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Original Research Article (Experimental)

Anticholinesterase activity and antioxidant properties of *Heinsia crinita* and *Pterocarpus soyauxii* in *Drosophila melanogaster* model

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ABSTRACT

Background: Plant alkaloids have become important sources of nutraceuticals owing to their pharmacological importance especially in the management of neurodegenerative diseases such as Alzheimer's disease. In assessing the therapeutic potentials of plant phytochemicals, the fruit fly (*Drosophila melanogaster*) has emerged as a very veritable tool and has been largely accepted as an alternative model in biomedical research.

Objectives: In this study, alkaloid extracts from bush apple (*Heinsia crinita* (Afzel.) G. Taylor and padauk (*Pterocarpus soyauxii* Taub.) leaves were assessed on *D. melanogaster* exposed to aluminum toxicity.

Materials and methods: Alkaloid extracts were prepared by solvent extraction method. Thereafter, the extracts were evaluated for their *in vitro* antioxidant properties, Fe^{2+}-chelating abilities and inhibitory effects on drosophila acetylcholinesterase (AChE) activity. The samples were also characterized for their constituent alkaloids via HPLC. Thereafter, effective safe dose of the extracts were determined in *D. melanogaster* (Harwich strain). Subsequently, flies assaulted with $AlCl_3$ were co-treated with the extracts (8.3 and 16.6 μg/g) for seven days, during which their survival rate was monitored. This was followed by assaying for the activities of AChE, antioxidant enzymes [superoxide dismutase (SOD), catalase and glutathione-S-transferase (GST)]. Also, the flies were assayed for levels of thiobarbituric acid reaction substance (TBARS) and reactive oxygen species (ROS).

Results: The results revealed that both extracts showed *in vitro* antioxidant properties with Padauk showing significantly higher antioxidant properties *in vitro*. However, there was no significant difference in their *in vitro* AChE inhibition. *In vivo*, Al-induced toxicity reduced survival rate, elevated AChE, SOD and GST activities, as well as TBARS and ROS levels which were ameliorated by the extracts. It was also revealed that piperine was predominant in PA, while 1-cyclohexen-1-yl-pyrrolidine was predominant in BA.

Conclusion: Our data suggest that the protective abilities of these extracts against Al-induced toxicity can be primarily associated with their anticholinesterase and metal chelating abilities. Thus, these vegetables can be potential sources of nutraceuticals against aluminum toxicity and associated diseases.

1. Introduction

Aluminum (Al) is an highly ubiquitous and abundant environmental element. Despite its ubiquity, studies have not shown its usefulness in biological systems, rather it has been considered an environmental toxicant when exposed in excess to humans [1]. Since 1886 when Al was first used, it was broadly used industrially for various purposes including cooking wares, while it served as antacids in its alkali form. Other means of Al exposure includes water and food packages [2,3]. Severe Al exposure leads to its accumulation in organs and could eventually cause series of cytotoxic effects; for example Al is found in brain hippocampus and frontal cortex which has been associated with onset of neurodegeneration [3]. Consequently, several neurodegenerative diseases, including Alzheimer's disease (AD) have been associated with Al-toxicity [4–6].

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The role of Al in AD has been associated with oxidative stress and cholinergic impairments. Through accumulation of iron and reactive oxygen species (ROS) production, Al has been shown to induce cytotoxicity and neurodegeneration in primary human neuronal tissues [7]. Furthermore, Al is implicated in the increase of brain lipid peroxidation which is associated with increase in iron. Oxidative stress caused by increased free radicals results in loss of cell homeostasis and neurotoxicity [8]. The distortion in redox homeostasis is majorly characterized by elevated lipid peroxidation and impaired antioxidant system. The cholinergic system of neurotransmission is critical to neuronal function and development. The system is mediated by the neurotransmitter-acetylcholine (ACh), which undergoes enzymatic hydrolysis by acetylcholinesterase (AChE) [2]. Al has been shown to elicit anticholinergic properties and impair noradrenergic neurotransmission [2]. Furthermore, accumulation of Al in the brain coupled with the ability of Al to form high affinity complex with enzyme's anionic site can induce accumulation of free radicals and potentiate oxidative stress [2].

