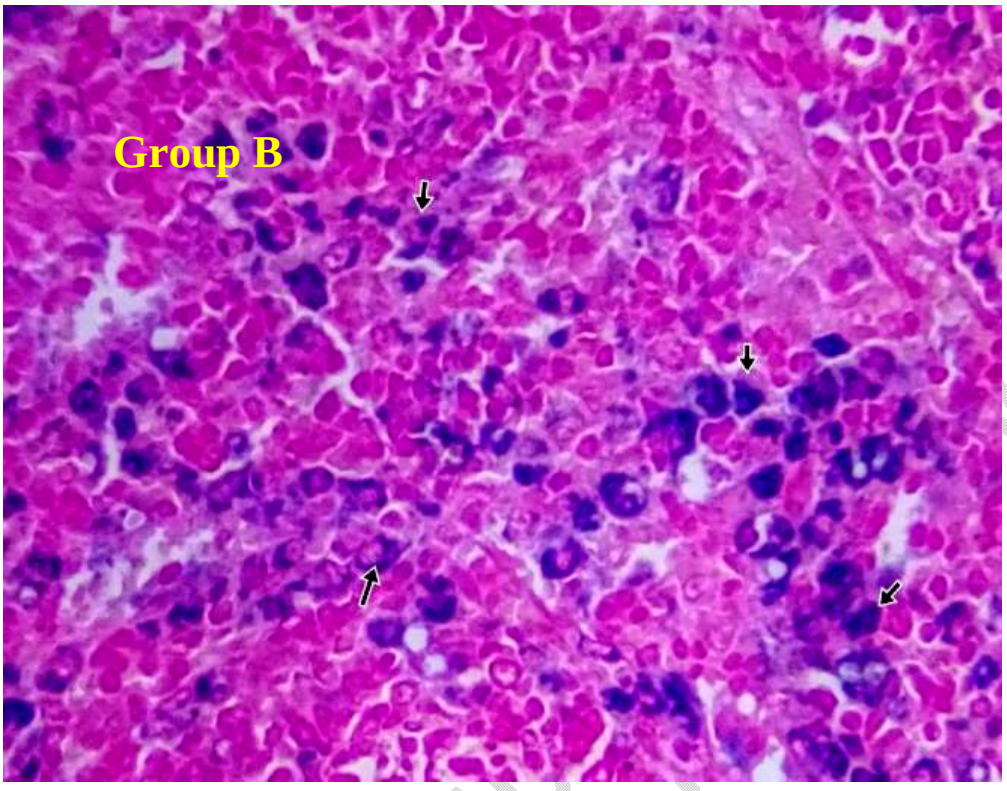

PLATE 8: Photomicrograph of spleen of Wistar rats in group B treated with 45 mg/kg Streptozocin only showing intense Perl's staining of hemosiderin deposits [arrows] within the red pulp. Perl's Prussian stain [mag x 100]

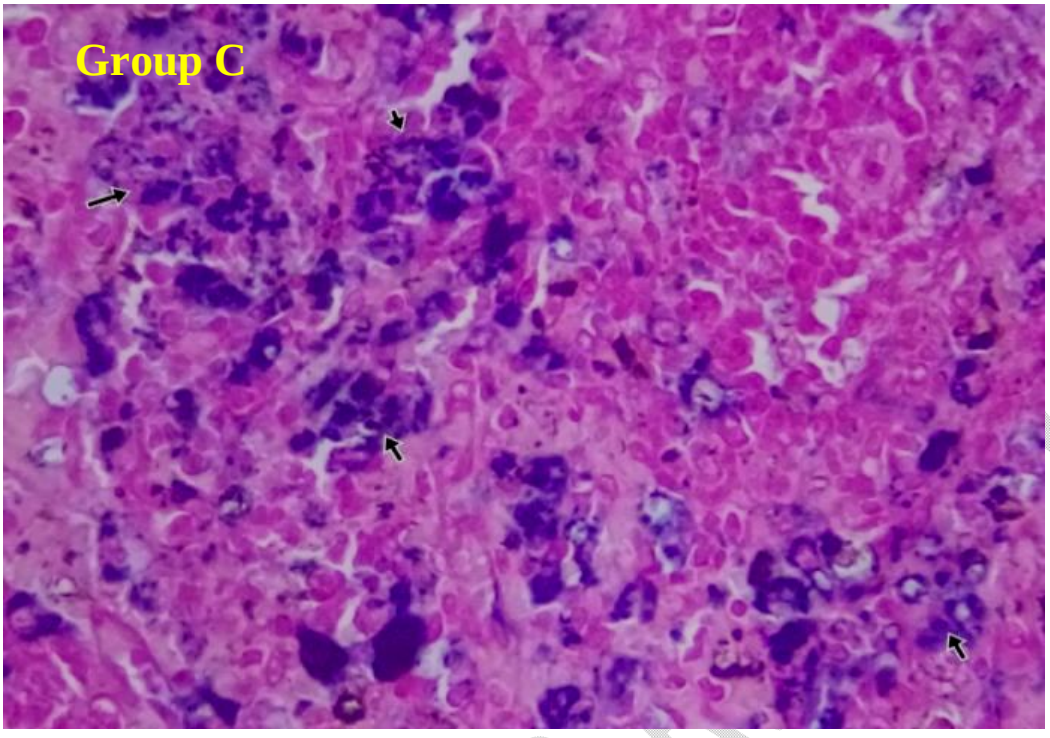

PLATE 9: Photomicrograph of Group C splenic tissue treated with 45 mg/kg streptozocin and 100 mg/kg of VA only showing moderate staining of hemosiderin deposits [bluish colouration] within the red pulp [Perl's Stain X 100].

