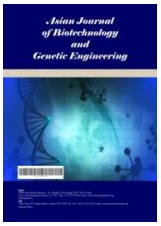

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Butyryl-cholinesterase Inhibitory Effect of *Ficus platyphylla* Root Methanolic Extract on Catfish Exposed to Arsenic

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Authors' contributions

This work was carried out in collaboration between both authors. Author IMH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IMH and AIJ managed the analyses of the study. Author AIJ managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to test the effect of *Ficus platyphylla* ROOT on Butyryl-cholinesterase in *Clarias gariepinus*. Extraction of the root was carried out with 80 percent methanol. Initially, adult catfish were subjected to a toxicity analysis at the doses of 0.5, 1 and 2 M for arsenic and 62.5, 125, 250 and 500 g/L for the crude extract. This is followed by chronic toxicity and determination of Butyryl-cholinesterase inhibitory effect using Ellman's approach in the fishes. Fishes where divided into 4 groups, group 1 was treated with 125 g/L of the extract, group 2 was treated with 0.5 M arsenic, group 3 was treated with 125 g/L of crude extract for 24 hours and the exposed to arsenic, group 4 was maintained in 0.1% Dimethyl sulfoxide (DMSO). Results showed the $LC_{50,\ 70,}$ and $_{90}$ of 0,53, 0,67 and 0,95 M for arsenic and 346.7, 1148.2 and 6760 g/L for the crude extract. There is elevated crude extract cholinesterase inhibitory activity with high significant different at $p<0.05$) between the group treated with crude extract alone, the group treated with crude extract and exposed to arsenic, the group exposed to arsenic alone and the group maintained in tap water. It

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can be inferred that the medicinal effects of this crude extract are caused by low toxicity and the high cholinesterase inhibitory activity of the crude extract. Toxicity screening of this crude extract on mammals such as mice and rats is recommended in order to reaffirm their toxicity profile.

Keywords: Arsenic; catfish; cholinesterase; extracts; toxicity study.

1. INTRODUCTION

Structural modifications resulting in changes contributing to atrophy and degradation of neurons and glial cells in particular limbic regions and circuits are characteristic of neurodegenerative diseases. In the pathogenesis of these disorders, stress and depression have played a significant part [1]. Dysregulation of neuroprotective and neurotrophic signaling pathways needed for the maturation, development and survival of neurons and neuroglia is part of the fundamental changes that triggered this disease [2]. Several studies have demonstrated that behavioral and life-style changes as well as therapeutic interventions can counteract these systemic changes by activating neuroprotective and neurotrophic pathways as well as suppressing the harmful, excitotoxic and inflammatory effects of the stress [3,4,5]. Factors that predisposed to this disorder include prolong exposure to cellular and environmental stressors as well as mutation due to gene interactions [6]. Medical treatments are primarily targeted at calming down the effects of these diseases. No successful therapeutic records are available that improve full rehabilitation or avoid recurrence. Therefore, none of the conventional medications used to date have been confirmed to treat or reverse the disease's clinical syndrome. The effectiveness of these diseases in therapy is not sufficient, much of the treatment required high financial costs and prolonged treatment length [7]. In developed countries, many people afflicted by this condition were discourages from taking synthetic medications due to inefficiency [8].

Since time immemorial, traditional Chinese medicines (TCM) have been in use and have been reported to produce promising results in the treatment of many diseases. Research studies have shown that bioactive herbal extract compounds are effective in neurodegenerative disease control [9]. Neurodegenerative disease pathogenesis is complex, but a successful therapeutic solution to these disorders may be a mixture of bioactive substances from various herbs. In certain disease cases, herbal decoctions may function on several goals to synergistically improve the medicinal effects (Babazadeh et al., 2019).

In order to find a solution to these issues, the *Ficus platyphylla* root was obtained, extracted and tested for its neuroprotective effect. *F. Platyphylla* belongs to the Ficus genus and to the Moraceae family, which consists of no fewer than 850 species. In the forests of tropical and subtropical areas, over 200 different varieties of Ficus are widely used as woody trees, shrubs and vines [10]. In Asian countries, such as Malaysia and Europe example Australia, around 500 species of Ficus are found. In Asia, many varieties of Ficus are cultivated as indoor and outdoor ornamental plants [11]. This plant species is very rich in nutritious components and is used in Egypt, India, South China, Turkey and Malaysia as a source of food [12]. For the cure of various diseases, parts of this herb have been used as a medicinal plant. The presence of phenolic acid and flavonoids in high concentration in Ficus plants was recorded in several literatures that contributed to it therapeutic power [13,14].