Drosophila melanogaster is a good animal model for Al toxicity in comparison to other animal models; the short life span, no ethical concerns, adaptability as alternative model, ease of handling, small genome size and homologs of human disease-causing genes have made the fly very useful in biomedical research [9–11]. Previous studies have shown that features of neurodegeneration such as reduced longevity, locomotion impairment, as well as deficiencies in olfactory learning and brain vacuolization have been reported in *Drosophila* fed with excess dietary aluminum [7]. Al treatment in *Drosophila* have been observed to elicit several behavioral changes and general neurodegeneration (deficit in learning, memory, locomotor activity) as well as accumulation of large amount of iron, ROS and increased SOD activity.

Alkaloid is a class of naturally occurring nitrogen − containing compounds. Alkaloids have several pharmacological properties as they exhibit broad-range activities in living system including the cardiovascular and neuronal systems [12]. They have also been reported as pain killers, antimalarial drugs, muscle relaxant and lots more. Interestingly, alkaloids have been discovered to alleviate symptoms of AD, with some major drugs used for the management of AD such as galantamine and rivastigmine, being alkaloid derivatives [13]. This breakthrough fueled interest in identifying other sources of alkaloids that can manage AD. Padauk (*Pterocarpus soyauxii*) belongs to the genus *Pterocarpus* and family Leguminosae. It is distributed in both tropical and subtropical regions. It is locally called Oha among the Igbo tribe. The leaves are consumed mostly as vegetables and it retains high ascorbic acid content even after cooking. The medicinal properties of padauk include the management of kidney and skin diseases and diabetes. Bush apple (*Heinsia crinita*) belongs to the genus *Heinsia* and family Rubiaceae. It is locally referred to as "Atama" in the South-eastern part of Nigeria. The leaves are consumed either as vegetable in preparation of local cuisine or as component of alcoholic concoction for the treatment of some diseases such as bacterial infections, diabetes, hypertension and infertility. Both plants have been known in folklore for the management of various diseases and have also been studied to be rich in alkaloids. Therefore, this study evaluated the antioxidant and ameliorative effect of the alkaloid extracts from both padauk and bush apple leaves on Al-induced toxicity in *D. melanogaster* as a model of neurotoxicity and neurodegeneration.

2. Materials and methods

2.1. Sample collection

Padauk (*P. soyauxii* Taub.) and bush apple (*H. crinita* (Afzel.) G. Taylor) leaves were sourced from Abba, Abia State (South East), Nigeria. The samples were authenticated in the Herbarium, Centre for Research and Development, Federal University of Technology, Akure, Nigeria (Voucher numbers 0235 and 0236 respectively). The leaves were washed, sliced, air dried and blended to fine powder using a stainless steel blender. The blended samples were kept dry at room temperature for further analysis. The *D. melanogaster* (wild type, Harwich strain) stock culture used for this study was maintained on standard fly diet as previously reported [10].

2.2. Preparation of alkaloid extract

The alkaloid extract of the samples was prepared using standard solvent extraction method as previously reported by Ademiluyi et al. [14]. Subsequently, extracts were kept in the refrigerator at 4 °C for further analysis.

2.3. In vitro analysis

2.3.1. Antioxidant assays

The extracts were assessed for their antioxidant assays by the 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTS) radical scavenging assay, ferric reducing antioxidant property, and hydroxyl (OH) scavenging ability using previously reported methods [15]. The nitric oxide (NO) scavenging ability was determined according to the method reported by Marcocci et al. [16], while the Fe (II) chelating ability was determined as reported by Puntel et al. [17]

2.3.2. AChE and TBARS assay

The tissue homogenate of the flies was prepared as previously reported [15]. The effect of the extracts on AChE activity was assessed by the method of Ellman et al. [18], as reported by Abolaji et al., [19]. The assay to quantify the thiobarbituric acid (TBARS) production in the flies using the method of Ohkawa et al. [20], as previously reported by Ogunsuyi et al. [21], was used to monitor the effect of the extracts on lipid peroxidation level in the presence of 250 μM $FeSO_4$, 40 mM $AlCl_3$ and 13 mM quinolinic acid as prooxidants.

2.4. Bioassay

2.4.1. Survival study

The effect of the extracts on seven days survival rate of the flies was determined. Flies (both genders, 3–5 days old) were divided into four groups containing 40 flies each (n = 5) and exposed to varied concentrations of the samples (16.6–1666.6 μg/g). Thereafter, flies were monitored for incidence of mortality and counted daily to pool their survival rate at the end of the experiment and expressed as percentage of the total fly population per group.

