Received: нр !LJWXf 2021 | Revised On WdzyŜ 2021 | Accepted лр Wdzfê 2021

Research Article
Journal of Research and Review in Science, 31-39
Volume 8, June, 2021

DOI:10.36108/jrrslasu/1202.80.0140

ORIGINAL RESEARCH

Modulation of the Complement system by Zingiberacea Aframomum Melegueta in mice-induced Schizophrenia

Thona Wusu [1], Victoria Ajile [1], Segun Adeola [1], Adedoja Wusu [1], Akintayo L. Ogundajo [2]

[1]*Department of Biochemistry, Faculty of Science, Lagos State University, Nigeria*

[2]*Department of Chemistry, Faculty of Science, University of Lagos, Nigeria*

Correspondence
Segun Adeola, Department of Biochemistry, Faculty of Science, Lagos State University, Nigeria.
Email:Segun.adeola@lasu.edu.ng

Abstract:

Introduction: Schizophrenia is a severe mental disorder among nationalities of the world, with a vast social and economic effect. The complement system, a central part of innate immunity and an adjuvant of adaptive immunity, has been implicated in the pathogenesis of Schizophrenia. However, the pathway to the inappropriate or chronic complement activation associated with several neurodegenerative disorders, such as Schizophrenia, are yet to be understood, thus complicating scientific therapeutic or antipsychotic drugs with evidence of adverse effects on the complement system linked to Schizophrenia. *Zingiberaceae Aframomum Melegueta* is used for various pharmacological purposes in Nigeria.

Aims: This study aimed to investigate the effect of *Zingiberacea Aframomum Melegueta* in modulating the complement system linked to Schizophrenia.

Materials and Methods: Male mice were induced with Schizophrenia using ketamine and dexamethasone, after which they were treated with an aqueous extract of Zingiberacea Aframomum Melegueta (200mg/ml and 400mg/ml) respectively for 7 days. Blood and brain samples were obtained, and biochemical parameters of the complement system were analysed spectrophotometrically.

Results: The aqueous extract of Zingiberacea Aframomum Melegueta contained significant phytochemical which includes flavonoids, tannins, saponins, steroids, phlobatannins, terpenoids, and cardiac glycoside. Treatment with the plant extract initiated a significant decrease in the level of C-reactive protein, complement component 3, complement component 4 and dopamine, in a dose-dependent manner in the subject compared to control.

Conclusion: Our study findings suggest that the antipsychotic property of alligator pepper may be used in the management and treatment of Schizophrenia. Thus, a probable natural therapy for Schizophrenia.

Keywords: Schizophrenia, complement system, ketamine, dexamethasone, alligator pepper

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Schizophrenia is considered to be a rare disorder in childhood, but it becomes increasingly common in adolescence. Schizophrenia is a severe psychiatric disorder that leads to chronic symptoms [1]. The positive and negative signs of schizophrenic patients include thinking disorder, blunted effect, attention, or memory problems, due to the distortion of reality [2], hallucination, delusions which is as a result of increased sub-cortical release of dopamine, which augments D2 receptor activation [3]. Consequently, patients with Schizophrenia lack coping skills and have social and occupational functioning failings [4] resulting in a low level of quality of life [5]. It is plausible that the multicomponent complement system has more than one association with schizophrenia susceptibility, pathophysiology, and illness course, understanding of which will bring a new perspective for possible immunomodulation and immunoprotection of the disease [4].

Complement is a central part of the innate immunity that serves as the first line of defense against foreign and altered host cells [6]. Complement proteins collaborate as a cascade to opsonize pathogens and induce a series of inflammatory responses helping immune cells to fight infection and maintain homeostasis. The complement system can be initiated depending on the context by three distinct pathways classical (CP), lectin (LP), and alternative (AP), each leading to a common terminal pathway from C5 to C9. These complement components form the final membrane attack complex [7]. Several lines of evidence suggest that immunological factors contribute to Schizophrenia. Since 1989, the role of complement, a major effector of innate immunity and an adjuvant of adaptive immunity, has been explored in Schizophrenia. Two or more groups have reported increased activity of C1, C3, C4 complement components in Schizophrenia.

Furthermore, many cultures widely use alligator pepper, whose scientific name is Zingiberaceae Aframomum Melegueta, in Nigeria for various purposes. The pharmacological usage of the plant includes serving a potential antihypertension role` in young and elderly hypertensive patients [4]. It has also been shown to inhibit the growth of many bacterial organisms suggesting that the plant extract has broad-spectrum activity on bacteria [8]. Other laboratory-based studies have shown that Alligator pepper can halt the multiplication of virus [9], while Inegbenebor and Ebomoyi, (2012) have suggested that fetal macrosomia (birth weight >4kg) could be prevented by the use of an intraperitoneally administered aqueous extract of alligator pepper as a vaccine or food supplement). Also, the aqueous extract of the leaves of Alligator pepper has been found to lower blood glucose in alloxan-induced diabetic rats faster than the conventional anti-diabetic drugs such as diabenese [10].