Therefore, the ability of Ficus plants to defend against oxidative stress disorders is high. In the field of traditional medicine, the plants of the Ficus species are well known. Effectiveness treatment of diabetes, stomach ache, piles, ulcer, dysentery, asthma, oxidative stress and cancer was demonstrated by studies on the medicinal value of the Ficus plant [15]. Anti-cancer, anti-inflammatory and anti-diabetic ability of this plant's promotes it ethno-medicinal application in medicine, making it popular and sold worldwide among other medicinal plants [16]. The purpose of this study, therefore, was to determine the neuroprotective effects of *F. platyphylla* methanolic root extract by testing its effect on the acetyl-cholinesterase enzyme of fish.

2. MATERIALS AND METHODS

2.1 Animal Model

At More wards, Sokoto South L.G.A, Sokoto State, a total of 250 adult catfish of average length 8-10 cm, weighed 1.8-2.5 kg and aged 1

month was purchased from a fish farm. At the Veterinary Biochemistry Lab, Usmanu Danfodiyo University Sokoto, fish were acclimatized for 2 weeks in the aquarium glass with tap water filled to one third. The fishes were fed with essential fish feed pellets purchasing from the feed seller in the metropolis of Sokoto. They were fed 3 times a day, and water was change at 8 a.m. after 24 hours [17].

2.2 Plants Collection and Identification

The leaves and roots of the plant were obtained from the Department of Veterinary Pharmacology and Toxicology Garden at Usmanu Danfodiyo University City Campus, Sokototo (UDUS). The plant leaf and the root were identified and validated by a botanist at the Department of Biological Science, UDUS, and voucher specimen number UD 009 was given and kept in the Departmental Herbarium.

2.3 Plant Extraction

The *F. platyphylla* root was washed and allowed to dry at room temperature (26 ± 1 ^{o}C) for two weeks before being crushed into a semi-powdered form. A total of 10 kg of the root sample was immersed in 1000 mL of 80% methanol in smooth bottom flasks for 3 days (Sigma Aldrich, USA). In order to obtain high crude extract, the powdered root methanol mixture was shaken every day for three days at 26 ^{o}C; this process was repeated three times to extract all the bioactive components of the root. The extract obtained was then filtered with 25 mm Whatman filter paper (Sigma Aldrich, USA) and then condensed with a rotary evaporator (IKA® RV 10, USA) to a semi-solid form at 42 ° C. The resulting crude extract was then weighed and transferred to sample bottles and kept at 4 ° C until needed [18].

The percentage yield of the crude extract was determined by multiplying by 100 the weight of the filtrate divided by the total weight of the seed ground in the semi-powder. Yield (percent) = [wt of plant content (g)/wt of extract (g)] x 100.

2.4 Plants Sample Dilution and Dose Preparation

The stock solution was prepared with 1 g of *F. platyphylla* crude root extract dissolved in 10 mL of 100 percent DMSO (1 g/10 mL), the use of DMSO was to solubilize the crude extract, as the extract is not fully soluble in aqua solvent. Preparation of the milliliter (g/mL) sub-stock solution was carried out by diluting the stock solution with tap water at the concentration of interest. Using double serial dilution with tap water at eight concentrations ranging from 7.81 to 1000 g/mL, the working solution was prepared from the sub-stock solution.

2.5 Acute Toxicity Test of Arsenic

Acute arsenic toxicity testing in adult catfish (length 8-10 cm, weight 1.8-2.5 kg and age 1 month) was performed in chlorine-free tap water for 2 days, as recorded by the OECD. Five fishes of both sexes were randomly picked and subjected to concentrations of formulated arsenic dose (0.5, 1 and 2 M) in a rectangular glass tank of 3 liters size. The test was used with three replicates each along with control group. Mortality was constantly monitored and the fish were declared dead when they did not respond to contact with glass rod, and there was no proof of respiration. Immediate removal of the the dead fish from the tank was carried out. The exposed fish were moved after 24 hours to new tanks containing chlorine-free tap water. Prior to and during the experimental time, the fish were fed. The fish were closely supervised during the experiment [18].