2.4.2. Experimental layout

Flies (both genders, 3–5 days old) were divided into 10 groups with 40 flies per vial (n = 5). Group I was placed on normal diet while group II–X were placed on normal diet containing aluminum chloride, the alkaloid extracts (8.3 and 16.6 μg/g) and aluminum chloride plus the alkaloid extracts (8.3 μg/g and 16.6 μg/g) as described below:

Groups	Treatment
I	Basal diet
II	Basal diet + 40 mM aluminum chloride
III	Basal diet + 8.3 μg/g padauk
IV	Basal diet + 16.6 μg/g padauk
V	Basal diet + 8.3 μg/g bush apple
VI	Basal diet + 16.6 μg/g bush apple
VII	Basal diet + aluminum chloride + 8.3 μg/g padauk
VIII	Basal diet + aluminum chloride + 16.6 μg/g padauk
IX	Basal diet + aluminum chloride + 8.3 μg/g bush apple
X	Basal diet + aluminum chloride + 16.6 μg/g bush apple

The choice of concentrations of the alkaloid extracts was based on the survival study where no significant mortality was observed in the flies. The choice of dose for aluminum chloride was based on previous studies on aluminum toxicity in *D. melanogaster* [7,21,22]. The flies were maintained on these treatments at room temperature for seven days. All experiments were carried out in triplicate (n = 6).

2.4.3. Biochemical assays

The tissue homogenate of the flies was prepared as previously reported [15]. The effect of the extracts on AChE activity was assessed by the method of Ellman et al. [18], as reported by Abolaji et al., [19]. The assay to quantify the thiobarbituric acid (TBARS) production in the flies using the method of Ohkawa et al. [20], as previously reported by Ogunsuyi et al. [21], was used to monitor the lipid peroxidation level in the flies. Reactive oxygen species was quantified in the flies via the dichlorofluorescein fluorescence assay which monitored the rate of oxidation of dichlorofluorescein diacetate [23,24]. The flies were also assayed for endogenous antioxidant enzymes; superoxide dismutase activity was assayed by the method of Alia et al. [25], catalase activity in the homogenate samples was determined according to the method of Sinha et al. [26], while the assay for glutathione-S-transferase (GST) activity was carried out according to the method of Habig et al. [27].

2.5. HPLC characterization

Characterization of alkaloid extracts was carried out in an HPLC system, using standard procedures as recently reported [22].

2.6. Statistical analysis

Results were expressed as mean ± standard deviation (S.D) of all experiments. For in vitro experiments, EC_{50} values of extracts representing extract concentration causing 50% inhibition was determined and compared with student t-test (significance was accepted at $p < 0.05$). All bioassay data were appropriately analyzed using one-way ANOVA coupled with Tukey's post hoc test (significant level of mean difference was accepted at $p < 0.05$). Graph pad PRISM (V.5.0) was used for all data analysis.

3. Results

Results for the crude alkaloid yield for bush apple (BA) and padauk (PA) leaves are 2.8 g/100 g and 5.6 g/100 g respectively. The ABTS radical scavenging ability of the alkaloid extracts from padauk and bush apple is presented in Table 1. It reveals that both extracts have the radical scavenging ability with the alkaloid extract from padauk (10.47 ± 0.02 μM TEAC/100 g) exhibiting the higher scavenging ability than bush apple (6.48 ± 0.57 μM TEAC/100 g). The ferric reducing antioxidant property of the alkaloid extracts (Table 1) revealed that the extract of padauk (13.26 ± 0.53 mgAAE/g) had a higher reducing property compared to bush apple (1.06 ± 0.01 mgAAE/g).

Table 1
In vitro antioxidant properties and AChE inhibitory effects of alkaloid extracts of padauk and bush apple leaves.

	Padauk	Bush apple
ABTS (μMTEAC/100 g)	10.47 ± 0.02^{a}	6.48 ± 0.57^{b}
FRAP (mgAAE/g)	13.26 ± 0.53^{a}	1.06 ± 0.01^{b}
	EC_{50} (mg/mL)	
Fe^{2+} chelation	0.38 ± 0.01^{b}	0.45 ± 0.01^{a}
OH scavenging ability	0.41 ± 0.05^{a}	0.64 ± 0.01^{b}
NO scavenging ability	1.45 ± 0.03^{a}	0.94 ± 0.01^{b}
AChE inhibition	0.58 ± 0.05^{a}	0.62 ± 0.02^{a}

Values represent mean ± standard deviation of replicate readings. Mean values with different superscript letters are significantly different at $p < 0.05$.