Although antipsychotic medication is the mainstay of treatment for people with Schizophrenia, which can be helpful, still, it leaves some people with distressing symptoms or disabling adverse effects. Traditional herbs are now serving a tremendous advantage in treating Schizophrenia; their usefulness dates back to 2000 years [11] is may be due to the presence of active ingredients, antipsychotic properties of some herbs that have complement inhibitory properties. Therefore, our study investigated the effects of alligator pepper on the complement systems of Schizophrenia induced mice.

2. MATERIAL AND METHODS

2.1 Plant Materials

Alligator pepper fruits were obtained at Iyana-Iba market in Ojo Local Government area of Lagos State, South-Western part of Nigeria. It was prepared using hot water extraction (HWE). The alligator fruit was processed and ground. Sixty grams of the ground sample was suspended in 600ml of water, then autoclave at 121 ^{0}C for 15 min. which then was centrifuged and the supernatant was lyophilized (freeze-dried).

2.2 Animals

Adult male Swiss albino mice weighing 20–25 g used for this study were obtained from the Central Animal House, College of Medicine, University of Lagos. The animals were housed five per plastic cage (42×30×27cm) in a controlled environment at room temperature (25 ± 1 0C) with a 12:12 h light/dark cycle. They were fed with standard rodent pellet food and water ad libitum throughout the experimental period. They were acclimatised for at least one week before the commencement of the experiments.

2.3 Experimental Design

Animals were divided into nine groups with 5 animals each. While one group served as control and received normal saline, the remaining groups were further divided into eight. Animals were induced with ketamine and dexemethasone for 30 minutes and confirmed schizophrenic. Thereafter, treated with aqueous extract of alligator pepper according to the following groups for seven days; Ket group (ketamine), Ket + 200mg AP group (Ketamine + 200mg of Alligator Pepper), Ket + 400mg AP group (Ketamine + 400mg of Alligator Pepper), ket + STD (Ketamine + Haloperidol: standard drug), Dex group (Dexamethasone), Dex + 200mg AP group (Dexamethasone + 200mg of Alligator Pepper), Dex + 400mg AP group (Dexamethasone + 400mg of Alligator Pepper), Dex + STD (Dexamenthasone + Haloperidol: standard drug. After treatments, blood samples were collected through ocular puncture using a capillary tube then transferred into a non-heparinized tube and the brain removed. All samples were stored at -4˚C until analysed for biochemical analysis.

2.4 Phytochemical Screening

2.4.1 Qualitative Phytochemical Screening

Phytochemical screening was carried out to establish the presence of phytochemicals such as saponins, tannins, alkaloids, flavonoids, steroids, glycosides, and cardiac glycosides.

2.4.1.1 Test for Tannins
Tannins were tested by the method of Siegelman and colleagues [12]. The water extract of crude dry powder of the alligator pepper extract was treated with alcoholic FeCl3 reagent. The blue colour indicated the presence of tannins.

2.4.1.2 Test for glycosides
Glycosides were tested by the method of Hikino and colleagues [13]. About 5ml H2SO4 was added to a 0.2g sample of crude extract, and the mixture was heated in boiling water for 15 minutes. Fehling solution was then added, and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.

2.4.1.3 Test for Saponins
Saponins were tested by the method of Kapoor and associates [14]. The presence of saponins was determined by frothing analysis. The crude extract was vigorously shaken with distilled water and was vigorously stirred with purified water and was allowed to stand for 10min and classified for saponin content as follows: No froth indicates the absence of saponins and stable froth more than 1.5cm indicated the presence of saponins.

2.4.1.4 Test for Steroids
Steroids were tested by the method of Lieberman-Burchard [15]. A chloroform solution of the crude dry extract of the alligator pepper was treated with acetic anhydride, and a few drops of concentrated H2SO4 were added down the sides of the test tube. A blue, green ring indicated the presence of steroids.

2.4.1.5 Test for flavonoids
Flavonoids were tested by the method of Sofowara and colleague [16]. A small quantity of the extracts was dissolved in dilute NaOH. A yellow solution that turns colourless on the addition.

2.4.1.6 Test for cardiac glycosides
Cardiac glycosides were tested by the method of Ayaiyeobu [17]. Keller kiliani test was performed to assess the presence of cardiac glycosides. The crude extract was treated with 1ml of FeCl3 reagent (a mixture of 1 volume of 5% FeCl3 solution and 99 volumes of glacial acetic acid). To this solution, a few drops of concentrated H2SO4 was added. The appearance of a greenish-blue colour within a few minutes indicated the presence of cardiac glycosides.

