2020-01-15

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International Journal of Biosciences

INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 1, p. 1-13, 2020

RESEARCH PAPER OPEN ACCESS

Recovery of acetyl cholinesterase inhibition by Methanolic Bark Extract of *Acacia nilotica* from Organophosphate Pesticides Exposure in mice model

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Key words: Antioxidant, Organophosphate Pesticides (OP), Radical Oxygen Species (ROS), Acetyl cholinesterase Enzyme (AChE), *Acacia nilotica*.

http://dx.doi.org/10.12692/ijb/16.1-13 Article published on January 15, 2020

Abstract

Organophosphates (OPs) pesticides are reported to cause acute poisoning because of their ability to inhibit acetyl cholinesterase enzyme (AChE). Available antidotes drugs are atropine sulfur, Pralidoxime (2-pyridine aldoxime methyl chloride) and diazepam, which act to recover OP-AChE inhibition. These are controlled drugs not easily accessed and very expensive. In this present study *Acacia nilotica* was assessed for its antioxidant activity, and *in vivo* AChE depression and recovery from OP-AChE inhibition. The mice were exposed in three different OPs including chlorpyrifos 480g/l (CPF), Fenitrothion 10g/l (FNT) and Profenophos 720g/l (PFP). The methanolic bark extract of *A. nilotica* had a substantial increase of absorbance readings from 2.895±0.0032 to 3.716±0.0259 compared to standard (ascorbic acid) from 0.108±0.0033 to 1.468±0.0297 at $P<0.05$. AChE depression and recovery were assessed by using the AChE test mate kit to analyze blood collected from the mice's tail. Recovery effect under crude methanolic extract from *A. nilotica*, ascorbic acid and normal feeding were compared with the untreated group. Results have shown that there is a significant decrease of AChE level from Day zero to 14th day in all treated groups of CPF, PFP and FNT which indicate poisoning. Significance of AChE recovery observed only in male mice in all treatment groups. This is a first study to assess and report the antioxidant activity of stem bark methanolic extracts of *A. nilotica* in controlling organophosphate pesticide toxicity in mice, hence further studies on isolation of active compounds are recommended.

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Introduction

Pesticides exposure cause adverse effects on human health (Elibariki & Maguta, 2017). Worldwide, approximately 200,000 cases are due to acute poisoning that leads to deaths each year (UN, 2009). 99% of acute poisoning occurs in developing countries (WHO, 2014). Statistics show that about 700 cases of death related to pesticide poisoning may occur annually (Gupta & Sharma, 2006). This shows the need to prevent people from the adverse effects and death associated with pesticide exposure.

In Tanzania, a recent report shows that the prevalence of occupational acute pesticide poisoning range from 50% to 96% (Lekei *et al.*, 2016). The current treatment of pesticide poisoning cases available in Tanzania's hospital are an antidote, which includes a drug such as atropine, Pralidoxime (2-pyridine aldoxime methyl chloride) and diazepam which are controlled drugs. The drugs are unavailable in rural settings and are very expensive, (Eddleston, *et al.,* 2008). Alternative drug product from natural plant should be searched and developed in order to protect people who are exposed to pesticide and who are at risk to get pesticide poisoning.

Presence of OP in the human body triggers the production of reactive oxygen species (ROS), which induces Oxidative Stress (OS) such as lipid peroxidation, it also induces neurotoxic action and cause inhibition of Acetylcholinesterase Enzyme (AChE) and a decrease in the antioxidant enzyme (Verma *et al.*, 2007; Oruc, 2012). These antioxidants are essential for neutralizing ROS (Sultana, *et al.,*2007), meanwhile, AChE is an essential enzyme in neuro-system which play a great role of converting acetylcholine to Acetate and Choline, finally Choline taken back to the neural cell. Acetyl CoA from mitochondria combines with choline to form Acetyl-choline (Ach). Acetate at the ring is released as well as CoA in the neural (Akefe, 2017). Inhibition of AChE results into increase of acetylcholine in the body which cause decrease of AChE level (u/mL) result into acute health effects (headache, dizziness, abdominal pain, death) or chronic health effect (cancer, loss of coordination, loss of vision) (Fayuk & Yakel, 2004). Hence there is a need to prevent the AChE level depression.

Studies have observed that antioxidant derived from vitamins have the capability to fight ROS induced by OPs (Verma, *et al.,* 2007). Also, some studies reported that ROS induced by Chlorpyrifos OP can be scavenged by vitamins enriched antioxidants (A, C and E) (Verma *et al.*, 2007). However, other antioxidant-enriched plants should be searched and assessed their antioxidant property to fight against ROS induced by other OPs.

