Nigerian Journal of Pharmaceutical and Applied Science Research, 11(3):13-19; September, 2022. ISSN: 2971-737X (Print); ISSN: 2971-7388 (Online). Available at www.nijophasr.net

Assessment of the Therapeutic Potential of the Synergy of *Solenostemous monostachyus* and Vernonia amygdalina in Fish induced with Oxide of Calcium

[*1]Eneni Roberts Inala and [2]Thomas Ohwofasa Ikpesu

[1]Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria,
[2]Department of Chemistry, Federal University Otuoke, Bayelsa State, Nigeria.

ABSTRACT

Background: The therapeutic capability of the synergy of *Solenostemous monostachyus* and *Vernonia amygdalina* against calcium oxide was investigated.

Methods: One hundred and fifty post juveniles of *Chrischthys nigrodigitatus* were purchase from a fish farm in Otuoke, Bayelsa State. The fish were acclimatized for fourteen days, divided into five groups, and exposing to different ranges of treatments; Group A received 250mg/L CaO; group B received 250mg/L CaO and 1500mg/L *V. amygdalina*; group C received 250mg/L CaO and 1500mg/L *S. monstachyus*; group D received 250mg/L CaO and 750mg/L each of (*V. amygdalina* and *S. monstachyus*) and the control was left untreated. Water quality were kept within normal ranges by using an aerator. The fish were removed from each aquarium on the 28th day of the experiment, blood sample was taken from the caudal vein, dissected and the brain was carefully removed, and homogenized in a bottle. The blood was centrifuged for 15 minutes to ensure that the serum was clearly separated, and was then kept at -80 °C until analysis, while the brain was centrifuged and supernatant collected in a tube and used as an enzyme source. All the markers were measured spectrophotometrically.

Results: The activity of acetylcholinesterase and glutathione- s transferase were interfered in the oxide-induced fish, although it was improved when treated with each plant's extract alone, but restored in the synergy therapy. Similar observations were noticed in glucose, protein, urea, creatine, and globulin levels.

Conclusion: The present breakthroughs in phytochemical research are driven by the discovery of novel physiologically active compounds with potential medicinal applications. While the high chemical density and variety of phytochemical components in plant extracts have been credited with natural product effectiveness in pharmaceutical research. These findings are encouraging; nonetheless, these plant extracts should be used with caution because the abuse n misuse can be hazardous to one's health.

Keywords:- ***Solenostemous monostachyus, Vernonia amygdalina, Chrischthys nigrodigitatus*** **Synergy, Markers, Phytochemical**

1. INTRODUCTION

Modern research has demonstrated that a wide variety of plants can neutralize or detoxify poisons and protect the body from the toxic effects of pharmaceuticals and other chemicals. Plants have been used to treat a wide range of ailments for thousands of years. In many impoverished nations and rural areas today, medicinal plants remain the main source of healthcare [1]. It has long been known that medicinal herbs or plants can be a valuable source of therapies or curative help. In health systems around the world, the use of medicinal plants has assumed a dominant position; they are not only used to treat illnesses but also as possible materials for preserving health and conditions [2]. Plant extracts stand out as potential synthetic drug substitutes because they deliver beneficial biologically active metabolites with a range of advantages, including immune-modulating, growth-promoting, antioxidant-enhancing, antidepressant, digestive-enhancing, appetite-stimulating, and hepato-protective effects when used as directed [3,4]. Compared to synthetic medications, medicinal herbal extracts are similarly accessible, affordable, and frequently more biodegradable [5]. The Silver Catfish (*Chrischthys nigrogitatus*) is one of the most significant groups of farmed fin fish in freshwater aquaculture and is very useful in toxicological