2.6 Experimental Design for Arsenic Acute Toxicity Test on Adult Catfish

Group-I –subjected to 0.5 M arsenic for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-II – subjected to 1 M of arsenic for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-III – subjected to 2 M of arsenic for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-IV –maintained in tap water with 0.1% DMSO (control group) for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.

2.7 Acute Toxicity Test of Plant Extract on Adult Catfish (*Clarias gariepinus*)

The crude extract acute toxicity test on adult catfish (length 8-10 cm, weight 1.8-2.5 kg and age 1 month) was performed in chlorine-free tap water for 2 days as stated by the Hassan et al*.,* 2020. At a stocking density of five fish per liter, a random collection of catfish of both sexes was carried out and preserved. Five separate concentrations (62.5 to 1000 mg/L) were used, with three replicates of each dose alongside the

control group. Continuous mortality monitoring and confirmation of dead fish were conducted after the operculum activity was no longer moving and the fish were unable to respond when touched with a glass rod.

2.8 Experimental Design for Acute Toxicity Test of Plant Extract on Adult Catfish

Group-I – subjected to 62.5 g/L of crude extract for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-II – subjected to 125 g/L of crude extract for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-III – subjected to 250 g/L of crude extract for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-IV – subjected to 500 g/L of crude extract for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-V – subjected to 1000 g/L of crude extract for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.
Group-VI –maintained in tap water with 0.1% DMSO (control group) for 2 days in glass aquarium, 3 replicate with five fishes per replicate were used.

2.9 Chronic Toxicity Test of the Arsenic and Crude Extract on Adult Catfish

Chronic toxicity testing of crude extract and arsenic on adult catfish (length 8-10 cm, weight 1.8-2.5 kg and age 1 month) was performed in chlorine-free tap water for 10 days as recorded by OCED. This is to assess the modifications caused by extended exposure to crude extract and arsenic in the total protein and butyryl-cholinesterase. The fish were split into four groups of 10 fish per group followed by 2 weeks of acclimatization. The fishes were managed in separate 14 liter aerated tanks and after every 24 hours, water was replaced. The fish were initially exposed for 24 hours to a safety dose (250 g/L) of crude extract and subsequently exposed to 0.5 M of arsenic. These concentrations were selected on the basis of previous studies of acute crude extract and arsenic toxicity tests. The control groups were kept under the same conditions for 10 days, except without arsenic and crude extract. For each concentration, three replicates, along with control groups, were used.

2.10 Experimental Design for the Chronic Toxicity Test of the Crude Extract and Arsenic on Adult Catfish

Group-I subjected to 250 g/L of crude extract for 10 days in glass aquarium, 3 replicate with 10 fishes per replicate were used.
Group-II – subjected to 0.5 M of arsenic for 10 days in glass aquarium, 3 replicate with 10 fishes per replicate were used.
Group-III – subjected to 250 g/L of crude extract for 24 hours and then 0.5 M of arsenic for 9 days in glass aquarium, 3 replicate with 10 fishes per replicate were used.
Group-IV – maintained in tap water with 0.1% (control group) for10 days in glass aquarium, 3 replicate with 10 fishes per replicate were used.

The fish were cryo-anesthetized at the conclusion of the experiment by exposing them to ice for 60 seconds. The head of the fish was gently dissected and the brain was carefully retrieved without injury. The sampled brain tissue was weighed, cleaned and homogenized in a tissue homogenizer bottle with a 50 M Tris-HCl buffer (Polytron PT-6100, USA). As a homogenizing solvent, a Tris-HCl buffer (1% Triton X and 0.1% PMSF) (Sigma Aldrich) was used. For 20 minutes, the sample was centrifuged at 12,000 x g with (GRACE High Speed Refrigerated Centrifuge, India). The supernatant was transfered into a different tube and used as a source of enzymes [18].

2.11 Total Protein Estimation

The protein content was measured using the Bradford procedure on the homogenized fish brain [18]. As standard, Bovine Serum Albumin (BSA) 7.81-1000 µg/mL was prepared and used. A 1 ml stock solution was diluted with Bradford reagent and transfered into a 96-well micro plate with 1000 µg BSA, 200 ul Tris-HCl (10 mg/200 mL) at pH 7.4. Using a micro-Plate reader (U.K), the reading was taken at 590 nm and the normal curve was plotted. For the calculation of total protein in the brain, the same was method was repeated. Dilution of the Tris-HCl pH 7.4 with the brain sample at various concentrations from 0 to 1000 µg/mL, 200 µL of sterile phosphate saline (PBS) was carried out, Bradford reagent was later applied and then stored for 30 minutes. Reading was taken using a micro-plate reader at 590 nm (U.K). The standard curve was used to extrapolate the protein concentration according to OD values after performing the assay.