A. nilotica is multi-medicinal plant found in kingdom Plantae, division Mangnoliophyta, Family Fabaceae and is widely found in Africa and Asia (Harmacy & Ciences, 2011). It is reported to have polyphenol antioxidant property (Johns *et al.*, 1999). The medicinal property of the plant may vary depending on part of plant taken. Barks from *A. nilotica* was shown to contain polyphenol and flavonoids compared with leaves and roots (Sadiq *et al.,* 2015). The presence of polyphenol in *A. nilotica* gives the plant an ability to scavenge ROS induced by chemicals and protect from oxidative stress in human body (Del *et al.*, 2008): Duganath *et al.*, 2010: Ravikumar & Angelo, 2015). This study was aimed to assess antioxidant activity of *A. nilotica* in controlling the effects of Chlorpyrifos, Profenophos and Fenitrothion organophosphate pesticides poisoning in mice.

Materials and methods

Sample Collection and Identification

A.nilotica stem barks were collected at the duka bovu Village, in Monduli district with geographical coordinates of at -3°30'2.35"S, 36°44'5.04"E, Arusha region. Plant stem bark pieces were collected 5 samples per population for conservation purpose (Figure1) and submitted to National Herbarium of Tanzania (NHT), TPRI, Arusha and given Specimen voucher RJE001 after Confirmation be done by a plant taxonomist. Collected stem barks were air dried at room temperature before extraction and *in vitro*

study was conducted.

Study design

Study design involved both *in vitro* and *in vivo* experiments. *In vitro* study involved evaluation of reducing power of extracts while *in vivo* involved assessing efficacy of crude methanolic stem bark extract of *A. nilotica* on recovery of AChE from OP inhibition in mice. Stem barks contain antioxidants compounds of Phenolic (Phenolic acid, Flavonoid and Tannins) (Anjum *et al.*, 2013). Hence, Phenolics are often extracted in higher amount in more polar solvent like methanol (Ismail *et al.*, 2016). A pilot study was conducted in mice prior the experiment to develop tolerance and toxicity doses of Chlorpyrifos, Profenophos and Fenitrothion pesticides used in this study including from lowest to highest dose.

Laboratory work

Preparation of Plant Extracts and Extraction

Stem barks of *A. nilotica* were prepared by using Soxhlet extraction method as described by Singh, Singh, & Kumar, (2012) (Figure 5) which is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent (Ravikumar & Angelo, 2015; Sultana *et al.*, 2007). About 20 g of powdered plant barks were uniformly packed into a thimble and extracted with 180 mL of 100% methanol. Methanol is used because of high polarity and ability to extract both polar and non-polar compounds and followed by 180mL of 50% Methanol. Extracts were taken into beakers and kept on a hot plate and heated at 30 – 40° C till all the solvent got evaporated. Dried extracts were kept in a refrigerator at 4°C for their future use.

Evaluation of R*educing Power of Methanolic Bark Extract of A. nilotica*

Reducing power was performed based on method described by Duganath *et al.*, (2010) whereby 2mL of crude sample was taken and diluted with normal saline as vehicle from 2, 4, 6, 8, up to 20mL of 10 samples in serial dilution. The aim of this dilution is to observe at what concentration or dilution ratio extract against vehicle used will show a high absorbance reading. 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric-cyanide [K_3Fe (CN) $_6$] (2.5 ml, 1%) of each sample point (Figure 6). The mixture of each sample was incubated at 50°C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to mixtures, followed by centrifugation for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) of each sample was taken and mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). Prepared samples were measured absorbance in triplicate at 700 NM against a blank using UV-Vis spectrophotometer (Elico –SL 196) with compared with ascorbic acid as a standard (Figure 7).

Experimental animals

Albino mice of both sexes, weighing between 25 and 30 g and aged 8 to 12 weeks were obtained from the Plant Protection Division at TPRI. Arusha, Tanzania. The animals were allowed to stay in cages with sawdust litters in a controlled temperature environment of about 23 °C. Lighting was controlled to supply 12 h of light and 12 h of darkness for each 24 h period. Animals were handled ethically in an experimental animal room maintained temperature range 22° C (± 3). Animals were fed with conventional rodent laboratory diets with an unlimited supply of drinking water. Animals were fasted prior to dosing.