[*] Corresponding author: Email: ikpesuto@fuotuoke.edu.ng; Phone: +234803231141

research [6]. It is an omnivorous fish that consumes debris, seeds, insects, and bivalves [7]. However, the catfish is one of the most conspicuous species on the decline in terms of population density and catch in Nigerian rivers and lakes. The species is found in tropical rivers and freshwater lakes throughout Western and Central Africa [8]. *C. nigrodigitatus* is a valuable commercial fish due to its high protein content and hardy meat, and it is a key component of many Nigerians' diets. Despite its significance, *C. nigrodigitatus* has received little research in terms of biology [8], and little or no attention is devoted to its dwindling population in freshwater ecosystems and the need for protection. According to [9], fish is one of the most endangered categories of animals, with some of the greatest extinction rates. Dudgeon [10] asserts that freshwater fish in Asia and Africa are more vulnerable to extinction than in other emerging countries. To prevent the extinction of this fish, researchers must investigate its biology, assemblage, catch statistics, and habitats in lakes and rivers. These would serve as a guide for understanding the causes of its diminishing population and determining appropriate conservation and management methods. *Solenostemous monostachyus* is a significant herb found in West and Central Africa, Asia, and Europe. The plant is an important herb found throughout West and Central Africa. It grows as an annual weed in cultivated areas and rocky savannahs. It is aromatic and slightly succulent, growing up to 100 cm tall [11]. The aerial parts of the plant are traditionally used in various decoctions by the Ibibios of Nigeria's Niger Delta to cure stomach ulcers, fever/malaria [12] hemorrhoids, and other inflammatory disorders. Water, proteins, lipids, calcium, phosphate, essential oil, and Phyto components such as diterpenoids, flavonoids, coumarin, and polyphenols have all been discovered in the leaves of *S. monostachyus* [13]. *V. amygdalina* is an African native that grows in tropical and subtropical climates. It has a pleasant perfume, and its leaves are used as a spice and in traditional medicine [14].*V. amygdalina* can develop dense monospecific thickets, out-competing native plants and reducing natural biodiversity. The essential oil of *V. amygdalina* includes eugenol and appears to have some therapeutic action [15]. For fish farmers to profit economically from intensive farming techniques, they began utilizing synthetic antibiotics and other chemotherapeutic medications to keep farmed fish healthy. Likewise, to avoid economic losses due to sanitary deficiencies, veterinary medications such as calcium oxide are frequently employed in aquaculture to prevent and treat disease outbreaks simultaneously [16]. The use of these medications in aquaculture seems to be purely commercial and unsustainable, as they also present other challenges like the development of drug resistance in fish pathogens, immune suppression, environmental pollution, and the buildup of chemical residues that may be dangerous to human health [17]. As a result, several countries around the world, including those in the United States, the European Union, and Asia [18], have strong requirements for aquatic items that are free of chemicals and medicines. The need to substitute dietary supplements or components that can improve fish health, growth, and feed utilization—and ultimately guarantee safety. The number of studies that have shown that plant extracts can replace manmade chemicals like antibiotics and other chemotherapeutic medications in aquaculture have increased over the past two decades. Medical herbal extracts stand out as potential substitutes for synthetic drugs in aquaculture because, when administered properly, they produce beneficial biologically active metabolites with a range of benefits, including immune modulation [19], growth promotion, antioxidant enhancement, antidepressant, digestive enhancement, appetite stimulation, and hepato-protective effects. Typically, plants have secondary metabolites that can enhance health either singly, in combination or both. In contrast to pharmaceutical medications, medicinal plants frequently contain many compounds that operate catalytically and synergistically to produce a combined impact that is greater than the sum of the activities of the individual constituents [20]. By accelerating or delaying the major medicinal component's absorption into the body, the combined effects of these plants tend to boost the active ingredient's activity. Although *S. monostachyus* and *V. amygdalina* are both potent pharmacological agents, little is known about their combined detoxifying and stress-relieving actions. This should contain the background of the subject, statement of the problem, and relevant literature on the subject.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

The materials used are; De-chlorinated water, Oxide of calcium, Insulin syringe, EDTA bottle, Tissue paper, Weighing balance, Heating mantle, Filter paper, Condenser, Thimble jacket, Volumetric flask, Spectrophotometer, Bunsen burner, Autoclave, Spatula, Beaker, Test tube, Micropipette, Cork-borer, Conical flask, Retort stand, Flat bottom flask, Plain sterile container, Refrigerator, Petri dish, Rotary evaporator, Aerators, Ethanol, Aquaria.