2.4.1.7 Test for phenols
Phenols were tested by the method of Mc Donald and his co-workers [18]. Test extract was dissolved in a ferric chloride solution. Blue-Black or brown coloration indicates the presence of phenol.

2.4.1.8 Test for Phlobatannins
The analytical method is, according to Ejikeme and associates [19]. To each sample (0.30 g) weighed into a beaker was added 30 cm3 of distilled water. After 24 hours of extraction, aqueous extract (10 cm^3) of each wood sample was boiled with 5 cm3 of 1% aqueous hydrochloric acid. Deposit of red precipitate showed a positive test.

2.4.2 Quantitative phytochemical screening

2.4.2.1 Determination of phenolic content
The total phenolic content was estimated by the Folin Ciocalteu method as described by Singleton and associates [20] with slight modifications. The extract (1 mg. mL^{-1}) was mixed with 5 mL of distilled water, 1 mL of sodium carbonate (20%), and 1 mL of Folin Ciocalteu reagent. The mixture was allowed to stand in a water bath for 30 min at 40°C. The content of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per g dry matter (mg GAE. g-1DM). The absorbance was measured at 765 nm using a UV-Vis spectrophotometer T60 U.

2.4.2.2 Determination of total flavonoid content
The flavonoids content was determined by the aluminum trichloride method using catechin as a reference compound by Zhishen and colleagues [21]. A volume of 125µL of the extract is added to 75 µL of a 5% NaNO2 solution. The mixture was allowed to stand for 6 min, then 150 µL of aluminum trichloride (10%) was added and incubated for 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500µL with distilled water. After 15 min of incubation, the mixture turned pink, and the absorbance was measured at 510 nm.

2.4.2.3 Determination of total condensed tannin contents
The tannin contents or Proanthocyanidin were determined by the method of Broadhurst and colleagues [22] with slight modification, using catechin as a reference compound. A volume of 400µL of the extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5mL of concentrated hydrochloric acid. After 15 min of incubation, the absorbance was read at 500 nm.

2.4.2.4 Estimation of Dopamine
Dopamine was estimated by the method of Wenger and associates [23]. The catecholamine is first oxidized to red indole derivatives, adrenochrome, and nonadrenochrome respectively, which are then rearranged in alkali to strongly fluorescent trihydroxyindole, adrenolutine, and noradrenaline respectively. Previous attempt to estimate dopamine according to this principle has not been successful. The fluorescence obtained has been weak, with activation and fluorescent peaks undisguisable from

noradrenolutine. With the modification of technique, however, it has been possible to raise the fluorescence obtained from dopamine considerable and to obtain spectra which differ markedly from those of adrenolutin and noradrenolutin.

2.4.2.5 Estimation of C - reactive protein

CRP was estimated by the method of Kindmark [24]. Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particle is proportional to the CRP concentration and was measured by a spectrophotometer at a wavelength of 540nm.

2.4.2.6 Estimation of Complement Component C3

Complement C3 was measured by the method of Price and colleagues [25]. Complement component C3 in the sample precipitates in the presence of anti-human C3 antibodies. The light of the antigen-antibody complexes is proportional to the C3 concentration and can be measured by spectrophotometer.

2.4.2.7 Estimation of Complement Component C4

Complement C4 was measured by the method of Price and colleagues [25]. Complement component C4 in the sample precipitates in the presence of anti-human C4 antibodies. The light scattering of the antigen-antibody complexes is proportional to the C4 concentration and can be measured by spectrophotometer reading 540nm.

2.4.2.8 FTIR Spectrophotometry

The FTIR spectrophotometry was by Ismail and co-workers [26]. FTIR relies on the fact that most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds precisely to the bonds present in the molecules. The frequency ranges are measured as wavenumbers typically over the range 4000-600cm-1. FTIR is particularly useful for the identification of organic molecular groups and compounds due to the range of functional group side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

2.4.3 Statistical Analysis

Data were digitally analysed using statistical software package Prism Graph Pad (version 6.0). All values were expressed in ±SEM. Results were analyzed statistically using analysis of variance one-way ANOVA to identify possible differences of the samples and biochemical values. P values were considered statistically significant at $P < 0.05$.

3. RESULTS

The preliminary qualitative phytochemical screening of the aqueous extract of alligator pepper was conducted to access its phytochemical constituents. The different phytochemical constituents present in alligator pepper extract are shown in Table 1.