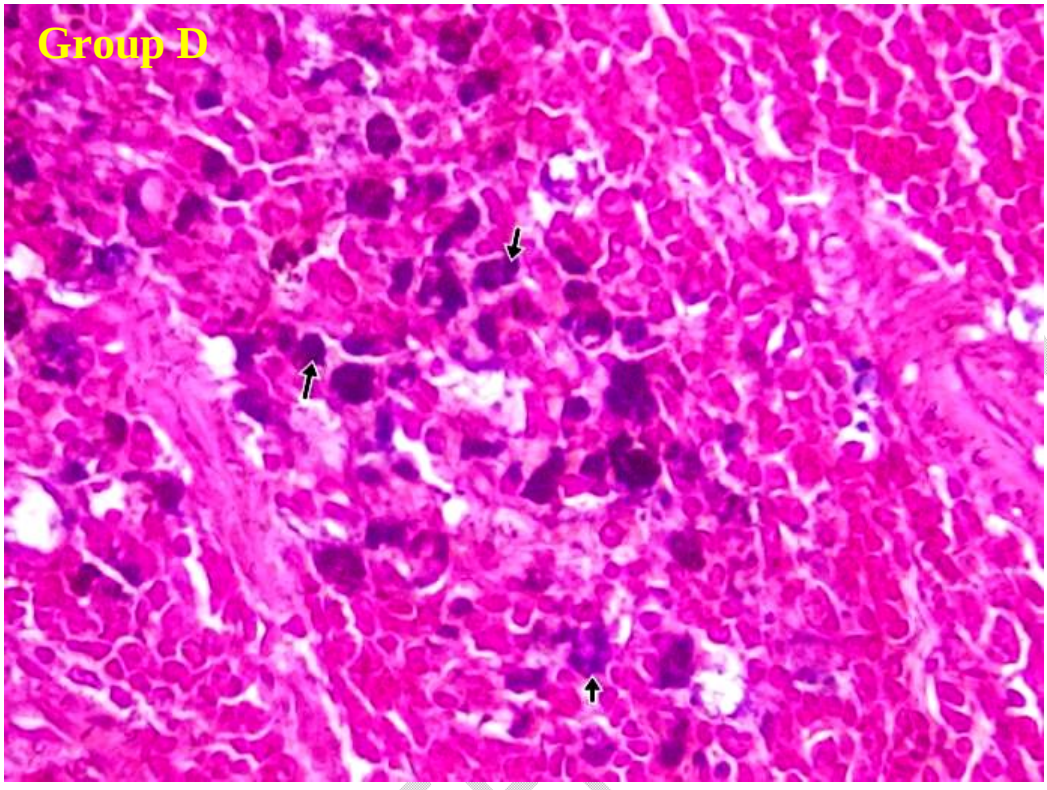

PLATE 10: Photomicrograph of spleen of Wistar rats ingroup D treated with 45 mg/kg streptozocin and 100 mg/kg of GL onlyshowing moderate Perl's stain with moderate hemosiderin deposits [arrow] within the red pulp. Perl's Prussian stain, [mag x 100]

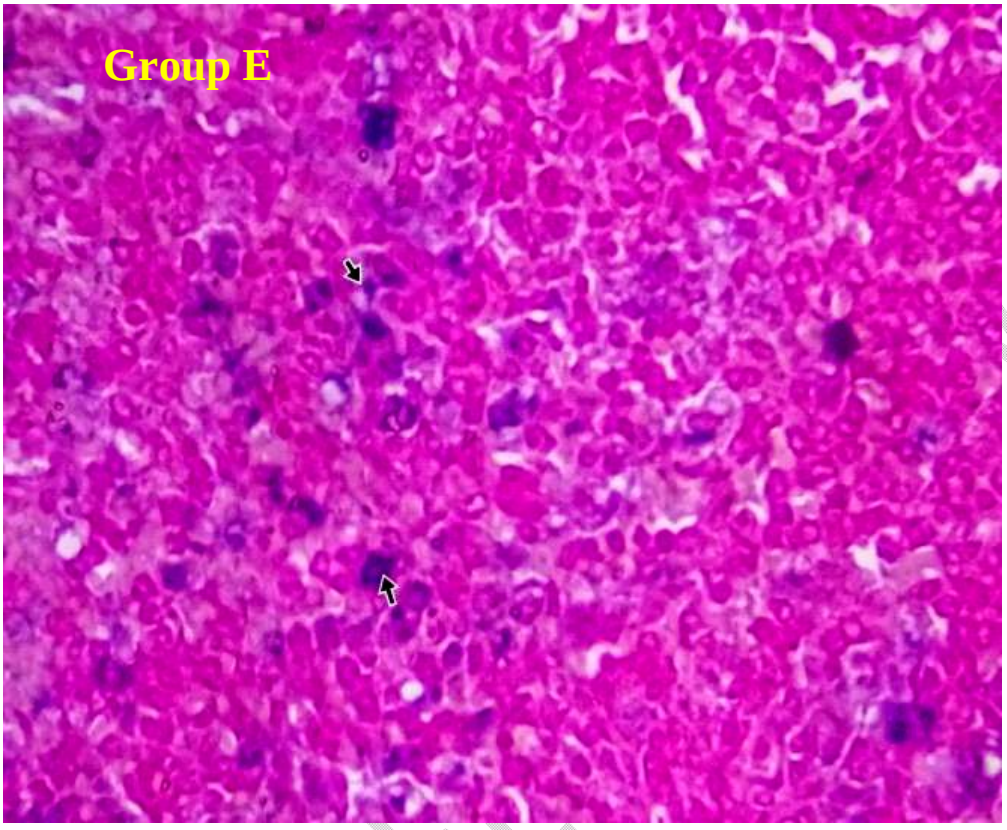

PLATE 11: Photomicrograph of the spleen of Wistar rats in group E treated with 45 mg/kg streptozocin and 5mg/kg metformin showing moderatePerl's stain of hemosiderin deposits[arrow] within the red pulp. Perl's Prussian stain, [mag x 100]