2.1.2 Biological Materials

The biological materials used were fresh stems of *S. monostachyus* and *V. amygdalina*, as well as *C. nigrodigitatus*,

2.2 Methods

2.2.1 *Collection and Handling of Experimental Fish*

150 post-juvenile of C. *nigrodigitatus* utilized in the experiment was purchased from a fish pond farm in Otuoke, Bayelsa state. To avoid hyperactivity, injury, and shock, the fish weighing an average of 23gm and 17cm in length were placed in well-aerated containers, and were checked for pathological signs before being rinsed with a 1% $KMnO_4$ solution and moved to glass aquaria (100x50x50cm) containing de-chlorinated tap water and acclimatized for fourteen days. The fish were given 30% protein pellets, the water was changed regularly, and the immunological parameters of the water were kept within the approved range using aerators. About 20 fish died in the first seven days, but no deaths were detected after that. After the acclimation, 130 fish were in good health, with no death.

2.2.2 *Cultivation of the plants*

A private garden in Otuoke provided the seeds for *S. monostachyus* and *V. amygdalina* which were then planted in the botanical gardens of Federal University Otuoke. The plants were grown in a well-drained and sunny site with the pH and nutrient contents of the garden soil determined. The herbs were grown in a garden 20 by 20 feet in size. After receiving twice daily irrigation for three months, the plants were fully developed and ready for toxicological and therapeutic research.

2.2.3 *Crude Extraction*

Fresh *V. amygdalina* and *S. monostachyus* were taken from the school garden, cleaned, and air dried separately in the laboratory for 30 days at room temperature (22 °C). They were grounded separately and then blended to a fine powder.50g each of the dry powder from *S. monostachyus* and *V. amygdalina* were measured into flasks A, B, and C using an electronic weighing scale. A = dry powder of *S. monostachyus*, B = dry powder of *V. amygdalina*, and C = an equal blend of both plants. Utilizing the volumetric flask, 500 ml of distilling water was dispensed into A, B, and C and extracted for three days. thereafter, the three samples were filtered separately with filter paper. The filtrates were then dried in a rotary vacuum evaporator. The crude extract was transferred to the beaker and refrigerated until needed. The phytochemical compositions of the plants were analyzed in the laboratory, and the following chemical compositions were determined quantitatively; Alkaloid, Terpene, Flavonoids, Saponins, Anthraquinones, Tanin, Phlobatanins, Phenol, and Cardiac glycoside.

2.2.4 *Bioassay test*

The fish were divided into five groups of five fish apiece, with replicates of each group. They were treated to calcium oxide concentrations commonly used in aquaculture, as well as the other treatments listed below. Group A received 250mg/L CaO; group B received 250mg/L CaO and 1500mg/L *V. amygdalina*; group C received 250mg/L CaO and 1500mg/L *S. monstachyus*; group D received 250mg/L CaO and 750mg/L each of (*V. amygdalina* and *S. monstachyus*) and the control was left untreated. Water quality readings were kept within normal ranges by using an aerator. The fish were removed from each aquarium on the 28th day of the experiment, and immediately, a blood sample was taken using a technique that has been shown to minimize dilution by tissue fluids: puncturing the caudal vessels with an insulin syringe needle and aspirating 0.2–0.4ml of mixed arterial and venous blood into a heparinized insulin syringe and kept in heparinized blood collecting duct. The blood was centrifuged for 15 minutes to ensure that the serum was clearly separated, and it was then kept at -80 °C until analysis The fish was dissected immediately after the blood was collected, and the brain was carefully removed, weighed, rinsed with 50 M Tris-HCl buffer, and homogenized in a bottle with a tissue homogenizer (Polytron PT-6100, USA). (1% Triton X and 0.1% PMSF) Tris-HCl buffer As a homogenizing solvent, Sigma Aldrich was used. The sample was centrifuged for 20 minutes at 12,000 x g. (GRACE High-Speed Refrigerated Centrifuge, India). The supernatant was transferred into a separate tube and used as an enzyme source [21].

2.2.5 *Biochemical Analysis*

The approach of [22] was used to measure blood glucose, in which a drop of fresh blood from a caudal decapitated fish was touched with a glucose strip inserted in a standard glucometer. The total protein content of the fish was determined using the Kjeldahl method, which was adapted from AOAC International method 981.10 [23]. In summary, 2.5 mL of raw blood sample was hydrolyzed for 2 hours in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) with 3.5 mL hydrogen tetraoxosulphate (iv) acid and two copper catalyst tablets. After cooling, H_2O was added to the hydrolysates before neutralization and titration. Total protein content was determined by multiplying the total nitrogen in the sample by both the standard conversion factor of 6.25 and the species-specific conversion factor of 5.6 [24]. Serum urea and serum creatinine and serum bilurubin were measured spectrophotometrically. The BCG (Bromocresol Green) albumin test kit was used to assess the albumin concentration of the fish serum, and absorbance at 628 nm was measured against a blank. Subtracting albumin from total protein content yielded globulin content. Acetylcholinesterase activity was measured with a commercial kit developed by Bohringer Mannhim that was based on the Ellman spectrophotometric assay technique adapted for micro plates as reported by [24] utilising acetylthiocholine iodide as a substrate. Using the GST Assay Kit, the total GST activity in the fish plasma was calculated. The Assay Kit makes use of CDNB, which works with the