Also presented in Table 1 is the Fe^{2+} chelating ability of the extracts. This, according to the EC_{50} values, revealed that the alkaloid extract from PA extract showed a significantly higher percentage inhibition. Similarly in Table 1, the EC_{50} values for OH and NO radical scavenging abilities revealed that while PA extract showed significantly higher OH scavenging ability, BA extract showed significantly higher NO radical scavenging ability. Furthermore, the EC_{50} for AChE inhibitory effects of both extracts (Table 1) revealed PA extract as having significantly higher AChE inhibitory effect.

In order to determine a safe and effective dose of the alkaloid extracts, *D. melanogaster* (Harwich strain) were treated with varied concentrations (16.6–1666.6 μg/g) of the alkaloid extract of both samples and their mortality rate was monitored for seven days (Supplementary Figures). It was observed that there was no significant difference between the control group and the group treated with 16.6 μg/g. Thus, the maximum safe dose was set at 16.6 μg/g.

In the subsequent bioassay, the percentage survival and locomotor performance of the flies fed with diet supplemented with alkaloid extracts (16.6 μg/g and 8.3 μg/g) of both samples, aluminum and aluminum + safe concentrations of the alkaloid extract from both samples (Fig. 1), reveal that there is no significant mortality and locomotor impairment between the control group and the varied concentration of the alkaloid extracts, but the group treated with Al had significantly reduced survival rate and locomotor performance compared to the control, with the alkaloid extracts reducing the mortality and restoring their survival, as well as the impairments in locomotor performance.

The ameliorative effect of the alkaloid extracts on the acetylcholinesterase (AChE) activity of control flies and aluminum assaulted flies are presented in Fig. 2. The result shows that aluminum assaulted flies have higher AChE activity in comparison to the control, while all the alkaloid extracts ameliorate the elevated AChE activity in aluminum assaulted flies with 16.6 μg/g alkaloid extract from PA having the most significant ameliorative effect, restoring the activity to almost that of the control.

From this study, an elevated level of TBARS was observed in aluminum assaulted flies, which indicates that increased lipid peroxidation occurred (Table 2). It is impressive to observe that all the extracts were very effective in lowering the rate of lipid peroxidation while the control flies treated with the alkaloid extracts