Table 1: Qualitative result of the phytochemical constituents in Alligator Pepper

Phytochemicals	Status
Flavonoids	+
Tannins	+
Saponin	+
Steroids	+
Phlobatannins	+
Terpenoids	+
Cardiac glycoside	+

Key: + indicate present

The quantitative phytochemical screening of antioxidants properties (flavonoids, phenol, and tannins) was conducted on an aqueous extract of alligator pepper. The phytochemical composition is illustrated in Table 2. A variable concentration of the phytochemicals was observed, which is as a result of the concentration of the aqueous extract, i.e., the higher the concentration, the higher the level of phytochemicals.

Table 2: Quantitative Result of Phytochemical Composition of Alligator Pepper

Phytochemicals	**200mg**	**400mg**
Flavonoids (mg/ml)	100.0 ± 0.2	105.3 ± 0.1
Phenol (mg/ml)	94.2 ±0.1	221.7 ± 0.2
Tannins (mg/ml)	726.8 ±0.2	1292.6 ±0.3

Table 3 shows the functional group present in alligator pepper. It was observed after the analysis that alligator pepper contains the following active group: OH, COOH, CH_2, NH, CH_3, NH_2, NO, which was obtained from peaks shown in Figure 1.

Table 3: FTIR Spectroscopy Determination of Alligator Pepper

Functional group	Frequency range (cm^{-1})
O-H bonded	3400-3200
O-H Carboxylic acid	3400-2400
C-H Alkane stretch	3000-2850
N-H stretch	3500-3100
N-H bend	1640-1550
C-CH_3 bend	1450-1375
C-N Amine	1350-1000
N=O Nitro	1550-1350

Table 4 shows the absorbance band spectra of the various functional group and also their %frequencies. This was observed by FTIR.

Table 4: Absorbance Band Spectra and Percentage Frequency

Peak	Frequency (cm^1)	%Frequency (%T)
1	3304.65 (Carboxylic acid)	32.17
2	2929.84(alkane stretch)	54.66
3	1607.59 (Nitro bend)	33.88
4	1384.28(Alkane bend)	53.03
5	1325.34 (Amine)	59.38
6	1021.2 (Alkene)	11.34
7	861.56 (Aromatic compounds)	93.66
8	792.83 (halogen)	89.75
9	569.92 (halogen)	73.17
10	512.28 (halogen)	61.13

Figure 1 shows the results of FTIR spectroscopic studies and has revealed the presence of the various functional group in the crude extract of alligator pepper.

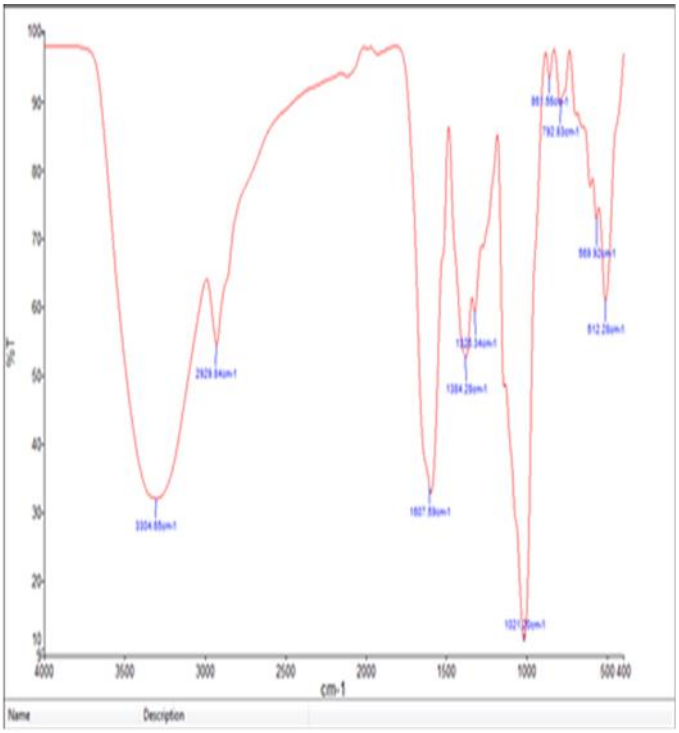

Figure 1: Graphical representation of the FTIR result of alligator pepper

Figure 2 shows the effect of aqueous extract of alligator pepper on CRP following exposure to ketamine and dexamethasone in male Swiss mice. Ketamine caused a significant increase in the level of CRP as compared to the control. Treatment with alligator pepper extract, caused a significant reduction in the level of CRP as compared to the untreated group. In fact, the level of CRP was far lower than the mice in the control group.

Also, dexamethasone caused a significant increase in the level of CRP. Treatment with alligator pepper caused a significant decrease in the level of CRP to about 65%, compared to the untreated group.