broadest range of GST isozymes. Using the thiol group of glutathione, GST catalyses the conjugation of L-glutathione to CDNB. GS-DNB Conjugate, the reaction's end product, absorbs at 340 nm. The sample's GST activity directly correlates with the rate of increase in absorption.

2.3 Statistical Analysis

The therapeutic effects of various treatments on the fish were presented as mean ± SE. The difference between the control and the various treatments, as well as within the treatments, was examined using a 95% confidence level using student's t-test and one-way analysis of variance SPSS (14.0 version), with $P < 0.05$ being significant.

3. RESULTS

There were no significant different ($p \geq 0.05$) in the water quality metrics (pH, temperature, dissolved oxygen, alkalinity, and hardness) between the three experimental groups and the control group or within the experimental groups during the investigation (Table 1). The therapeutic properties of *V. amygdalina*, *S. monstachyus* and their synergistics in *C. nigrodigitatus* exposed to the oxide of calcium in is shown in Figure 1 and 2. The AChE activity in the brain of control fish was 83 ± 0.60 mg-1 protein, whereas it was 47 ± 0.20, 67± 0.40, 69 ± 1.50, and 81± 3.20 mg-1 protein in the treatments A, B, C, and D. No significant different ($p > 0.05$) between the control and therapeutically treated fish ($p > 0.05$). Comparing the Cao-treated fish to the control and other treatments, the AChE activity was significantly ($p < 0.05$) affected in the induced fish (Figure 1). While in the blood of the control fish, the AChE activity was 42.50 ± 0.20 mg-1 protein. It was 38.10±0.11, 38.20 ±0.40, 39.90 ± 0.31, and 43.10 ± 0.50 mg-1 protein in the treatments A, B, C, and D. No significant different ($p > 0.05$) between the control and all the treatments (Figure 1). The GST activity in the brain of groups B, C, and D were 36 ± 0.60, 29 ± 0.30, and 21± 0.70 umg-1protein respectively. It was 67± 0.30 umg-1protein in group A fish and 25 ± 0.10 umg-1protein in the control fish. Between the control fish and group A fish, as well as between group A fish and other treatments, there are significant differences in the enzyme activity ($p < 0.05$) (Figure 1). The activity of GST in the blood of groups B, C, and D were 61.40 ± 1.30, 57.80 ± 2.10, and 43.10 ± 0.80 umg-1protein respectively, it was 68.30 ± 1.40 umg-1protein in group A fish and 40.80± 0.54 umg-1protein in the control fish. GST activity varies significantly ($p < 0.05$) between the control fish and group A,B and C fish, as well as between group A fish and group D fish ($p < 0.05$). (Figure 1).

Table 1: Physiochemical parameters of the test media during sub-lethal exposure of *C. nigrodigitatus* to different concentrations (mg/l) of Ca after 28 days of exposure

Parameters	Control Mean ± SE	A Mean ± SE	B Mean ± SE	C Mean ± SE	D Mean ± SE
pH	6.95 ± 1.02 [a]	6.80 ± 0.20[a]	7.10 ± 0.20[a]	6.90 ± 0.10[a]	7.20 ± 0.30[a]
Temperature (^{O}C)	26.10 ±0.40	26.07± 0.01[a]	26.40± 0.06[a]	26. 80± 1.30[a]	25.80 ± 1.20[a]
Alkalinity (mg/l)	14.30±1.06[a]	15.30 ±0.30[a]	15.2±0.30[a]	14.50 ±0.20[a]	14.90 ± 0.50[a]
Total hardness (mg/l)	27.20 ±0.10[a]	25.20 ± 0.10[a]	25.20 ± 0.10[a]	26.20 ±0.20[a]	25.10 ± 0.70[a]
Dissolve Oxygen (mg/l)	8.20 ±0.35[a]	25.20 ± 0.10[a]	8.23 ±0.20 [a]	8.50 ±0.01[a]	7.80 ± 0.10[a]