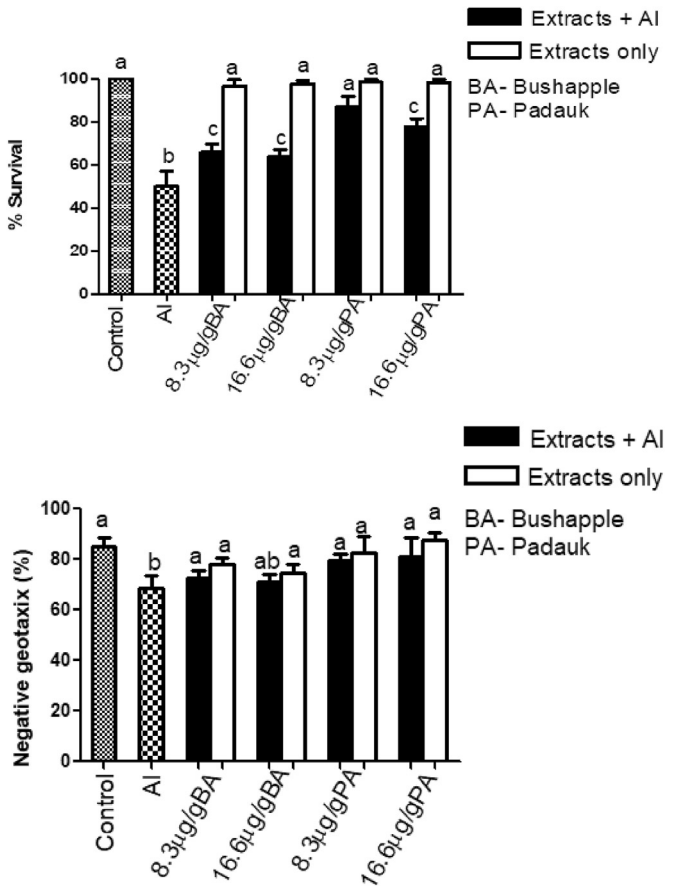

Fig. 1. a: Percentage Survival of normal and aluminum assaulted flies treated with alkaloid extract from padauk (PA) and bush apple (BA) leaves. Values represent mean ± standard deviation. Mean values with difference letters are significantly different at $p < 0.05$. b: Percentage locomotor performance of normal and aluminum assaulted flies treated with alkaloid extract from padauk (PA) and bush apple (BA) leaves. Values represent mean ± standard deviation. Mean values with difference letters are significantly different at $p < 0.05$.

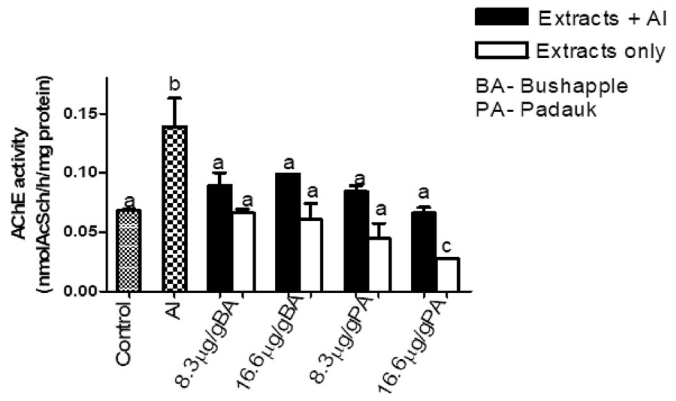

Fig. 2. Acetylcholinesterase (AChE) activity of normal and aluminum assaulted flies treated with alkaloid extract from padauk (PA) and bush apple (BA) leaves. Values represent mean ± standard deviation. Mean values with difference letters are significantly different at $p < 0.05$.

only have no significant difference between the assaulted flies treated with the alkaloid extracts in comparison to the control. Fig. 3 presents effect of the alkaloid extract on the ROS level in control flies and aluminum assaulted flies. An increased level of ROS was observed in aluminum assaulted flies which was ameliorated (except for 16.6 µg/g PA) by the alkaloid extracts. For the control flies treated with the alkaloid extracts only, there was no significant difference in comparison to the control.

Table 2 also reveals the effect of alkaloid extracts on GST activity of control flies and aluminum assaulted flies. There was no significant difference between the control flies treated with alkaloid extracts only and the control, but there was an observed increase in the enzyme activity of aluminum assaulted flies with the alkaloid extract having an ameliorative effect on assaulted flies (except for 16.6 µg/g PA). The effect of alkaloid extracts on SOD activity of control flies and aluminum assaulted flies is represented in Table 2. An elevated increase was observed in the group assaulted with aluminum, while the alkaloid extracts significantly ameliorated the elevated enzyme activity in aluminum assaulted flies (except for 16.6 µg/g BA). There was no significant difference observed in the control flies treated with alkaloid extracts and the control group. Lastly, Table 2 also shows the catalase activity of control flies and aluminum assaulted flies treated with alkaloid extracts of both samples. It was observed that the activity of the enzyme was reduced in the aluminum assaulted flies in comparison to the control but the extracts showed no significant amelioration.

The result of the HPLC alkaloid characterization (Table 3 and Fig. 4) revealed two predominant alkaloids namely 1-1-cyclohexen-1-yl-pyrrolidine and piperine; PA leaf had abundance of piperine (1 mg/100 g), while BA had abundance of 1-1-cyclohexen-1-yl-pyrrolidine (0.73 mg/100 g).