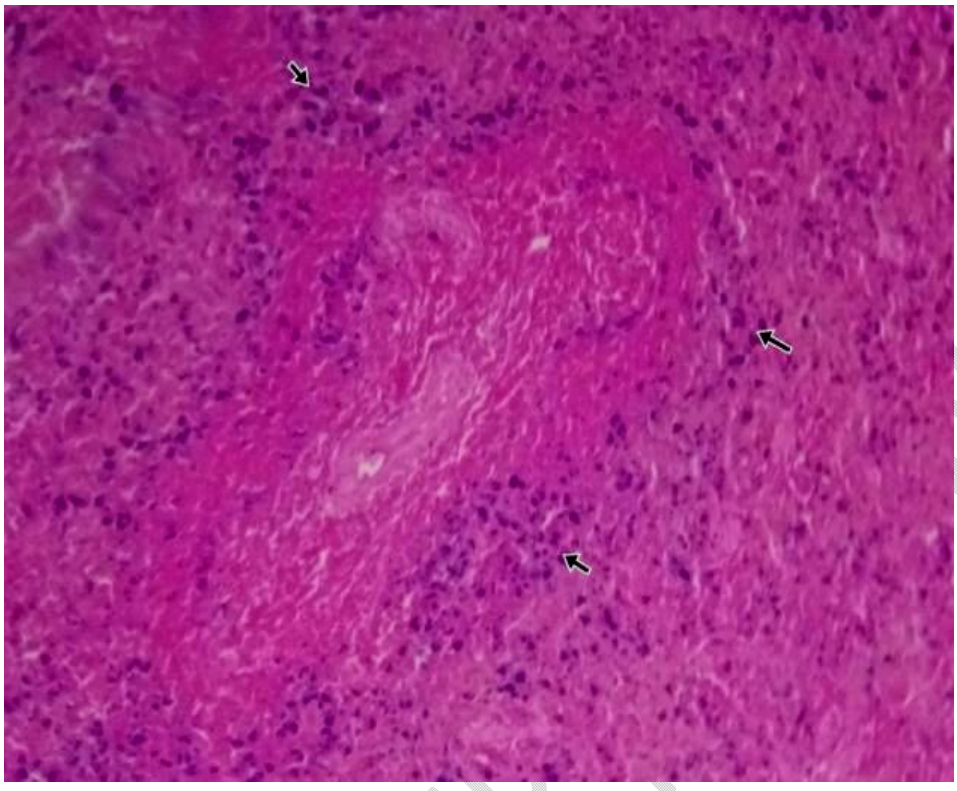

PLATE 12: Photomicrograh of spleen of Wistar rats in group F treated with 45 mg/kg streptozocin and combined extract of 200 mg/kg VA and GL showing weak Perl's stain with moderate hemosiderin deposits [bluish coloration] within the red pulp. Perl's Prussian stain, [mag x100]

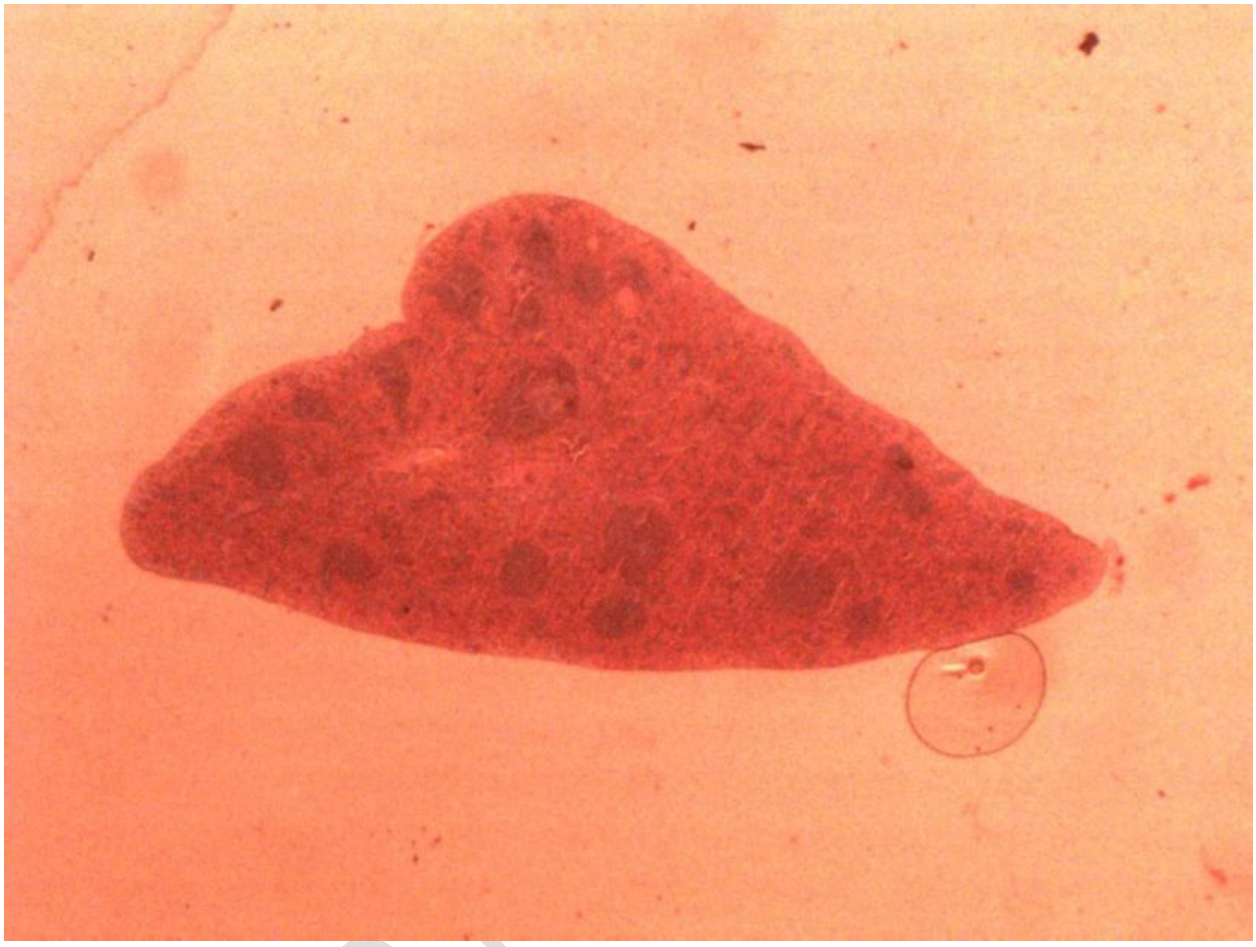

Plate 13: Stereological image of the spleen in normal control group of wistar rat showing normal morphology and splenic volume.

Plate 14: Stereological image of splenic tissue of diabetic group that received 45mg/kg of STZ only, showing splenic tissue atrophy with distorted morphology and reduced spleen volume.

Plate 15: Stereological image of splenic tissue of animals that received 45mg/kg of STZ and treated with 100mg/kg of VA only showing increased splenic volume compared to the diabetic control group.