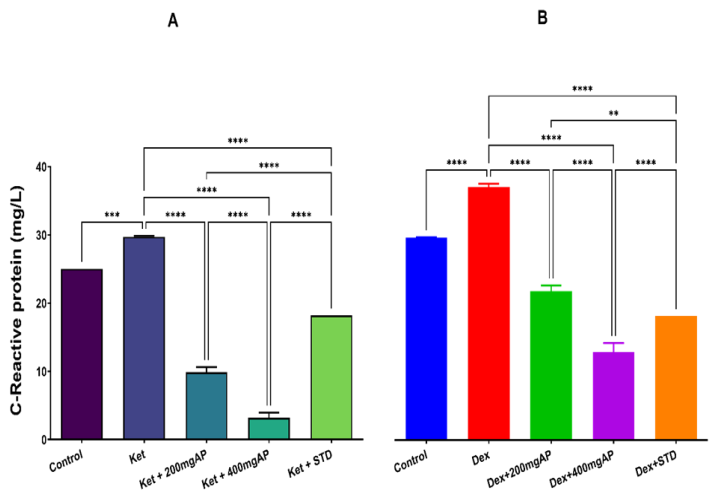

Figure 2: The effect of AP on CRP following ketamine and dexamethasone injection in male Swiss mice. The result is expressed as mean ± SEM. Bar with different alphabet are statistically different from each other (p<0,05); control vs induced * (p<0.001) ***(p<0.0001) = control vs treatment(n=5).**

Figure 3, shows a high level of complement 3 protein, in ketamine and dexamethasone schizophrenia induced group. The ketamine schizophrenia treated group has a significant reduction, with a 92% decrease at 200mg of alligator pepper, whereas the 400mg and standard drug caused a significant decrease the level of C3 to 95%. Showing the effectiveness of the 400mg of the alligator pepper extract.

Accordingly, in the dexamethasone schizophrenia induced group, a significant decrease of about 79% was observed in 400mg dosage of the alligator pepper extract. Similar, the standard drug had a significant reduction.

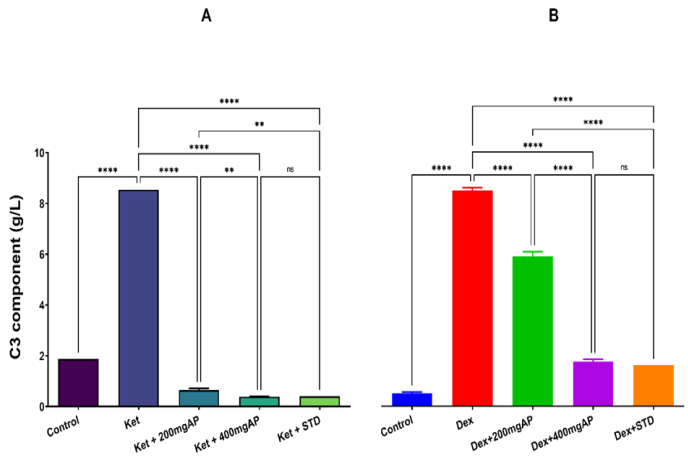

Figure 3: The effect of AP on C3 following ketamine and dexamethasone injection in male Swiss mice. The result is expressed as mean ± SEM. Bar with different alphabet are statistically different from each other (p<0,05); control vs induced * (p<0.001) ***(p<0.0001) = control vs treatment(n=5).**

Figure 4 shows the level of C4 in mice groups and treated with aqueous extract of alligator pepper at different concentrations. In the dexamethasone induced schizophrenia group, the alligator pepper extract has a significant effect of more than 50% reduction. The 200mg does not have a significant decrease, thus the 400mg has a considerable difference, as compared with the standard drug which has 83% decrease.

However, in the ketamine induced schizophrenia group, the 200mg alligator pepper extract has a reduction of 55%. In comparison, the 400mg alligator pepper extract has a 78% decrease. In contrast, the standard drug has a 20% decrease on the level of C4 protein.