4. Discussion

Aluminum has been used to model neurotoxicity and AD-like neurodegeneration in various animal models. In *D. melanogaster*, Al toxicity has been associated with impaired locomotor performance, learning and memory, as well as redox imbalance [7,21,22,28] which are synonymous to neurodegeneration. One of the key hypotheses of Al toxicity is the cholinergic impairment. Cholinergic impairments is often connected with depletion in acetylcholine (Ach) neurotransmitter in the synapse of AD brain. This decline in the level of ACh could be attributed to the elevated activity in the cholinergic enzyme, AChE, which hydrolyses ACh, thereby, inhibiting the pivotal function of ACh as neurotransmitter. Hence, there is loss of communication between neurons leading eventually to neuronal death. Due to this fact, AChE becomes a target in the management of AD and other neurodegenerative diseases. This study revealed an increase in the AChE activity of Al-assaulted flies, and this agrees with other studies that reveal that Al can induce elevated AChE activity [21,22,29]. Wei et al. [30], and Adedayo et al. [22], reported an elevation in the activity of AChE in $AlCl_3$ treated mice and *Drosophila* respectively. In addressing the possible underlying mechanism of Al induced increase in AChE activity, Kaizer et al. [31], suggested that it may be as a result of peripheral sites of AChE interacting with Al^{3+}, as well as modifying AChE secondary structure. It was further revealed from this study that the alkaloid extracts of both PA and BA ameliorate the elevated AChE activity, this further confirms the *in vitro* result which shows that both alkaloid extracts inhibit AChE activity, with PA leaf extract showing better anticholinesterase effects. Since, AChE inhibitors are often recommended to AD patients, these alkaloid extracts can also have potential as anticholinesterase nutraceuticals in managing cholinergic mediated neurodegenerative diseases like AD.

Oxidative stress is another hypothesis underlying Al toxicity. In simple words, oxidative stress results from imbalance in the oxidant and antioxidant system, favoring the former. Al toxicity induces oxidative stress and it is reported to mediate several Al-induced pathological conditions including neurotoxicity.

Table 2
Thiobarbituric acid reactive substance (TBARS) level, as well as antioxidant enzymes' [glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase]. Activity of normal and aluminum assaulted flies treated with alkaloid extracts from padauk (PA) and bush apple (BA) leaves. Values represent mean ± standard deviation. Mean values along the same column with difference letters are significantly different at $p < 0.05$.

Groups	TBARS (mmol/mg protein)	GST Activity (μmol/min/mg protein)	SOD activity (mmol/min/mg protein)	Catalase activity (μmol H_2O_2 consumed/mg protein)
Control	0.12 ± 0.02^a	0.57 ± 0.05^a	0.39 ± 0.04^a	1.50 ± 0.17^a
8.3 μg/g BA	0.13 ± 0.01^a	0.56 ± 0.05^a	0.35 ± 0.03^a	1.56 ± 0.05^a
16.6 μg/g BA	0.12 ± 0.002^a	0.53 ± 0.07^a	0.38 ± 0.02^a	1.59 ± 0.06^a
8.2 μg/g PA	0.10 ± 0.01^a	0.56 ± 0.03^a	0.39 ± 0.03^a	1.52 ± 0.07^a
16.6 μg/g PA	0.12 ± 0.01^a	0.58 ± 0.08^a	0.38 ± 0.04^a	1.54 ± 0.05^a
$AlCl_3$ (40 mM)	0.46 ± 0.08^b	0.86 ± 0.02^b	1.10 ± 0.07^b	0.60 ± 0.01^b
Al + 8.3 μg/g BA	0.14 ± 0.02^a	0.76 ± 0.01^c	0.88 ± 0.04^c	1.03 ± 0.14^c
Al + 16.6 μg/g BA	0.17 ± 0.04^a	0.69 ± 0.10^c	1.03 ± 0.05^b	0.66 ± 0.01^b
Al + 8.2 μg/g PA	0.21 ± 0.06^c	$0.64 \pm 0.08^{b,c}$	0.67 ± 0.03^c	0.75 ± 0.03^b
Al + 16.6 μg/g PA	0.19 ± 0.11^c	0.75 ± 0.10^b	0.84 ± 0.01^c	0.66 ± 0.02^b