Assessment of the Recovery Efficacy of AChE by Methanolic Bark Extract of A.nilotica Caused by Chlorpyrifos, Profenophos and Fenitrothion poisoning in mice

Three different active ingredients of OPs were assessed in mice, including chlorpyrifos 480g/l (CPF), fenitrothion 10 g/L (FNT) (Figure 13) and profenophos 720g/L (PFP) (Figure 12). These OP are reported to cause severe effects to human health (Kapeleka *et al.,* 2001). OPs were prepared by dissolving into normal saline where 3 mL of CPF in 200 mL, 3 mL of PFP in 200 mL and 3g of FNT in 200 mL, differently. This 3 ml or 3g in 200 ml doses were established as tolerance dose for mice to have effect without getting harm that was developed during

the pilot study. Three groups of each CPF, FNT and PFP were grouped based on recovery, treatment including normal feeding, ascorbic acid and methanolic crude extract. Blood samples were collected from the mice tail before and after treatments (Figure 9). Blood samples were analyzed for the level of AChE (u/ml) by using AChE test mate kit (Figure 10 and 11) as described in Test-mate ChE Cholinesterase Test System (Model 400) - Instruction Manual.," (2003) where 10 ul of blood filled in capillary and placed into assay tube followed by vigorously shaking before inserting into the analyzer. Mice were poisoned with CPF, FNT and PFP from day 0, 7 and 14.OPs (Figure 12 and 13) bottles were removed manually and induce AChE recovery treatments including methanolic crude extracts from *A. nilotica,* ascorbic acid and normal feeding for 21 and 28 days. The control group did not receive anything except food and water. The dose used to be 1500mg/kg per body weight (LD_{50} dose obtained during oral toxicity study) of both Crude extracts of *A. nilotica* and ascorbic acid.

Statistical analysis

The results were entered into an Excel spreadsheet and expressed as the mean ± STDEV. The comparison t-test was used to compare mean absorbance readings of crude methanolic extract of *A. nilotica* against ascorbic acid and acetyl cholinesterase level (AChE) of treated groups against untreated by using MedCalc ® Version 12.7.1.0.

Ethical consideration

Ethical approval was obtained from Kibong'oto Infectious Diseases Hospital, Nelson Mandela African Institution of Science and Technology, Centre for Educational Development in Health and Research Ethical committee (KNCHREC) and given Approval No. KNCHREC009.

Results and discussion

Results

Evaluation of reducing the power of A. nilotica extract

There is a significant difference in the increase of the mean absorbance readings of the crude methanolic extracting of *A. nilotica* and ascorbic acid at $P<0.05$. Absorbance readings of the crude methanolic extract of *A. nilotica* were observed to be increasing as diluted with normal saline decreasing from 1:10, 2.895 ± 0.006 up to the ratio of 1:1, 3.716 ± 0.045 (Table 1) meanwhile absorbance readings of ascorbic acid at dilution ratio 1:10 was 0.108 ± 0.006 and 1:1 was 1.468 ± 0.052 (Table 1).

Table 1. Mean Absorbance readings of Methanolic Bark Extract of *A. nilotica* and ascorbic acid.

				95% CI			
S.ID	G1	G2	G3	Mean diff	Sign Level	Lower Bound	Upper Bound
S1	1:1	3.716±0.045	1.468±0.052	2.248	0.0001	2.1378	2.3582
S2	1:2	3.394±0.057	0.648±0.003	2.746	0.0001	2.6545	2.8375
S3	1:3	3.236± 0.013	0.535± 0.006	2.701	0.0001	2.6780	2.7240
S4	1:4	3.366±0.011	0.425±0.003	2.941	0.0001	2.9227	2.9593
S5	1:5	3.254±0.011	0.347±0.006	2.907	0.0001	2.8869	2.9271
S6	1:6	3.217±0.003	0.323±0.003	2.894	0.0001	2.8872	2.9008
S7	1:7	3.117±0.001	0.286±0.005	2.831	0.0001	2.8228	2.8392
S8	1:8	3.066±0.002	0.215±0.001	2.851	0.0001	2.8474	2.8546
S9	1:9	2.979±0.006	0.186±0.008	2.793	0.0001	2.7770	2.8090
S10	1:10	2.895±0.006	0.108±0.006	2.787	0.0001	2.7734	2.8006

Value expression: Mean ± Standard deviation, S.ID: Sample Identification number,

G1: Volume ratio between crude extract / ascorbic and normal saline, G2: Absorbance readings of crude extract of *A. nilotica*, G3: Absorbance readings of ascorbic acid.