2.12 Determination of Acetyl-Cholinesterase Activity

During the entire procedure, a 50 M Tris–HCl pH 7.4 was used as a buffer. The Butyryl-cholinesterase (Ache) used in this assay was harvested from the homogenized experimental fish brain. In 96-well plates I, 50 M Tris-HCl was added to 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) followed by butyrylcholine (BTC) and incubated. Then the 210 µL (pH7.4) Tris-HCl buffer, 20 µL (0.1 M) DTNB, and 10 µL (54 mg/mL) brain butyryl-cholinesterase enzyme (AChE) were prepared to another 96-well plate II and incubated at 28 °C for 15 minutes. Then, in 96-well plate II, 10 µL of BTC (2.5 M) was added to the homogenized and incubated for 10 minutes. Anti-cholinesterase (ChE) activity was calculated based on Ellman's approach using an updated 96-well microplate assay. The hydrolysis by the cholinesterase enzyme of the substrate butyryl-thiocholine results in thiocholine production. Thiocholine responds to Ellman's reagent (DTNB) to generate 2-nitrobenzoate-5-mercaptothiocholine with a micro plate reader calibrated at 405 nm. Using a micro plate reader (U.K) at fluorescent excitation of 485 nm and 535 nm emission, the reading was taken at 405 nm (Hassan et al., 2020).

2.13 Statistical Analysis

Using probit analysis and variance analysis (ANOVA), data collected was collected, evaluated for their core preferences and differences. Using Dunnett's post hoc test at $p<0.05$, Significant means is separated. To compare the survival curves, the log rank (Mantel-Cox) test at df 1 and the Gehan Breslow-Wilcoxon test were used. The findings were viewed as a mean ± standard error of the mean (SEM). GraphPad Prism Version 5.0 was used to statistically evaluate all data [18].

3. RESULTS

3.1 Crude Ficus Extract Yield

The result of F. platyphylla root percentage yield of was 1988.07 g after extraction, evaporation and concentration with a rotary evaporator at 42^{o} C (10.06 percent).

3.2 Acute Toxicity Study of Arsenic

The findings of the acute arsenic toxicity test on catfish on day 2 show calculated LC_{50}, LC_{70}, LC_{90} of 0,53, 0,67 and 0,95 M. In groups exposed to 1 M, there is high mortality. In groups that were exposed to 0.5 M arsenic, up to 80% of the fish survived 24 hours after exposure and only 13.3% survived 48 hours after exposure. Survival study of groups exposed to 1 M showed that only 20% of fish survived day 1 post-exposure and all fish died 48 hours after exposure. All fishes exposed to 2 M of arsenic were died 24 hours after exposure. Comparison of Survival Curves with Log-rank (Mantel-Cox) test at df 1 and Gehan-Breslow-Wilcoxon test at df 4 reveals a high significant difference of $p< 0.05$ between groups maintained at 0.1 percent DMSO and groups exposed to various arsenic concentrations Fig. 1.

3.3 Acute Toxicity Study of Crude Extract

Calculated of LC_{50}, LC_{70}, LC_{90} of 346.7, 1148.2 and 6760 g/L were obtained following the acute toxicity examination of the extract on catfish on day 10. As in the result, it was observed that all fish exposed to 62.5 g/L of crude extract survived day 2 after exposure on. In group that were exposed to 125 g/L of crude extract, up to 70% of the fish survived 24 hours after exposure, and only 20% survived 48 hours after exposure. Survival study of group exposed to 250 g/L of crude extract reveals that only 20% survived 24 hours after exposure and all other fish died 48 hours after exposure. After 24 hours of exposure, all fish subject to 500 and 1000 g/L of crude extract die. Comparison of Survival Curves with Log-rank (Mantel-Cox) test at df 5 indicates a significant difference between the group maintained at 0.1 DMSO and the group exposed to various crude extract concentrations. The Gehan-Breslow-Wilcoxon test to compare survival curve at df 1 indicates a significant differential of $p< 0.05$ between groups maintained at 0.1 percent DMSO and groups exposed to varying extract concentrations Fig. 2.