Mean with the same superscript in the row are significantly different * ($p \leq 0.05$)
A: 2.50mg/l Cao; B: 2.50gm/l and 1500mg/l *V. amygdalina*, C: 2.50gm/l and 1500mg/l *S. monstachyus*, D: 2.50gm/l and 750mg/l of *V. amygdalina* and *S. monstachyus*

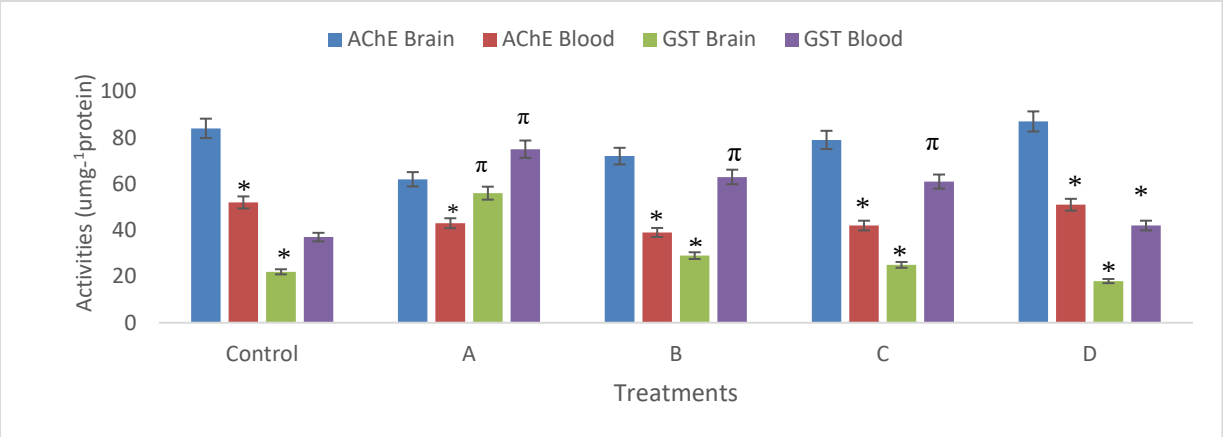

Figure 1: Efficacy of *S. monostachyus* and *V. amygdalina* on altered AChE and GST activities in *C. nigrodigitatus* induced with Calcium Oxide. Data presented as mean ± SE. Different symbol above bars indicate significant differences between the control and the experimental groups ($p \leq 0.05$).

The fish treated with toxicants only (group A) had the highest glucose levels (56 mg/100 ml). It was 26 mg/100 ml in the control fish; 32 mg/100 ml, 37 mg/100 ml, and 28 mg/100 ml in *V. amygdalina* (B), *S. monstachyus* (C), and their synergy (D) treated fish respectively. Except for the synergy therapy (Treatment D), the glucose level varies significantly ($p < 0.05$) between the control and other treatments. The marker level also differs considerably ($p < 0.05$) between pairs A and B, A and C, and A and D. No significant difference between treatments B and C ($p > 0.05$). D and the control are comparable. The total protein concentration was annihilated in the calcium oxide-treated fish, and it varies significantly ($p < 0.05$) between the CaO-treated fish and other treatments including the control (Figure 2). The serum urea level was highest in the group A-treated fish (60.40 mg/100ml), and lowest in the control fish (10.30 mg/100ml). Group B, C, and D treated fish had 40.20, 39.10, and 11.70 mg/100ml serum levels respectively. Except for the synergy treatment, the urea level varies significantly ($p< 0.05$) between the control and the other treatments. Similarly, the marker level differs significantly (p 0.05) between A and B, A and C, and A and D. There was no statistically significant difference ($p > 0.05$) between treatments B and C. Both the control and D are comparable. The globulin concentrations in the control was 0.39 mg/100ml, in the treatment A, B,C and D the concentrations were;1.30, 0.42,0.38 and 0.32 mg/100ml respectively. The globulin level in the fish in treatment A varies significantly ($p < 0.05$) with the other treatments including the control. The creatine content in the control was 0.33 mg/100ml, while in treatments A, B,C, and D were;0.73,0.62,0.40, and 0.33 mg/100ml respectively. The creatine level varies significantly ($p < 0.05$) between A and treatments C, D, and the control. The bilirubin concentrations in the control, treatments A, B,C, and D were 0.51, 1.13, 0.79, 0.72 and 0.52 respectively. The globulin level in the control fish varies significantly ($P < 0.05$) with treatments A, B, and C, and is comparable with the D treatments (Figure 2).