Assessment of AChE depression and recovery

Depression of AChE Level by Fenitrothion (FNT) and Recovery efficacy. A quantity of 3g of FNT diluted in 200ml of normal saline was used to poison mice both sexes, the results were compared with the untreated group (A1) (Table 2). The results from table 2 show that at day 0 (before poisoning with FNT), mean AChE level in both sexes, male and female mice were observed to be significantly decreased compared to the untreated group at P<0.05 in A2 (group poisoned with FNT from day 0 to day14 and recovery by methanolic stem bark extract of *A. nilotica* at the rate of 1500 mg/kg per body weight from 21st to 28th) group, while no significant differences of the means observed in groups A3 (a group poisoned with FNT from day 0 to day14 and recovery by ascorbic acid at the rate of 1500 mg/kg per body weight from day 21st to 28th) and A4 (a group poisoned with FNT from day 0 to day14 and recovery through normal feeding (food and water) from day 21st to 28th) at P>0.05. For the 7th day, in treatment groups, A2, A3, and A4 the mean AChE level was significantly decreased compared to the untreated group at P<0.05. But, in the 14th day, all treated mice were very weak. These findings indicate poisoning effect and depression of AChE in the mice.

Table 2. Mean AChE level (u/ml) of mice poisoned with fenitrothion (FNT) and treated by methanolic stem bark extract of *A. nilotica*, Ascorbic acid and normal feeding.

	Treatments		A1	A2	A3	A4
	Days	Sex				
Poisoning	0	F	1.735 ± 0.21920	0.915 ± 0.06364*	1.340 ± 0.31113	1.550 ± 0.16971
		M	1.705 ± 0.04949	1.235 ± 0.12021*	1.070 ± 0.25456	1.595 ± 0.37477
	7	F	1.710 ± 0.07071	0.285 ± 0.04949*	0.670 ± 0.28284*	0.545 ± 0.13435*
		M	1.390 ± 0.07071	0.375 ± 0.09192*	0.730 ± 0.26870	0.640 ± 0.11314*
	14	F	1.630 ± 0.05657	-0.165 ± 0.4030	0.330 ± 0.16971*	-0.15 ± 0.537401*
		M	1.450 ± 0.14142	-0.22 ± 0.01414*	0.005 ± 0.33234*	-0.065 ± 0.54447
Recovery	21	F	1.530 ± 0.09899	0.115 ± 0.00707*	0.330 ± 0.12728*	0.125 ± 0.007071*
		M	1.105 ± 0.17678	0.045 ± 0.38891	-0.160 ± 0.70711	-0.125 ± 0.75660
	28	F	1.520 ± 0.18385	0.665 ± 0.16264*	0.390 ± 0.08485*	0.325 ± 0.14849*
		M	1.090 ± 0.32527	0.225 ± 0.16264	0.330 ± 0.14142	0.250 ± 0.19799

Values are expressed as mean ± STDEV, STDEV: standard deviation, day 0: the day treatment started. A1- untreated group, A2 - The group poisoned with FNT from day 0 to 14th and recovery by methanolic stem bark extract of *A. nilotica* at the rate of 1500 mg/kg per body weight from day 21st to 28th. A3: The group poisoned with FNT from day 0 to day14th and recovery by ascorbic acid at the rate of 1500 mg/kg per body weight from day 21st to 28th, A4: The group poisoned with FNT from day 0 to 14th and recovery through normal feeding (food and water) from day 21st to 28th: Superscript "*" indicate significance at P < 0.05 compared to the untreated group for each treatment group.

The recovery effect was observed when poisoning (FNT) was removed after the 14th day. Whereby, on the 28th day observed that the mean AChE level of the A2 treatment was the same as the untreated group (A1) in male mice which is an indicator of recovery similar to A3 and A4 groups.

Hence, the recovery effect observed only in male mice was significant in all treatments including methanolic bark extract of *A. nilotica*, ascorbic acid, and normal feeding. Depression of AChE Level by Profenophos (PFP) and Recovery Efficacy. AChE depression was achieved through poisoning of mice of both sexes into a solution made by 3ml of PFP to 200 ml of normal saline from day 0 to 14th while AChE recovery from 21st to 28th day through administering 1500mg/kg body weight of crude methanolic extract of the stem bark of *A. nilotica* (B2), ascorbic acid (B3) and normal feeding (B4) (Table 3). All treatment groups were compared with the untreated group (B1).

Table 3. Mean AChE level (u/ml) of mice poisoned by profenophos (PFP) and treated by methanolic stem bark extract of *A. nilotica*, ascorbic acid and normal feeding.