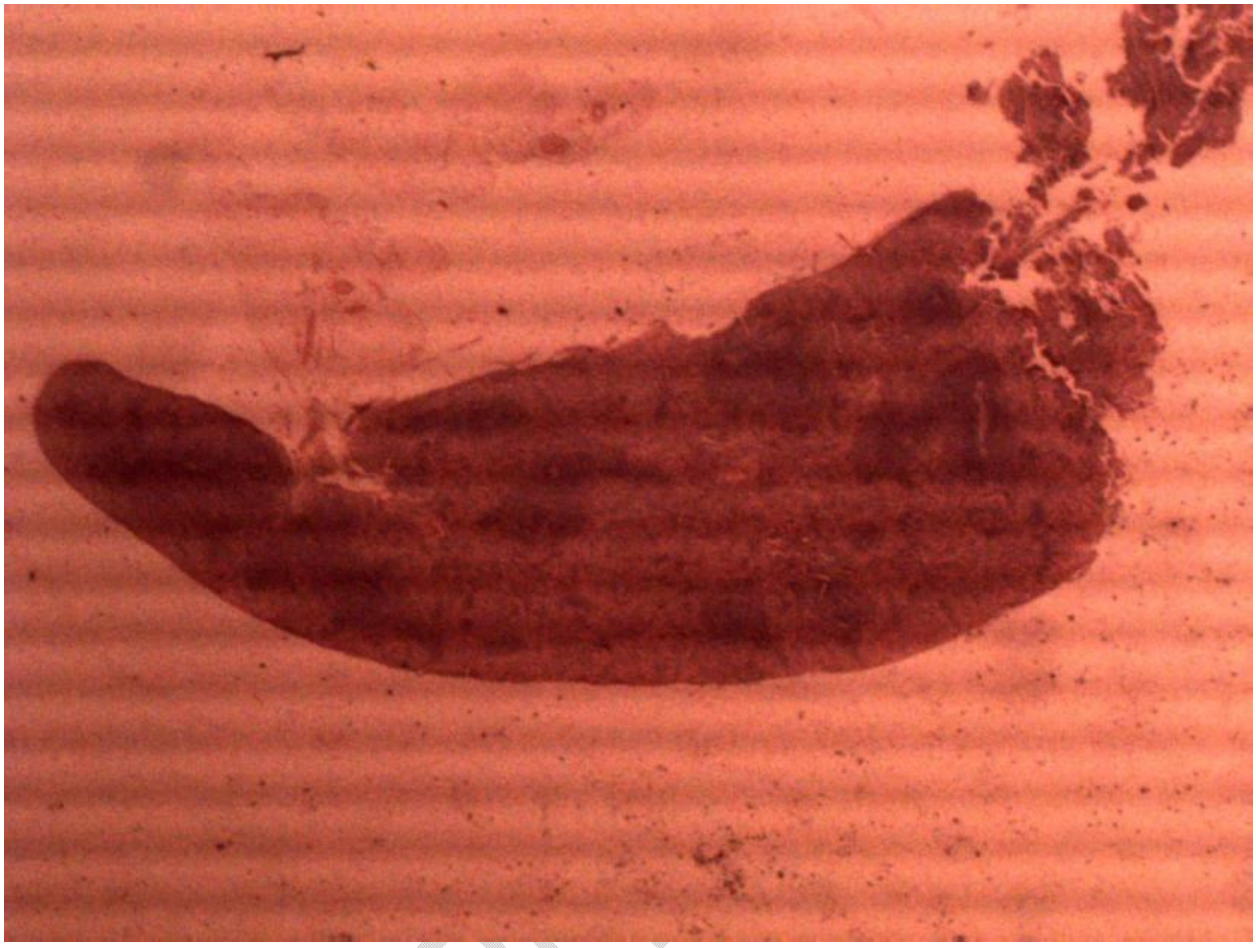

Plate 16: Stereological image of splenic tissue of animals that received 45mg/kg of STZ and treated with 100mg/kg of GL only showedincreased volume of the spleen when compared to the diabetic group.