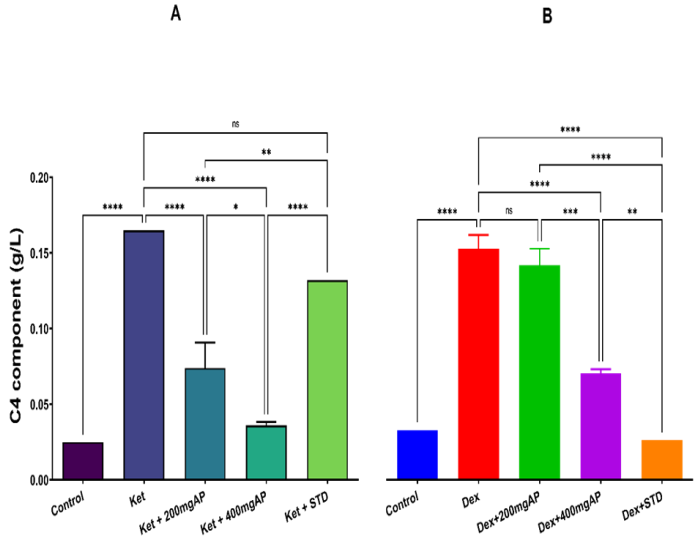

Figure 4: The effect of AP on C4 following ketamine and dexamethasone injection in male Swiss mice. The result is expressed as mean ± SEM. Bar with different alphabet are statistically different from each other (p<0,05); control vs induced * (p<0.001) ***(p<0.0001) = control vs treatment (n=5).**

Figure 5 shows the level of dopamine at a different concentration of aqueous extract of alligator pepper. There was a significant decrease in the level of dopamine at both the ketamine and dexamethasone treatment group in a dose dependent manner. The level of dopamine was reduced by 64% and 55% at 400mg and 200mg, respectively, while the standard drug has only 10% reduction.

Similarly in the dexamethasone group, 400mg dosage caused a reduction of 93%, also the 200mg caused a decrease of 89%, and thus the stand drug reduced the level of dopamine to about 55%.

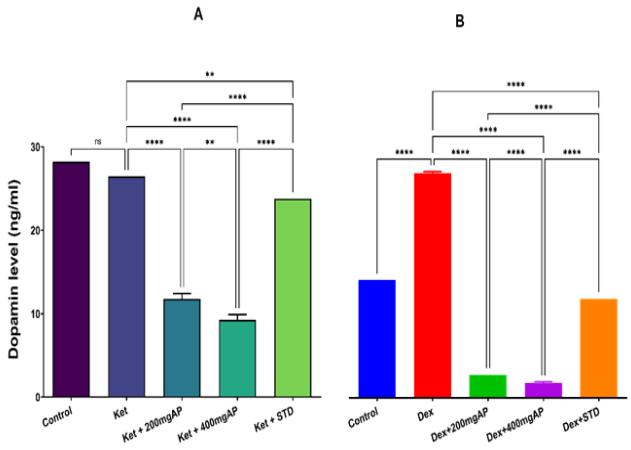

Figure 5: The effect of AP on dopamine following ketamine and dexamethasone injection in male Swiss mice. The results are expressed as mean ± SEM. Bar with different alphabet are statistically different from each other (p<0,05); control vs induced * (p<0.001) ***(p<0.0001) = control vs treatment(n=5).**

4. DISCUSSION

It is assumed that increased oxidative stress may be relevant to the pathophysiology of Schizophrenia [12]. Molecular mechanisms contributing to oxidative stress are very complex and not fully understood yet. Although oxidative stress may not be the main cause, oxidative damage to important biomolecules has been suggested to be a common pathogenic process contributing to deteriorating course and poor outcome [13]. Moreover, neurotransmitters (dopamine, adrenaline, and noradrenaline) present in excess in the brain can be autoxidized to form relatively large amount of hydrogen peroxide.

As experimented and shown in the result, the presence of phytochemicals in the alligator pepper extract, denotes a psychoactive property in the treatment of Schizophrenia and are attributable to the presence of secondary plant metabolites. Likewise, drugs that block dopamine reduce schizophrenic symptoms. In many cases, the effect of these phytochemicals on human CNS might be lined either to their ecological roles in the life of the plants [14]. Many phytochemicals have been shown to exert neuroprotective actions in animal and cell culture models of neurological disorders. For example, a chalcone (safflor yellow B) can protect neurons against ischemic brain injury, and piceatannol can protect cultured neurons against Aβ-induced death. Epidemiological studies of human populations and experiments in animal models of neurodegenerative disorders have provided evidence that phytochemicals in fruits and vegetables can protect the nervous system against disease [15], including Schizophrenia.

Polyphenolic compounds can participate in modulation of different signaling pathways, thus influencing the fate of cells [16] including nerve cells via influencing the neuronal survival, regeneration, development, or death [17]. Polyphenolic compounds possess also antimutagenic ability [18], vasodilating [18], antithrombic [19], antiapoptotic, and anti-inflammatory [19] effects.

The high content of flavonoids observed in the aqueous extract of alligator pepper in the study enhances its ability to modulate neuronal function and prevent neurodegeneration, which indicated its ability to improve memory and learning, by protecting vulnerable neurons, enhancing existing neuronal function, or stimulating neuronal regeneration. They are thus signifying that it may serve as a potential neuroprotective agent against the underlying pathology associated with Schizophrenia suggested by [20]. For flavonoids to access the brain, they must first cross the blood-brain barrier (BBB), which controls the entry of xenobiotics into the brain [21].