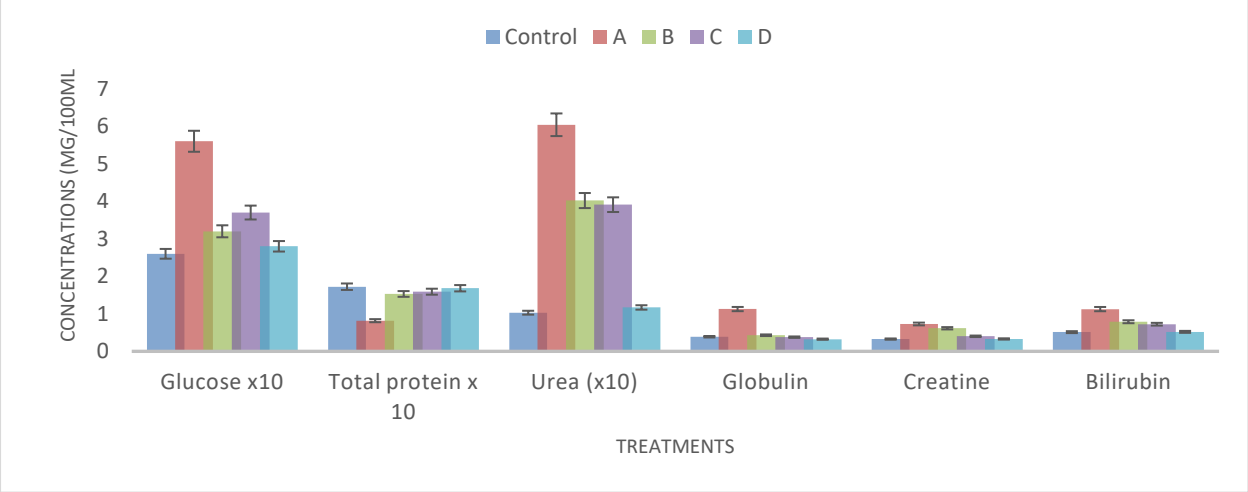

Figure 2: Synergistic effects of the aqueous leaves extract of *S. monostachyus* and *V. amygdalina* on altered biochemical parameters in *C. nigrodigitatus* induced with Calcium Oxide. Data presented as mean ± SE. The symbol above bars indicate significant differences between the control and the experimental groups ($p \leq 0.05$).
Important findings should be presented with clarity and precision. Where necessary, results should be illustrated with figures or tables, but this should be kept to a minimum.

4. DISCUSSION

The physicochemical measures used in the analysis did not show any appreciable variations in the water quality metrics between the experimental groups and the control group or among the experimental groups. Therefore, any noticeable changes in the fish would be attributed to the toxicity of calcium oxide. This study examined the blend of crude extracts of *S. monostachyus* and *V. amygdalina* in *C. nigrodigitatus* subjected to calcium oxide for its anticholinesterase, antioxidant, and antihyperglycemic properties. The findings indicate that the combination of these plant extracts may be able to lessen the negative effects of toxins on the body. The activity of AChE was inhibited in the oxide-induced fish, although it was improved when treated with each plant's extract alone, but restored, even enhanced in the synergy therapy. It has been observed that the essential oil of plants like *Rosmarinus officinalis* L affects mood and cognition, resulting in a considerable improvement in performance and general memory quality in healthy adults [25]. According to [26], the essential oil of *Turkish officinalis* exhibited a notable anti-BChE action, as did methanol preparations of *T. officinalis*. A better synergism was discovered by [27] between ethanolic extracts of *Syzygium aromaticum* (clove) and *Allium sativum* (garlic) against a bacterium. The role of oxidative stress in the generation of reactive oxygen species (ROS) has been implicated in the pathogenesis of diabetes and its consequences. Antioxidants can scavenge these ROS or neutralize these radicals, increasing oxidative stress, which is thought to be a common pathway linking many processes of diabetic problems development [28]. The effect of calcium oxide on GST activity, as well as changes in glucose, protein, urea,