3.4 Total Protein Content

The findings on total protein content in groups exposed to crude extract (250 g/L) alone indicated high protein content. Increased protein content was also found in groups maintained in tap water (0.1% DMSO) and in groups treated with crude extract (250 g/L) and exposed to arsenic (0.5 M), compared to groups exposed only to arsenic (0.5 M). At $p<0.05$, there was a significant difference between fish exposed to arsenic (0.5 M) compared to fish kept in tap water (0.1% DMSO), treated with crude extract (250 g/L) and those treated with crude extract

(250 g/L) and exposed to arsenic (0.1% DMSO) (0.5 M) Fig. 3.

3.5 Butyryl-cholinesterase Inhibitory Assay

Butyryl-cholinesterase inhibitory assay findings revealed elevated activity in groups maintained in tap water (0.1 percent DMSO). Similarly, elevated activity was also found in groups treated solely with crude extract and in groups treated with crude extract and exposed to arsenic as opposed to groups exposed solely to arsenic. At $p<0.05$, there was a significant difference between the fishes maintained in tap water (0.1 percent DMSO) and the other groups Fig. 4.

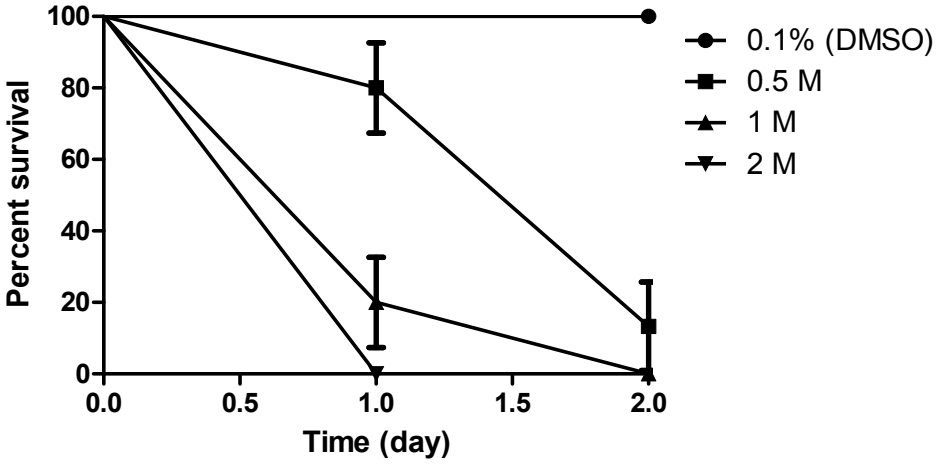

Fig. 1. Acute toxicity effect of arsenic on the survival rate of adult catfish exposed various concentrations (0.5-2 M). The survival percentage (n=3) against to the number of days exposure is shown in the above figure. From three separate experiments, the values reflect mean ± SEM

****P<0.05 accounted for significant different of the percentage survival values*

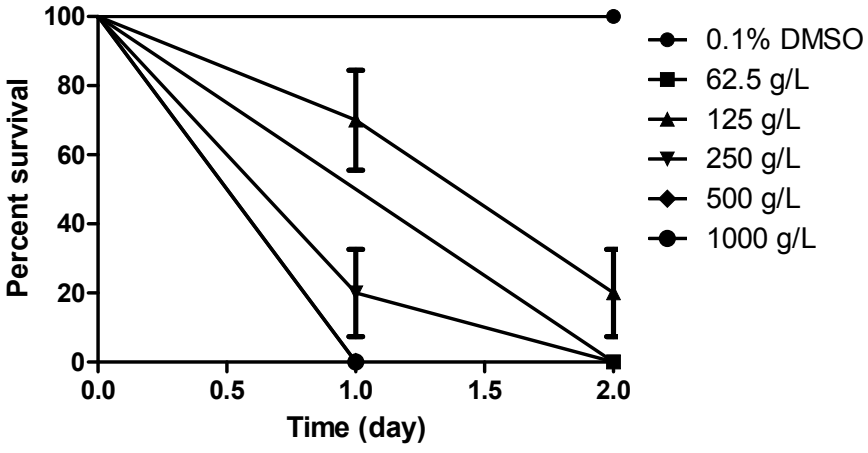

Fig. 2. Crude extract's acute toxicity impacts on the survival rate of adult catfish exposed at various concentrations (62.5-1000 g/L). The survival percentage (n=3) against to the number of days post exposure is shown on the above figure. From three separate experiments, the values reflect mean ± SEM