Phenolic compounds (catechins and epicatechins) which were also present in high proportion in alligator pepper have been reported to protect neurons against a range of oxidative and metabolic insults, which includes the protection of dopaminergic neurons from damage induced by 6-hydroxydopamine [22] and also protection of retinal neurons against ischemia-reperfusion injury; [23] and reduction of mutant huntingtin misfolding and neurotoxicity in Huntington's disease models. [21].

The reduction observed in dopamine level in this study by aqueous extract of alligator pepper in mice induced with Schizophrenia is in line with a report by Meltze, who reported that antipsychotic drugs are dopamine D2 receptor antagonists, and the dopamine hypothesis proposes that overproduction of dopamine or an increase in the number or sensitivity of dopamine receptors is responsible for Schizophrenia. According to this hypothesis, the excess dopamine or extra sensitivity to this neurotransmitter triggers a flood of unrelated thoughts, feelings, and perceptions.

Both C3 and C4 were related to independent components of neurological disorder, and elevated C3 or C4 was positively associated with the neurological disorder in the cross-sectional study. We also found that the high baseline C3 and C4 levels indicated an increased risk of Schizophrenia over a 4-year follow-up study which was also observed in this study [24].

4. CONCLUSION

The phytochemicals constituent reported in this study have positive potential and exert neuroprotective effects in various neurological disorders. Thus, alligator provides a new source of the beneficial neuropsychotropic drug. These studies provide a phytochemical basis for the effects of aqueous extract of alligator pepper on brain function and neuroprotection, including Schizophrenia. Moreover, alligator pepper has a suitable neuroprotective agent characteristic, and a fundamental property regards its

ability to cross the blood-brain barrier (BBB), to reach the target sites of the CNS.

Finally, the presence of phenols or other phytochemicals content in alligator pepper is likely responsible for the antipsychotic property of alligator pepper and thus can be utilized as a natural medicine for Schizophrenia.

ACKNOWLEDGEMENTS

The authors hereby gratefully acknowledge Dr. O. Okedeyi, and Mrs Babatimehin of the Department of Biochemistry, LASU and Mrs Shakunle of the Lagos State University College of Medicine, Ikeja for their technical assistance.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SAD designed the study and wrote the protocol. WDE and AVI managed the literature search, wrote the first draft of the manuscript and performed the phytochemical and in vivo studies supervised by SAD, AOG and WDO. AOG managed the interpretation of IR Spectrum. WDO performed the statistical analysis. All authors read and approved the final manuscript

REFERENCES

1. Bonnot, O. and N. Dumas, Schizophrenic disorders in adolescence. La Revue du praticien, 2014. 64(4): p. 499-504.
2. Tandon, R., H.A. Nasrallah, and M.S. Keshavan, Schizophrenia,"just the facts" 4. Clinical features and conceptualization. Schizophrenia research, 2009. 110(1-3): p. 1-23.
3. Stevens, B., et al., The classical complement cascade mediates CNS synapse elimination. Cell, 2007. 131(6): p. 1164-1178.
4. Chaudhuri, A., et al., Interaction of genetic and environmental factors in a Drosophila parkinsonism model. Journal of Neuroscience, 2007. 27(10): p. 2457-2467.
5. Pandey, G.N., et al., GSK-3β gene expression in human postmortem brain: regional distribution, effects of age and suicide. Neurochemical research, 2009. 34(2): p. 274-285.
6. Ricklin, D., et al., Complement: a key system for immune surveillance and homeostasis. Nature immunology, 2010. 11(9): p. 785.
7. Bayly-Jones, C., D. Bubeck, and M.A. Dunstone, The mystery behind membrane insertion: a review of the complement membrane attack complex. Philosophical Transactions of the Royal Society B: Biological Sciences, 2017. 372(1726): p. 20160221.
8. Ehiagwina, E. and U. Inegbenebor, Effect of aqueous extract of alligator pepper (Zingiberacea aframomum melegueta) on litter size of alloxan induced diabetic Sprague Dawley rats. International Journal of Community Research, 2016. 5(3): p. 90-94.
9. Olonisakin, A., E. Adebayo, and M. Ahile, TERPENOID CONSTITUENTS'CONCENTRATION OF AFRAMOMUM MELEGUETA USING HYDRODISTILLATION AND SOLVENT METHODS OF EXTRACTION. Trakia Journal of Sciences, 2012. 10(2).
10. Mojekwu, T., et al., Hypoglyceamic effects of aqueous extract of Aframomum Melegueta leaf on alloxan-induced diabetic male albino rats. Pacific Journal of Medical Sciences, 2011. 8(1): p. 28-36.
11. Kane, J.M., T. Kishimoto, and C.U. Correll, Non-adherence to medication in patients with psychotic disorders: epidemiology, contributing factors and management strategies. World psychiatry, 2013. 12(3): p. 216-226.
12. Bhattacharjee, A. and A. Das, Phytochemical screening of some Indian plants. Quarterly Journal of Crude Drug Research, 1969. 9(3): p. 1408-1412.
13. Hikino, H., et al., Antihepatotoxic actions of flavonolignans from Silybum marianum fruits. Planta medica, 1984. 50(03): p. 248-250.
14. Kapoor, L., A. Singh, and S. Kapoor, Survey of Indian plants for saponins, alkaloids and flavonoids. I. 1969.
15. Nath, M., M. Chakravorty, and S. Chowdhury, Liebermann-Burchard reaction for steroids. Nature, 1946. 157(3978): p. 103-104.
16. Sofowora, A., Medicinal plants and traditional medicine in Africa. 1996: Karthala.
17. Deepak, D., et al., Cardiac glycosides. Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products, 1996: p. 71-155.
18. McDonald, S., et al., Phenolic content and antioxidant activity of olive extracts. Food chemistry, 2001. 73(1): p. 73-84.
19. Ejikeme, C., C.S. Ezeonu, and A.N. Eboatu, Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to nigerdelta area of nigeria. European Scientific Journal, 2014. 10(18): p. 247-270.
20. Singleton, V.L. and J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 1965. 16(3): p. 144-158.
21. Zhishen, J., T. Mengcheng, and W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 1999. 64(4): p. 555-559.
22. Broadhurst, R.B. and W.T. Jones, Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture, 1978. 29(9): p. 788-794.