creatine, and globulin levels, demonstrated that the toxicant induces an imbalance in the fish system. However, as seen in treatment D, the plant's extract mixture provided an antidote to the toxicant's effects. The presence of aalkaloids, terpenes, flavonoids, saponins, deoxy-sugar, anthraquinones, tannins, phlorotannin, and cardiac glycoside was detected in the crude extracts of the two plants, which is indicative of their therapeutic imperious. It is well known that flavonoids interact with a variety of biomolecules to act as powerful antioxidants and to influence the activity of a number of enzymes [29]. Similarly, the lowering of blood glucose in treatments B, C, and D, with optimal synergy, is similar to the findings of [30] who acknowledged a hypoglycemic effect with flavonoids, steroids, and triterpenes.

5. CONCLUSION

The discovery of novel physiologically active chemicals with potential therapeutic applications is the driving force behind the current advances in phytochemical experiments involving herbal-drug interactions. While the great chemical density and variety of phytochemical components in herbal extracts have been credited with the success of natural products in medication discovery, their synergistic effects also make them effective for detoxifying a wide range of pollutants and treating a wide range of diseases. This study used calcium oxide as an aberration in a biological system to assess the therapeutic capability of the combination of *S. monostachyus* and *V. amygdalina* against a hazardous chemical. These results are encouraging in particular because scientists are very interested in the possible anticholinesterase and antioxidant properties of plant extracts to preserve health and protect against neurological and oxidative stress as well as diabetes. The use of these plant extracts should be done so carefully because misuse and abuse could be harmful to one's health. In developed communities, there have been a number of newsworthy incidents involving potentially fatal side effects from using herbal remedies or conventional medications.

Acknowledgments

We wish thank all laboratory staff in the Department of Biology, Federal University Otuoke for their assistance during sample collection and laboratory work.

Conflict of Interest

There was no conflict of interest between the two Authors.

Contribution of the Authors

Dr. Eneni Inala was fully in charge of the plant cultivation and phytochemical analysis while Dr. Thomas Ikpesu was in charge of biochemical and bioassay tests. The Compilation of this paper was organized collectively by the two authors.

6. REFERENCES

[1] Ferreira AC, Proença MLM, Serralheiro ME,M. Araújom. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J Ethnopharmacology. 2006; 108:31–37.

[2] Caruso DD, Lusiastuti AM, Taukhid SJ, Komarudin OO, Legendre MM. Traditional pharmacopeia in small scale fresh water fish farms in West Java, Indonesia: an ethno veterinary approach. Aquaculture. 2013; 416–417.

[3] Koffi NT, Marie–Solange AA, Emma ZG Noel H. Ethnobotanical study of plants used to reat arterial hypertension in traditional medicine, by abbey and Krobou population of Agboville (Cote d'ivoire). European Journal of Science Research. 2009;35:85–98.

[4] Xing BN, Jin SS, Wang HH, Tang QF, Liu JH, *et al.*, New diterpenoid alkaloids from Aconitum coreanum and their anti-arrhythmic effects on cardiac sodium current. Fitoterapia. 2014; 94: 120-126.

[5] Ogbonnia SO, Nkemehule FE, Emma N, Anyika EN, Evaluation of acute and subchronic toxicity of Stachytarpheta angustifolia (Mill) Vahl (Fam. Verbanaceae) extract in animals. African J Biotechnology. 2009;8:1793–1799

[6] Wen CC, Kuo YH, Jan JT, Liang PH, Wang SY, *et al.*, Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. Journal of Medical Chemistry. 2007; 50: 4087-4095.

[7] Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. Nat. Rev Drug Disc.2008; 7: 926-935.

[8] Ayinde BA, Omogbai EK, Amaechina FC. Pharmacognosy and hypotensiveevaluation of Ficusexasperata Vahl (Moraceae) leaf. ActaPoloniaePharmaceutica. 2007; 64:543-546

[9] Chakraborty SB, Hancz CC. Application of phytochemicals as immune stimulant, antipathogenic and antistress agents in fin fish culture. Reviews in Aquaculture. 2011; 3.103–119.