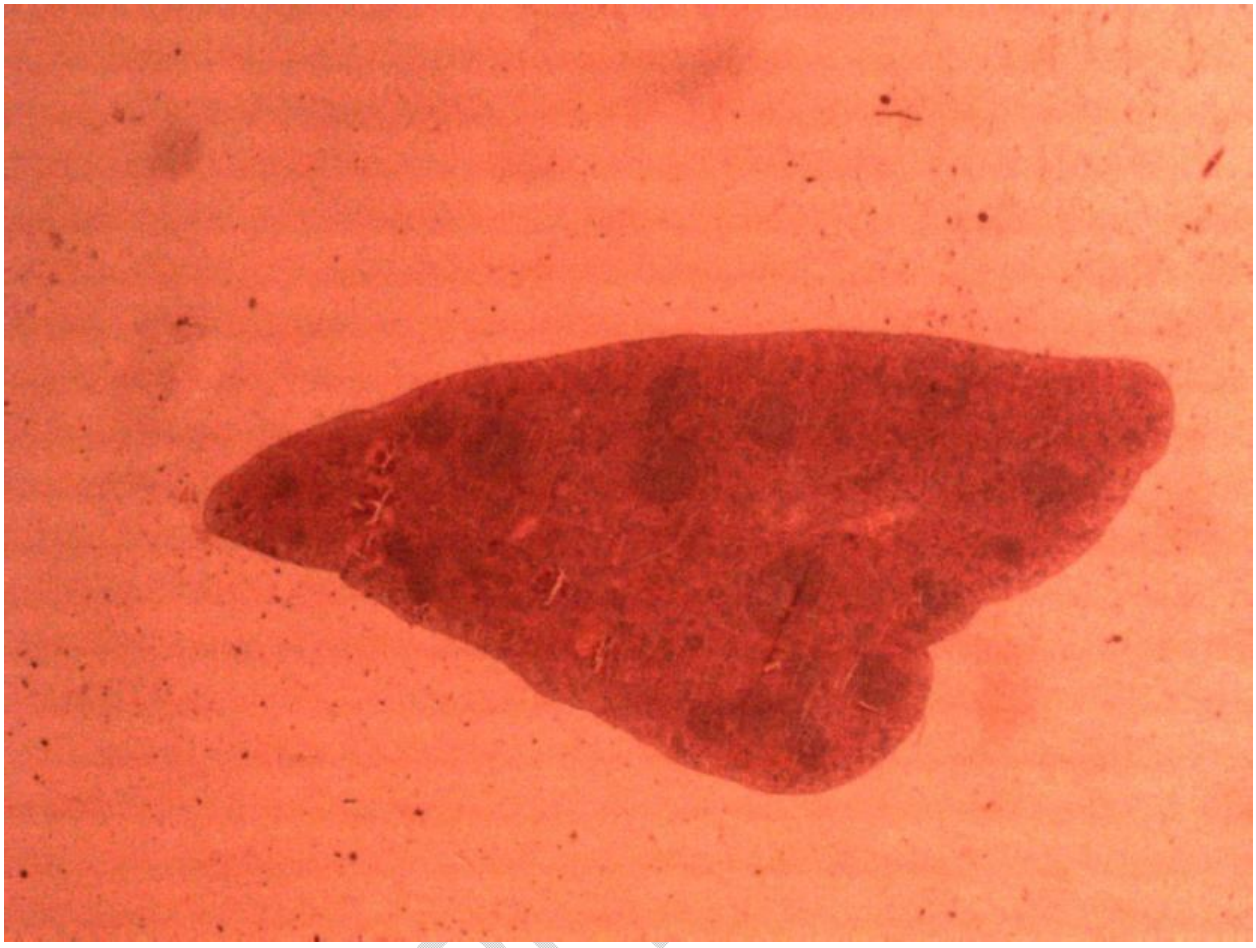

Plate 17: Stereological image of splenic tissue of animals that received 45mg/kg of STZ and treated with Metformin at 5mg/kg daily,showed significantly increased volume of the spleen when compared to the diabetic group.

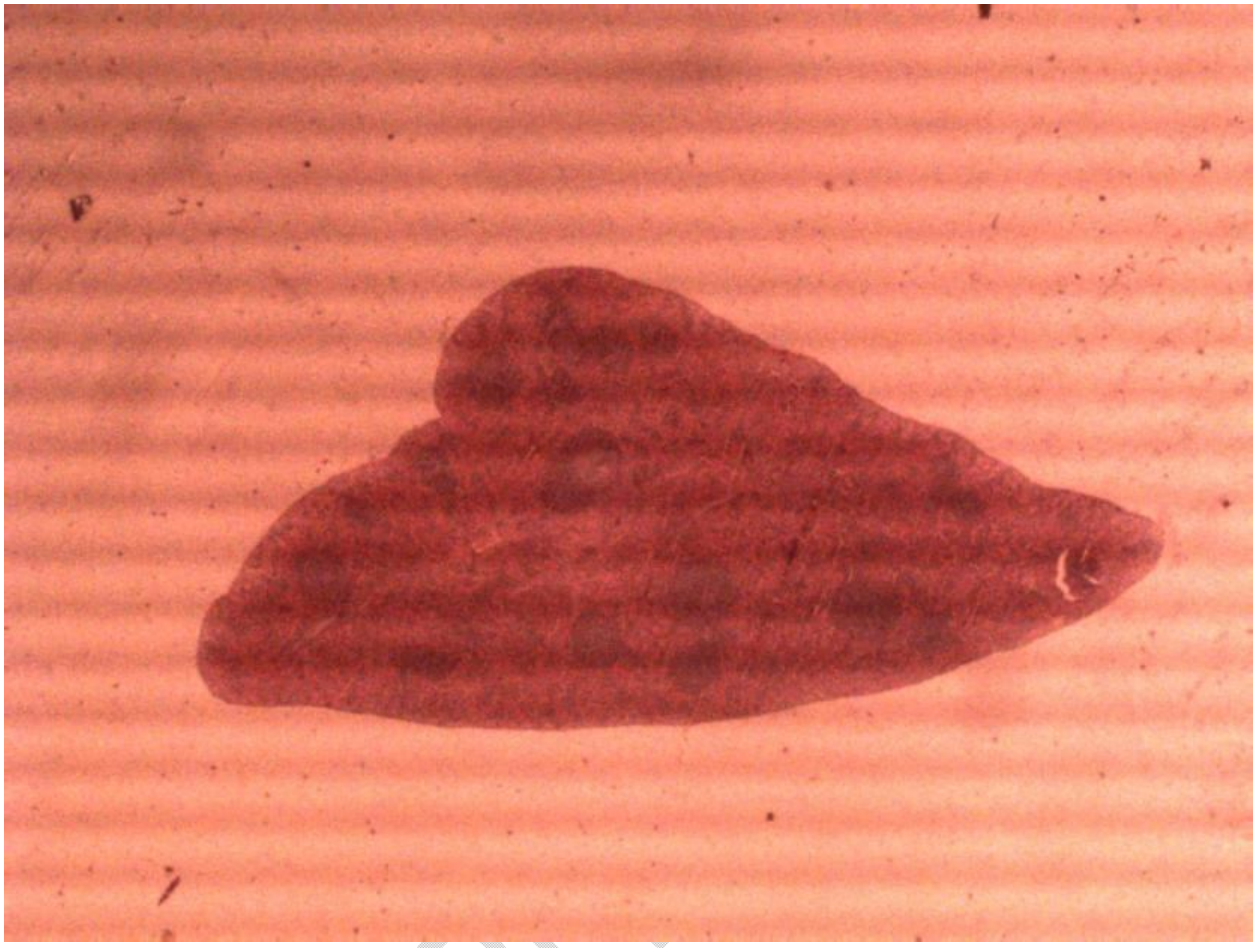

Plate 18: Stereological image of splenic tissue of animals that received 45mg/kg of STZ and treatedwith 200mg/kg of combined extract of VA+GL showed significantly increased volume of the spleen compared to the diabetic control group.

Discussion

Diabetes decreases immune response capacity, including the suppression of immune cell function, atrophy of immune organs [1,10]. Studies have been done to ascertain the potential utility of natural products as immunomodulatory agents to enhance hosts responses to diseases [26]. This study focuses on the effect of diabetes on one of the important immunologic organs-the spleen and the possible ameliorative effect of combined extract of *Vernonia amygdalina* and *Gongronema latifolium*.Diabetes-related spleen damage increases immune dysfunction, which often results in the high risks of infection, morbidity and mortality in diabetic patients.

"Herbs have been used in many capacities to improve human health and also serve as a source of natural templates for pharmaceutical synthesis of drugs. Many local communities across Afro-Asian regions of the world rely heavily on their ethno-botanical heritage to meet most of their primary healthcare needs" [20]. This study demonstrated that anti-diabetic effect of VA+GL in rats is mediated through complex multiple mechanisms.

“Immunodeficiency is one of the major causes of diabetic complications. The spleen, as a secondary lymphoid organ, acts as a site of initiation of most of the immune responses. Spleen harbors stem cells that act as precursors to insulin producing β cells of the pancreas. STZ selectively accumulates in the pancreatic β cells and induces hyperglycemic conditions through mitochondrial complication mediated glucotoxicity of the β cells”[36].