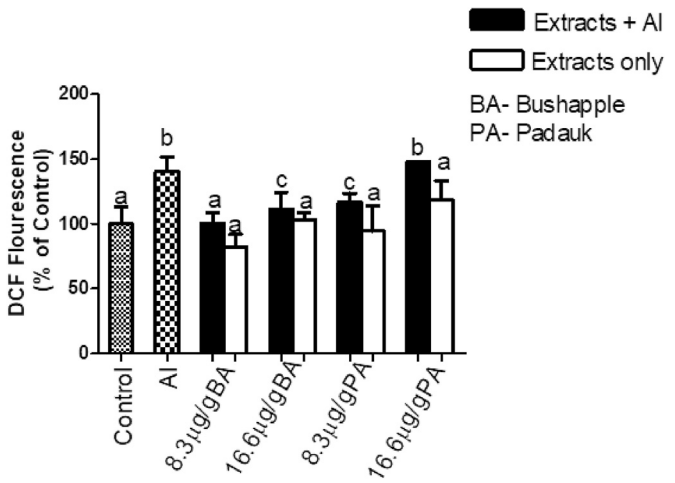

Fig. 3. Reactive oxygen/nitrogen species (RONS) content of normal and aluminum assaulted flies treated with alkaloid extract from padauk (PA) and bush apple (BA) leaves. Values represent mean ± standard deviation. Mean values with difference letters are significantly different at $p < 0.05$.

Elevated ROS level in the flies reflects a state of oxidative stress and Al stimulates increased production of ROS which likely results in the induction of iron-mediated lipid peroxidation [5,22,32]. As suggested by Exley [32], Al acts as a prooxidant by reacting with superoxides to form Al-superoxide radical ion, $AlO_2{}^{*2+}$. $AlO_2{}^{*2+}$ is central to the mechanism of action of Al, because it carries out the prooxidant activity of Al. It was further shown that $AlO_2{}^{*2+}$ can catalyze the formation of H_2O_2 and the reduction of iron (III) to iron (II) which facilitates the formation of OH* through the Fenton reaction. Hence, H_2O_2 and OH*, which are products of $AlO_2{}^{*2+}$, are highly implicative in lipid peroxidation.

Based on the mechanism of Al acting as a prooxidant as stated above, it can be suggested that Al elicit the production of ROS and this study supports that Al-assaulted flies have elevated ROS as expressed as DCF fluorescence. The result also showed the ameliorative effect of the alkaloid extracts on the ROS generated. Also another indicator of oxidative stress is the elevated production of thiobarbituric reactive substances (TBARS) expressed as MDA equivalent. An important diagnostic index for lipid peroxidation in a number of tissue damage is MDA [21,33]. The study also revealed an elevated TBARS production in the Al-assaulted flies with the alkaloid extracts ameliorating the level of TBARS produced. Interestingly, no significant difference was observed between the Al-assaulted flies treated with alkaloid extract and the control flies. The mechanism of ameliorating the generation of ROS and MDA in the Al-assaulted flies by the alkaloid extracts can be linked to their ability to reduce Fe^{3+} to Fe^{2+}, to chelate Fe^{2+}, scavenge OH*(a product of Fenton reaction) and scavenge NO* (which forms highly reactive radicals in the presence of superoxide radical) [21] *in vitro*, which all mediates lipid peroxidation.

Free radicals can induce cellular oxidative damage. In order to access the capability of the alkaloid extracts to scavenge radical species, ABTS and NO radical scavenging ability assays were used. The use of the ABTS radical model for assessing free radical scavenging ability has a number of advantages; it is more versatile in that it can be used for both polar and non-polar samples and there is also minimal spectra interference due to the fact that it absorbs maximally at 760 nm, of which at this wavelength most natural products do not absorb [34]. Overproduction of NO is often deleterious, in that, it is capable of forming very reactive peroxynitrite (ONOO−) in the presence of superoxides; ONOO− when elevated can cause severe mitochondria damage and contributes to several pathologies including neurodegeneration [35]. Thus, the nitric oxide radical (NO*) scavenging abilities of both extracts can also contribute to their overall antioxidant properties.

Generally, the cellular macromolecules are shielded from oxidative assaults by the endogenous antioxidant enzymes. Disruption/distortion in the cellular antioxidant status might be explained by increase in the ROS level as a signal of oxidative stress. Al toxicity is implicative in inducing increase in ROS level. This study focused on three of the endogenous antioxidant

Table 3
Quantitative analysis of padauk and bush apple leaves for alkaloids by HPLC.