****P<0.05 accounted for significant different of the percentage survival values*

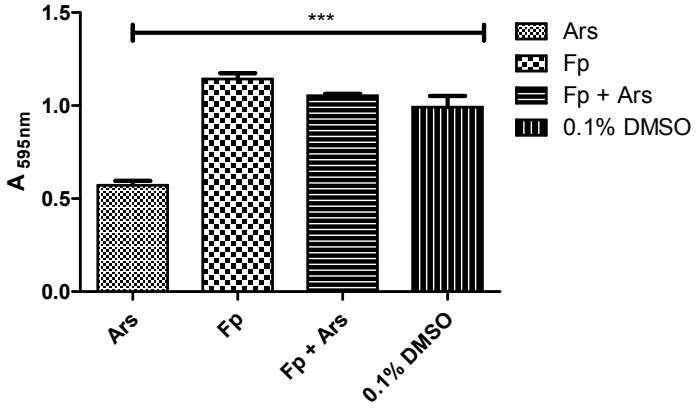

Fig. 3. Results of 250 g/mL of *Ficus platyphylla* (root) extract and arsenic (0.5 M) on total protein content derived from the brain of adult catfish. The ***P<0.05 represent significant different from the arsenic-treated group and other groups. From three separate experiments, the values reflect mean ± SEM. Ars = arsenic and Fp = extract of *Ficus platyphylla* (root)

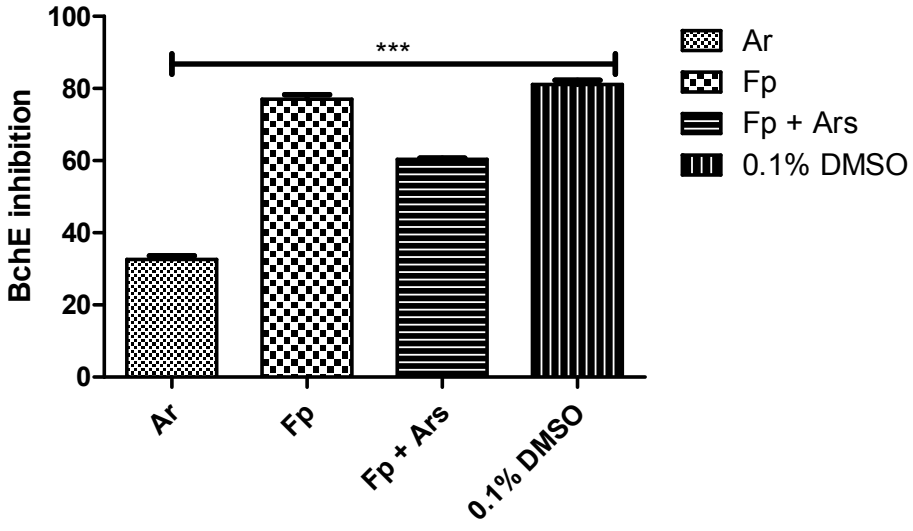

Fig. 4. The inhibitory effects of *Ficus platyphylla* root extracts on butyryl-cholinesterase against arsenic were tested using DTNB, Butyrylcholine iodide substrate read at 405 nm with microplate reader. The ***P<0.05 represent significant different from the Arsenic-treated group and other groups. From 3 separate experiments, the values reflect mean ± SEM. Ars= arsenic and Fp = extract of *Ficus platyphylla* (root)