In this study, administration of the combined extracts post diabetes induction was found to reduce the increased blood glucose level and restore the normal level of serum insulin, spleen size and the spleen weight in the treated rats compared to the diabetic rats.

In the diabetic control [Group B] induced with 45mg/kg of streptozocin, the spleen showed signs of depleted white pulp compared to the enlarged size in the normal control group which is in line with the work of[24] who reported that the lymphocyte number were dramatically declined in both peripheral blood and the spleen following a distortion of the white pulp, dilation in the blood vessels, an increase of collagen deposition and a depletion in the iron particles detected in the red pulp. This indicates that the lymphocytes are stressed by diabetic toxicity with high levels of free radicals which, increases the levels of pro-inflammatory cytokines leading to programmed cell death. The white pulp of the spleen is responsible for both adaptive and humoral immunity involving the B and T-lymphocytes.Shrunken white pulp leads to a reduction in immune response, thereby making the body susceptible to diabetic complications.

In groups C and D, which received 100mg/kg of Vernonia amygdalina [Bitter leaf]and Gongronema latifolium [Utazi] respectively showed significant reversible changes in the histological integrity of the spleen compared with the results in the diabetic group. This result suggests the extracts individually has effect on the diabetic alterations that occurred in the spleen after STZ induction to cause reversible changes in the splenic cytoarchitecture, with regenerating white pulp and presence of numerous normal lymphocytes in the spleen.In group E, treated with 5mg/kg of Metformin, changes in the histology of the spleen were observed. The shrunken white pulp seen in the diabetic group B was seen regenerating with increased size in this group. This shows that Metformin has anti-oxidant properties in line with [37]. There was a significant increase in the number of white blood cells which help to strengthen the body's immune response.

In group F that received 100mg/kg of both *V.amygdalina* and *G.latifolium* showed similar histological profile to that of the normal control. This suggests a possible regeneration of the splenic cell functions and a significant reversal of diabetic insults on the splenic cytoarchitechure by the combined extract.This observation is in line with previous studies on the potentials of Vernonia Amygdalina, Gongronema Latifolium and Azadirachta Indica to cause a regeneration of pancreatic beta cells of STZ induced diabetic rats. [2, 5,14]. The effects of the plant extracts were more pronounced when used in combination. This study observed that the improvement in the histology of the spleen was more remarkable in the group that received the combined extract [VA+GL] than in the groups that received individual extracts, [VA and GL]. In another study by [24], the administration of 100mg/kg of Whey Proteins restored the histological integrity of the spleen following STZ induced diabetic degenerative changes were seen. Plant extracts can be useful in reversing diabetic insults on the histology of the spleen as was discovered from this study.

The findings of this research are consistent with previous research on the regenerative potential of Vernonia Amygdalina, Gongronema latifolium, and Azadirachta indica in restoring pancreatic beta cells in STZ-induced diabetic rats [2]. Moreover, the synergistic impact of the mixed extract [VA+GL] was more pronounced in improving the histology of the spleen compared to the individual extracts [VA and GL] in this study. These results align with research by [24], which illustrated that the administration of whey proteins at a dosage of 100mg/kg restored the histological integrity of the spleen in the presence of STZ-induced diabetic degenerative changes. Therefore, the use of plant extracts shows promise in reversing diabetic-induced damage to the spleen's histology, as evidenced by the findings of this study.

Staining intensity of the white pulp by Perl's Prussian blue in the spleen sections of the normal control and the treated groups [C, D, E and F] groups were weakly positive, by contrast to the Diabetic group which showed higher intensity staining. The immunosuppressive states induced by hyperglycemia is largely responsible for the decline in lymphocyte population and subsequent increased hemosiderin deposition in the red pulp area as a consequence of impaired phagocytic activity [24]. The findings of [13] further support the current study's results, as they reported that the administration of whey protein to diabetic rats improved the lymphocyte population in the white pulp of the spleen. This improvement may be attributed to the inhibition cascade of the programmed cell death pathway, indicating a potential mechanism by which whey protein restores lymphocytic activity. Additionally, the restoration of iron deposition to approximately normal levels observed in the study suggests a further improvement in lymphocytic function. Therefore, the use of whey protein as a therapeutic agent holds promise for enhancing lymphocyte activity and restoring the normal functioning of the spleen in diabetic conditions. Changes observed in the cytoarchitecture of the spleen suggested that Whey Proteins could enhance immune response. This suggests that the ethanolic extract in single or combined doses administered reversed splenic cell destruction from STZ induced diabetes.

The immunosuppressive states induced by hyperglycemia is largely responsible for the decline in lymphocyte population and subsequent increased hemosiderin deposition in the red pulp area as a consequence of impaired phagocytic activity[24]. This agrees with the findings in another study by [13]who reported that the improvement of the lymphocyte population in the white pulp by Whey Protein treated diabetic rats may be due to the inhibition cascade of the programmed cell death pathway. Whey Proteins was found to restore the iron deposition to the approximately the normal level, indicating improvement of lymphocytic activity. Changes observed in the cytoarchitecture of the spleen suggested that Whey Proteins could enhance immune response. This suggests that the ethanolic extract in single or combined doses administered reversed splenic cell destruction from STZ induced diabetes.

The stereological analysis of the spleen showed significant decrease of the splenic pulp volume density in the diabetic group when compared to the control group and the treated group of Wistar rats. Reducing the presence of splenic pulp was mainly due to the decrease in the volume density of all structural components of the white pulp. This observation aligns with another study by [30],following administration of Dexamethasone at 150mg/kg body weight, which showed significant decrease of the splenic pulp volume density and significant increase of the connective tissue volume density.The cytoarchitecture of the spleen was restored following treatment with the combined extracts of VA+GL.