S/N	Compound		Sample	
			Padauk leaf	Bush apple leaf
1	1-Cyclohexen-1-yl-pyrrolidine	Retention time (min)	16.092	16.092
		Amount (mg/100 g)	8.10×10^{-4}	0.73
2	Piperine	Retention time (min)	17.094	17.094
		Amount (mg/100 g)	1.32	1.31×10^{-3}

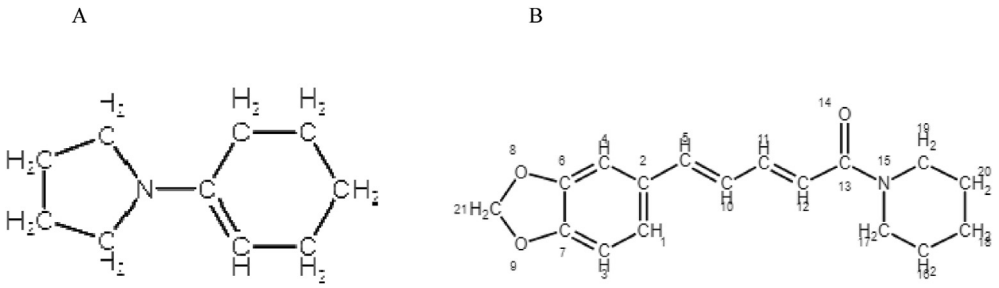

Fig. 4. Alkaloid characterized by HPLC from padauk and bush apple leaves. A = 1-cyclohexen-1-yl-pyrrolidine; B = piperine.

enzymes; SOD, CAT and GST. SOD functions to protect cellular macromolecules against free radicals by converting highly reactive superoxide anions to H_2O_2 and O_2, it often acts as one of the primary defence lines against ROS. Based on the proposed mechanism of action of Al as a prooxidant, SOD could competes with Al for the same substrate, which is, superoxide anions, while CAT acts on the hydrogen peroxide generated from SOD-catalyzed dismutation reaction on superoxide anions [36]. GST on the other hand is a vital enzyme that is involved in conjugation reaction during xenobiotic metabolism. It acts by catalyzing the reactions between a potentially toxic electrophilic compounds and reduced glutathione [37,38].

In this study, increased activity of SOD and GST was observed, in Al-assaulted flies. The increase in the activity of these two antioxidant enzymes can be associated with the elevation in production of ROS, this is often a process observed when there is oxidative stress [24]. The increase in the activities of the enzymes has been reported as an adaptive response possessed by *D. melanogaster* in order to counteract free radicals [24]. The alkaloid extract ameliorates the activity of these enzymes to almost that of the control. Catalase activity was observed to diminish in Al-assaulted flies, but was elevated in flies treated with the extracts. This result is in correlation with earlier the studies of Wei et al. [30], and Adedayo et al. [22], whereby catalase activity was reduced in Al-induced mouse and drosophila.

Control flies treated with the extract and aluminum assaulted treated with and without the extract were observed for mortality for 7 days and about 50% mortality was recorded in Al assaulted flies (without extract) relative to the control, while the alkaloid extract ameliorated the Al induced mortality. The mortality observed in Al assaulted flies can be attributed to impairment in cholinergic and redox systems. Although both extracts showed antioxidant properties *in vitro*, only mild antioxidant effects were observed *in vivo* in Al assaulted *D. melanogaster*. However, the anticholinesterase effect of both extracts observed *in vitro* were corroborated by their abilities to significantly ameliorate elevated AChE activities in Al-treated flies. This can also contribute to the ability of the extracts to ameliorate locomotor performance impaired in Al treated flies. Indeed, alkaloids are generally known for their limited antioxidant properties due to their structural characteristics, when compared to other phytochemicals like polyphenols with high reducing moieties [39]. On the contrary, plant alkaloids have been well known for their anticholinesterase properties [40–42]. However, the significant amelioration in elevated TBARS level shown in Al assaulted flies co-treated with the extracts could be associated primarily to their Fe^{2+} chelating abilities with possible contributory radical scavenging abilities. Therefore, we hypothesize that the ameliorative effects of both extracts on Al-toxicity in flies could be more associated with their anticholinesterase and metal chelating abilities.

5. Conclusion

This study reveals aluminum-induced toxicity in fruit flies evident from the observable reduced survival which can be associated with alterations in the antioxidant status and the cholinergic system of the flies. However, Al assaulted flies co-treated with alkaloid extracts from padauk and bush apple leaves were able to improve their survival rate. Our data showed evidence that the primary protective mechanism of the extracts against Al assault in the flies could be via their anticholinesterase and metal chelating abilities, and thus present them as potential sources of nutraceuticals. The padauk extract showed more potentials than bush apple extract at ameliorating Al-induced toxicity in the flies.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2020.10.004.

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