4. DISCUSSION

Incurable and debilitating conditions resulting in progressive degeneration and / or death of nerve cells are referred to as neurodegenerative diseases. This triggers movement disorders (called ataxia) or mental processing (called dementia) [19]. Treatment of these diseases with several conventional medicines may not produce any positive results [20]. To solve these challenges, *F. platyphylla* root metanolic extract was tested for toxicity and neuro-protective effects by anticholinesterase inhibitory assay on adult catfish. Solvent methanol is commonly

used in both plants and animals for the extraction of many bioactive compounds [21]. Hexane, water, ethyl acetate, acetone and ethyl acetate are other solvents used for the extraction of bioactive substances from live organisms [22]. The best polar solvents widely used by traditional herbalists for the preparation of traditional medicine are methanol and water [23]. Consequently, in this analysis, 80% methanol was chosen as an extraction solvent. Solvent polarity plays a very important role in assessing the form and concentration of the bioactive compound recovered after extraction. Increases in all phenolic compounds and their antioxidant effects were strong in high polar solvents [24]. In solvents with high polarities, acetone and methanol, high extract yields were recorded in fruit and vegetables following extraction [25]. The effects of acetone on the isolation of significant portions of non-polar herbs have also been recorded [26]. The main explanation for the high recovery of these compounds in the aforementioned solvent may be the high solubility of the nonpolar compounds in acetone [27]. Several studies have also reported high yields of phenolic compounds with ethanol and ethanol/water solvents from the roots of horseradish plants [28,29,30].

Similarly, *Moringa oleifera* leaf extraction demonstrated high methanol solvent extract yields relative to acetone, hot water and chloroform [31]. In the forms and quantities of bioactive constituents retrieved after laboratory extraction, time and procedures used during extraction also play an important role [32].

Causes of increased mortality for catfish exposed to crude extracts have been found to be correlated with the forms and amounts of bioactive compounds present in various parts of the plant. Most of the active substance used as a medicinal agent has a side effect, especially if it is given at high concentrations [33]. One of the main side effects of this extract, reported at higher concentrations, may be an increased mortality rate in catfish. Some medicinal plants are reported to have high therapeutic effects due to the presence of high phenolic compounds, such as *Digitalis purpurea, Hyoscyamus niger, Atropa belladonna, Physostigma venenosum, Podophyllum peltatum* and *Solanum nigrum* [34]. When taken at high doses, substances that are of medicinal value in the aforementioned plant also turn out to be poisonous to the animals. Alkaloid, which is among the bioactive compounds with high therapeutic values, has been documented by researchers to impair respiration in fish [35,36]. The effect of osmoregulation in fish by increased stimulation of opercula beat on gills has also been reported to be caused by the same compound [37]. Nerve malfunctions and or denaturation of the neurotransmitter by several phytochemical constituents of therapeutic significance have also been documented, particularly following high dose administration [38]. Sluggish motions, atypical heartbeats and death are one of the pathological changes found since the use of these compounds in fish.

Awareness of the influence of this plant extract on the enzyme acetylcholinesterase is not available. Generally, the biochemical catalyst that neutralizes the choline base esters is esterases. The enzymes group is referred to as cholinesterase or choline esterase [39]. Catalysis and conversion of the neurotransmitters acetylcholine or acetylcholine-like compounds to choline and acetic acid are main functions of these enzymes. To allow the cholinergic neuron to return to its resting state after excitation, the changes involved in this pathway are very important [40]. In order to increase contraction of muscle, the release of butyrylcholine at neuromuscular junctions it supports to the locomotive motion of the organs of the body is established. Neutralization of acetylcholine by the cholinesterase enzyme thus increases muscles relax from the contractile state for a time [41].

Most of the nonspecific cholinesterase enzymes that neutralize acetylcholine into choline-based ester are plasma cholinesterase or pseudocholinesterase, such as butyryl-cholinesterase (BChE, BuChE). This neurotransmitter is produced in the liver and transferred predominantly to various body tissues and organs by blood plasma [42]. It is very similar to neuronal acetyl-cholinesterase; it is also known as erythrocyte cholinesterase [43]. Another pseudo-cholinesterase present in the various organs and tissues of the body is propionyl-cholinesterase. Compared to Acetyl-cholinesterase and butyryl-cholinesterase, it is less widespread but has nearly the same biochemical effects [44].

5. CONCLUSION

It can be concluded that lack of adverse effects and high cholinesterase inhibitory effect of the crude extract is responsible for it therapeutic effects of this crude extract. Toxicity screening of

this crude extract on mammal such as mice and rat to reaffirm their toxicity profile are recommended. Antioxidant screening as well as isolation of bioactive compounds present in this plant part is strongly recommended.

ETHICAL APPROVAL

In compliance with the National Health Science Ethics Committee of Nigeria (NHREC) order, this work was scrutinized and accepted by the University Committee on Medical and Science Research Ethics. Animal Ethic committee approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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