Stereological studies also revealed that *combined extracts of VA+GL* treatment remarkably improved the volume of the splenic white pulp depleted by STZ induced diabetes.
The point-counting method was utilized to study the volume densities of the following tissue compartments: red pulp; white pulp [this compartment was divided in two sub compartments: follicles and per arteriolar lymphocyte sheath]; marginal zone; and connective tissue.
Stereology is a number of mathematical and statistical methods that permit the evaluation of 3-dimensional structural information from 2-dimensional sections [or histological slices]. Thus, researchers obtain important quantitative structural information, such as the volume, surface area or numbers of cells within described regional lines. The need for such quantitative information biological studies is of importance when evaluating the effect of various experimental treatments on any specific organs, tissues and cells in the body. Spleen volumes were significantly increased in animals treated with VA+GL and individual extracts of VA and GL.Stereological studies also revealed that *VA+GL* treatment remarkably improved the volume of the splenic tissues and the numerical density depleted by STZ diabetes.
Possible antidiabetic mechanism of *VA+GL* in STZ induced diabetes is through the regeneration of atrophic splenic structural integrity and its strong antioxidant potential. Diabetes is known to involve oxidative stress and changes in lipid metabolism. Gongronema latifolium has been shown to possess anti-hyperglycemic, anti-lipidemic, antioxidant and anti-inflammatory properties. [35], showed that ethanolic extracts of GL leaves have anti-hyperglycemic effects in Streptozocin induced diabetic rats, thought to be mediated through the activation of hexose and glucose -6-phosphate dehydrogenase, G6PDH. VA strengthens the immune system through its effect on cytokine regulation[33]. A study by [32], showed ferulic acid provides protection against hyperglycemia induced, oxidative stress mediated splenotoxicity by regulating the plasma insulin level, blood glucose level, intracellular inflammation and mitochondria dependent intrinsic pathway of apoptosis in the spleen.

This study highlighted that the spleen volume of diabetic control group [970±250.98][CE = 0.035] was significantly [$p<0.05$] lower when compared to normal control group [2398.7±352.70] [CE = 0.018] and group F [2548±871.34] were significantly [$p<0.05$] higher when compared to the volume of the diabetic control group [970±250.98] [CE = 0.023]

Conclusion

From this study, it could be inferred that combined and individual ethanolic extracts of ***VA+GL***possesses anti-diabetic and antioxidant properties that can mitigate the impact of STZ induced diabetes on the spleen of Wistar rats.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Abu-Taweel, G., Ajarem, J., Ebaid, H. and Rady,A. [2013]. Behavioral Changes Induced by Prenatal Acute Endotoxemia in Mice Offspring.*Pakistan Journal of Zoology*, 45, 359-369.
2. Akpaso, M. I., Atanghwo, I. J., Akpantah, A., Fischer, V. A., Igiri, A. O., and Ebong, P.E., [2011]. "Effect of combined Leaf Extracts of Vernonia amygdalina [Bitter Leaf] and *Gongronema latifolium* [Utasi] on the Pancreatic B-Cells of Streplozocin-Induced Diabetic Rats*". British Journal of Medicine and Medical Research, 1 [11]*, 24-34.
3. Alhassan, A.J., Sule, M.S., Aliyu, S.A. and Aliyu, M.D., [2009]. Ideal Hepatotoxicity in Rats Using Carbon Tetrachloride [CCL_4*]. Bayero Journal of Pure and Applied Science*, 2 [2], 185-187.
4. Anani SE, Eru EM, Okon DE, Uruakpa KC, Ugbem TI, Igiri AO [2020]. The Effect of Averrhoa Carambola (Star Fruit). Aqueous fruit extract on the Hippocampal Astrocyte Expression following Diazepam induced Neurotoxicity In Wistar rats. Trop J Nat Prod Res, 4[12]: 1170-1173
5. Atangwho, I.J., Ani, I. F., Agiang, M. A. Effiong, G. S., Ebong, P. E., [2010]. "Comparative Effect of Chlorpropamide and Combined Leaf Extracts of Azadirachta Indica and Vernonia amygdalina on Blood Glucose and Biochemical Parameters of Alloxanized Rats". *Journal of Medical Sciences*, 1[6], 248-253.
6. Atangwho, I.J., Ebong, P.E. Eyong, E.U. Eteng, M.U. Uboh, F.E. [2007]. *Vernonia amygdalina* Del: A potential prophylactic anti-diabetic agent in lipid complication. *Global Journal of Pure and Applied Science*. 18[1]: 103-106.
7. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweva, K., Ezennia, E.C., Atangbayila, T.O., [2008]. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South-western Nigeria. *Tropical Journal of Pharmaceutical. Research* 7, 1019-1024.
8. Boadu, A. A., Asase, A. [2017]. Documentation of Herbal medicines used for the treatment and management of human diseases by some communities in southern Ghana. Evidence Based complementary and Alternative Medicine, vol.2017, Article ID 3043061,12 pages
9. Cole, E.C [1943] Studies on hematoxylin stains. Stain Technol, 18:142
10. Ebaid, H., [2014]. Promotion of Immune and Glycemic Functions in Streptozocin Induced Diabetic Rats Treated with Un-Denatured Camel Milk Whey Proteins. *Nutrition and Metabolism [London], 11, 31.*
11. EBAID, H., 2014b. Neutrophil depletion in the early inflammatory phase delayed cutaneous wound healing in older rats: improvements due to the use of indentured camel whey protein. Diagnos. Pathol., 4:9:46