[10] Fabrini RA, Bocedi E, Del Grosso L, Morici G, Federici A, Palleschi A. Erythrocyte glutathione transferase: a novel biomarker to check environmental pollution hazardous for humans. Biochem Biophys Res Commun. 2012; 426: 71–75.
[11] Mba CE, Menut C. Aromatic plants of tropical Central Africa. Flav Frag J. 2004;9:315–317.
[12] Noce A, Fabrini R, Dessì M, Bocedi A, Santini S, Rovella V. Erythrocyte glutathione transferase activity: a possible early biomarker for blood toxicity in uremic diabeticpatients. Acta Diabetol. 2014; 51: 219 –224.
[13] Lee Y, Yuk D, Lee J, *et al.*, Epigallocatechin-3-gallate prevents lipopolysaccharide induced elevation of β-amyloid generation and memory deficiency. Brain Res. 2009;1250: 164–174.
[14] Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch. Biochem. Biophys. 2005;322:339–346.
[15] Bhuvaneswari R, Balasundaram C. Traditional Indian herbal extracts used invitro Against growth of the pathogenic bacteria–Aeromonas hydrophilia. Israeli Journal of Aquaculture–Bamidgeh. 2006;58:89–96.
[16] Jagetia GC, Reddy TK. The grape fruit flavonone naringin protects mice against doxorubicin induced cardiotoxicity. Journal of Molecular Biochemistry. 2014; 3: 34-49.
[17] Singh AB, Yadav DK, Mauria R, Srivastava AK. Antihyperglycaemic activity of a-amyrin acetate in rats and db/db mice. Natural Product Research. 2009; 23;876-882.
[18] Ayinde BA, Omogbai EK, Amaechina FC. Pharmacognosy and hypotensiveevaluation of FicusexasperataVahl (Moraceae) leaf. ActaPoloniaePharmaceutica. 2007;64:543-546.
[19] Adnan M, Rasul A, Hussain G, et al., Ginkgetin: a naturalbiflavone with versatile pharmacological activities, Food and Chemical Toxicology. 2020; 145, Article ID 111642.
[20] Kaur M, Jindal R. Oxidative Stress response in liver, kidney and gills of *Ctenopharyngodon idellus* (Cuvier&Valenciennes) exposed to chlorpyrifos. Biol. Med. 2017; 1: 103-112.
[21] Mariotti F, Tome D, Mirand PP. Converting nitrogen into protein—Beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. 2008;48:177–184.
[22] Stefani EC, Philipson LH, Prince VE, Kinkel MD. Blood sugar measurement in zebrafish reveals dynamics of glucose homeostasis. Zebrafish. 2010;7(2):205-213.
[23] Galloway TS, Handy RD. Immunotoxicity of organophosphorous pesticides. Ecotoxicology. 2003; 12: 344–363.
[24] Byers DM, Irwin LN, Moss DE, Sumaya IC, Hohmann CF. Prenatal exposure to the acetylcholinesterase inhibitor methane sulfonyl fluoride alters forebrain morphology and gene expression, Developmental Brain Research. 2005;158(1-2): 13–22.
[25] Ogbonnia SO, Nkemehule FE, Emma N, Anyika EN. Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Fam. Verbanaceae) extract in animals. African J Biotechnology. 2005; 8:1793–1799.
[26] Orhan IB, Sener MI, Choudhary A, Khalid B. Acetylcholinesterase and butyrylcholinester-ase inhibitory activity of some Turkish medicinalplants. J. Ethnopharmacol. 2004;91:57-60.
[27] Atteiaa HG, Husseinb EM. *In vitro* antibacterial and synergistic effects of some plant extracts against *Staphylococcus aureus* and *Klebsiella pneumonia*. J. Antimicrob. 2014;129: 338–346.
[28] Rahimi RN, Shekoufeh L, Bagher AA. Mohammed A. Review of the role of antioxidants in the management of diabetes and its complications Biomedicine & Pharmacotherapy. 2005; 59: 365-373.
[29] Exarchou V, Nenadis N, Tsimidou IP, Gerothanassis A, Troganis D, Boskou O. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. Journal Agricultural and Food Chemistry. 2002; 50(19):5294-5299
[30] Singh AB, Yadav DK, Mauria A, Srivastava AK. Antihyperglycaemic activity of a-amyrin acetate in rats and db/db mice. Natural Product Research. 2009; 23:876-882.