23. Wenger, C.B., et al., Thermoregulatory control of finger blood flow. Journal of applied physiology, 1975. 38(6): p. 1078-1082.
24. Kindmark, C.-O., In vitro binding of human C-reactive protein by some pathogenic bacteria and zymosan. Clinical and experimental immunology, 1972. 11(2): p. 283.
25. Price, J., et al., Immunological studies in asthmatic children undergoing antigen provocation in the skin, lung and nose. Clinical & Experimental Allergy, 1983. 13(5): p. 419-426.
26. Ismail, A.A., F.R. van de Voort, and J. Sedman, Fourier transform infrared spectroscopy: principles and applications, in Techniques and instrumentation in analytical chemistry. 1997, Elsevier. p. 93-139.
27. Do k., Schizophrenia: genes, environment and neurodevelopment. Revue Médicale de la Suisse Romande., 2013. 9(398): p. 1672-1677.
28. Pérez-Neri I., Ramírez-Bermúdez J., and R.C. Montes S., Possible mechanisms of neurodegeneration in schizophrenia. . Neurochemical Research, 2006. 10(1279-1294).
29. Erk, S., et al., Hippocampal Function in Healthy Carriers of the CLU Alzheimer's Disease Risk Variant. The Journal of Neuroscience, 2011. 31(49): p. 18180-18184.
30. Joseph, J.A., R.T. Bartus , and y.D.E. Clod, Reversing the deleterious effects of aging on neuronal communication and behavior: eneficial properties of fruit polyphenolic compounds. B Am J Clin Nutr. , 2005. 81(31): p. 3-6.
31. Ďuračková, Z., Some current insights into oxidative stress. Physiological research, 2010. 59(4).
32. Van Ommen, B. and R. Stierum, Nutrigenomics: exploiting systems biology in the nutrition and health arena. Current opinion in biotechnology, 2002. 13(5): p. 517-521.
33. Križková, L., et al., Antimutagenic in vitro activity of plant polyphenols: Pycnogenol® and Ginkgo biloba extract (EGb 761). Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2008. 22(3): p. 384-388.
34. Golański, et al., Does pycnogenol intensify the efficacy of acetylsalicylic acid in the inhibition of platelet function? In vitro experience. Postepy Hig Med Dosw.(online), 2006. 60: p. 316-321.
35. Youdim, K.A., r.M.Z. Qaise, and D.J. Begley, Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med, 2004. 36: p. 592–604. .
36. Ehrnhoefer, D.E., et al., Green tea (−)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum Mol Genet., 2006. 15(274): p. 43-51.
37. Guo, S., et al., Protective effects of green tea polyphenols in the 6- OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway. Biol Psychiatry., 2007. 62(13): p. 53-62.
38. Zhang, B., et al., Epigallocatechin gallate, an active ingredient from green tea, attenuates damaging influences to the retina caused by ischemia/reperfusion. Brain research bulletin, 2007. 11(59): p. 40-53.
39. Tsuang, M.T., et al., Gene-environment interactions in mental disorders. World Psychiatry, 2004. 3(2): p. 73.