12. Ebaid, H., Metwalli, A., Al-Tamimi, A. and Allam, A. [2015]. Effect of STZ-Induced Diabetes on Spleen Rats: Improvement by Camel Whey Proteins. *Pakistan Journal of Zoology*, 47[4],1109-1116.
13. EBAID, H., SALEM, A., SAYED, A. AND METWALLI, A., [2011]. Whey protein enhances normal inflammatory responses during cutaneous wound healing in diabetic rats. Lipids Health. Dis., 10:235.
14. Ebong, P. E., Atangwho. I. J., Eyong, E. U., Egbung, G. E. [2008]. The antidiabetic efficacy of combined extracts from two continental plants, Azadirachta indica and *Vernonia amygdalina*. *American Journal of Biochemistry and Biotechnology*, 43, 239-244.
15. Eru EM, Anani SE, Paulinus SO, Mesembe OE, Uruakpa KC, Umoh NM, Oku M E, Akpaso MI, Igiri AO. 2022. Scopolamine-Induced Alzheimer's Disease in Wistar Rats; Aqueous *Talinum Triangulare* Potency on the Hippocampal Nissl Bodies and Long-Term Learning and Memory. *Trop J Nat Prod Res*, 6[1]:117-122.
16. Eru ME, Gabriel UU, Samson OP, Kelechi CU, Michael EO, Sadeyeng EA, Nwosu CB, Theresa N, Nsikak MU and Anozeng OI. 2021a. Restorative Potentials of Aqueous *Telfairia occidentalis* Seeds Extract on the Hippocampal Nissl Granules and Short-Term Memory in Scopolamine Hydrobromide-Induced Alzheimer's Type Cognitive Dysfunction Rats. Trop J Nat Prod Res, 5[1]:182-187
17. Eru EM, Paulinus SO, Udo-Affah GU, Uruakpa KC, Oku ME, Anani SE, Umoh NM, Akpaso MI and Igiri AO l. 2021b. Hippocampal Astrogliotic Reduction in Scopolamine Hydrobromide-Induced Alzheimer's Cognitive Dysfunction Wistar Rats Following Administration of Aqueous Extract of *Telfairia occidentalis* [Hook F.] Seeds. Niger J Physiol Sci, 36: 241 – 244
18. Eru EM, Paulinus S O, Igiri AO, Akpaso MI. 2020a. Enhanced effect of aqueous extract of *Telfairia occidentalis* seed on the microstructure of the hippocampus of scopolamine hydrobromide-induced cognitive dysfunction rats. Asian J of Res in Neuro, 3[1]: 5-10.
19. Eru EM, Paulinus SO, Gabriel UU, Kelechi CU, Michael EO, Edet UI and Igiri AO. 2020b. Neurobehavioural enhancement in scopolamine hydrobromide-induced alzheimer type cognitive dysfunction in rats following administration of ethanol seedextractof *telfairia occidentalis*[hook.f.] cucurbitaceae. Trop J Nat Prod Res, 4[7]:282-285
20. Farombi, E. O., [2003]. African indigenous plants with chemotherapeutic potentials and biotechnological plants with production of bioactive prophylactic agents. *African Journal of Biotechnology*, 2, 662-671.
21. Fowler, M.J. [2008]. "Microvascular and macrovascular complications of diabetes." *Clinical Diabetes*, 26[2], 77–82.
22. Gundersen, H. J. G., BENDTSEN, T. F., KORBO, L., MARCUSSEN, N., Møller, A., Nielsen, K. & West, M. J. [1988]. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Apmis*, *96* [1-6], 379-394.
23. Gwarzo, M.Y., Ahmadu, J.H., Ahmad, M.B and Dikko, A.U. [2014]. Serum Glucose and Malondialdehyde levels in Alloxane Induced Diabetes Rats Supplemented with Methanolic Extract of Tacazzea apiculata. *International Journal of Biomedical Sciences*, 10 [4], 236-242.
24. Hossam Ebaid, Al-Timma, J. Ali Metualli, [2015]. Effect of size induced diabetes on spleen of wistar rates improvement by Camel Whey proteins.

25. Jana Besser, Maren Stropahl, Emily Urry, Stefan Launer. Hearing Research. Volume 369, November [2018], pgs 3-14
26. Krifa, M., Bouhlel, I.,Ghedira-Chekir, L &Ghedira,K. [2013]. Immunomodulatory and cellular anti-oxidant activities of an aqueous extract of Limoniastrum guyonianum gall. J. ethnopharmacol., 2: S0378-8741.
27. L. K. Nguma, *Health Seeking and Health Related Behaviour for Type 2 Diabetes Mellitus among Adults in an Urban Community in Tanzania*, University of Otago, [2010].
28. Luo, X., Jiang, Y., and Lee, K. S., Lin, C., Izevbigie, E. B., Lee, S., and K. S., [2017]. Lactone (Vernodalinol) from Vernonia amygdalina Extracts". Pharmaceutical Biology, 49 [5], 464-479.
29. Maregesi, S. [2016]. *Traditional Medicines and Treatment of Diabetes in Tanzania*.
30. Mitevska, E., Petrova I. K., &Kostovska N. [2015]. Changes of spleen in wistar rats Exposed to Therapeutic Doses of Dexamethasone and Medoxyprogesterone Acetate Evaluated by Stereological Parameters .
31. Okolie, U.V., Okeke, C.E, Oli, J.M., Ehiemere, I.O., [2008]. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. *African Journal of Biotechnology*, 7, 4581-4585.
32. Sumit Ghosh, Parames C, [2018]. Ameliorative role of ferulic acid against diabetes associated oxidative stress induced spleen damage. Food and Chemical Toxicology vol.118, pg272-286.
33. Sweeney, C. J., Mehrotra, S., Sadaria, M.R., Kumar., S., Shortle, N.H., Roman Y., Sheridan, C., Campell R.A,, Murray, D.J., Badve, S & Nakshatri, H [2005]. The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograftnmodel of breast cancer. Molecular Cancer Therapeutics, 4[6]: 1004
34. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. [2014]. The many faces of diabetes: a disease with increasing heterogeneity. Lancet. 2014;383:1084–94.
35. Ugochukwu, N.H., Babady, N.E, Cobourne, M., Gasset, S.R., [2003]. The effect of *Gongronema latifolium*extracts on serum lipid profile and oxidative stress indices in hepatocytes of diabetic rats. *Journal of Biosciences*, 28,1-5.
36. Wu, J., Yan Liang-Jun [2015]. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, metabolic syndrome &obesity . Volume 8: pgs 181-188
37. Yoshifumi S. [2015]. Metformin and Inflammation: its potential beyond Glucose-lowering effect. Endocrine metabolism immune disorders. Volume 15[3]: 196-205
38. Zimmet, P., Alberti, K.G.M.M., Shaw, J. [2001]. Global and societal implications of the diabetes epidemic. *Nature*, 414, 782–787.
39. Eru EM, Gabriel UU, Ifiok FB, Kelechi CU, Samson OP, Michael EO et al. Efficacy of aqueous extract of *talinum triangulare* on the microanatomy of the hippocampus and short-term memory of scopolamine hydrobromide-induced alzheimer's type cognitive dysfunction rats. *Niger. J. Physiol. Sci.* (2024): 39(in press).
40. Ifiok FB, Eru EM, Mathias OA, Nsikak MU, Williams AN, Florence NO et al. Effect of *musa paradisiaca* stem juice on acute status epilepticus, hippocampal histology and behaviour in pentylenetetrazole – induced wistar rats. *Niger. J. Physiol. Sci.* (2024): 39(in press).

41. Ifiok FB, Gabriel UU, Eru EM, Nsikak MU, Mathias OA, Williams AN et al. Histochemical study of Nissl substance and astrocytes in a pentylenetetrazole-induced model of epilepsy treated with *musa paradisiaca* stem juice. *Niger. J. Physiol. Sci.* (2024): 39(in press).