	Treatments		B1	B2	B3	B4
	Days	Sex				
Poisoning	0	F	1.735 ± 0.21920	1.025 ± 0.02121*	1.265 ± 0.47376	1.150 ± 0.35355
		M	1.705 ± 0.04949	1.050 ± 0.63639	1.010 ± 0.26870	1.150 ± 0.07071*
	7	F	1.710 ± 0.07071	-0.15 ± 0.07071*	0.450 ± 0.21213*	0.100 ± 0.28284*
		M	1.390 ± 0.07071	-0.05 ± 0.63639	0.100 ± 0.70711	0.100 ± 0.56569
	14	F	1.630 ± 0.05657	-0.15 ± 0.35355*	0.100 ± 0.28284*	-0.10 ± 0.28284*
		M	1.450 ± 0.14142	-0.25 ± 0.63639	0.050 ± 0.35355*	-0.20 ± 0.14142*
Recovery	21	F	1.530 ± 0.09899	0.200 ± 0.14142*	0.100 ± 0.42426*	-0.05 ± 0.35355*
		M	1.105 ± 0.17678	0.250 ± 0.21213*	0.350 ± 0.07071*	0.150 ± 0.07071*
	28	F	1.520 ± 0.18385	0.250 ± 0.07071*	0.250 ± 0.21213*	0.200 ± 0.14142*
		M	1.090 ± 0.32527	0.450 ± 0.35355	0.350 ± 0.21213	0.150 ± 0.07071

Values are expressed as mean ± STDEV, STDEV: standard deviation, day 0: the day treatment started. B1- untreated group, B2 - The group poisoned with PFP from day 0 to 14th and recovery by methanolic stem bark extract of *A. nilotica* at the rate of 1500 mg/kg per body weight from day 21st to 28th. A3: The group poisoned with PFP from day 0 to 14th and recovery by ascorbic acid at the rate of 1500 mg/kg per body weight from day 21st to 28th, B4: The group poisoned with PFP from day 0 to 14th and recovery through normal feeding (food and water) from day 21st to 28th: Superscript "*" indicate significance at $P < 0.05$ compared to an untreated group for each treatment group.

Results from table 3 show that there is a significant decrease of the mean AChE level of female mice during poisoning with PFP in the crude methanolic extract of the stem bark of *A. nilotica* group (B2) compared to the untreated group at days 0, 7th and 14th while no significance was observed in male mice, but on 14th day all treated mice were very weak. Results show that there is sex variation.

Table 4. Mean AChE level (u/ml) of mice poisoned with chlorpyrifos (CPF) and treated by methanolic stem bark extract of *A. Nilotica*, ascorbic acid and normal feeding.

	Treatments		C1	C2	C3	C4
	Days	Sex				
Poisoning	0	F	1.735 ± 0.21923	1.285 ± 0.06362	1.600 ± 0.39598	1.600 ± 0.08485
		M	1.705 ± 0.04952	1.450 ± 0.49492	1.410 ± 0.05657*	1.375 ± 0.06364*
	7	F	1.710 ± 0.07071	0.210 ± 0.04243*	0.805 ± 0.19092*	0.275 ± 0.07778*
		M	1.390 ± 0.07071	0.305 ± 0.28993*	0.400 ± 0.05657*	-0.21 ± 0.45962
	14	F	1.630 ± 0.05657	-0.03 ± 0.06364*	0.170 ± 0.08485*	-0.11 ± 0.32527*
		M	1.450 ± 0.14142	-0.12 ± 0.15556*	0.010 ± 0.18385*	-0.54 ± 0.16264*
Recovery	21	F	1.530 ± 0.09899	0.340 ± 0.11314*	0.235 ± 0.14849*	-0.2 ± 0.50912
		M	1.105 ± 0.17680	0.275 ± 0.09192*	0.315 ± 0.28991	-0.38 ± 0.06364*
	28	F	1.520 ± 0.18385	0.440 ± 0.14142*	0.380 ± 0.24042*	0.120 ± 0.09899*
		M	1.090 ± 0.32527	0.495 ± 0.09192	0.375 ± 0.33234	-0.17 ± 0.24749

Values are expressed as mean ± STDEV, STDEV: standard deviation, day 0: the day treatment started. C1- untreated group, C2 - The group poisoned with CPF from day 0 to 14th and recovery by methanolic stem bark extract of *A. nilotica* at the rate of 1500 mg/kg per body weight from day 21st to 28th. C3: The group poisoned with CPF from day 0 to 14th and recovery by ascorbic acid at the rate of 1500 mg/kg per body weight from day 21 to 28, C4: The group poisoned with CPF from day 0 to 14th and recovery through normal feeding (food and water) from day 21st to 28th: Superscript "*" indicates significance at $P < 0.05$ compared to the untreated group for each treatment group.

The recovery effect was observed when poisoning (PFP) was removed after the 14th day. Whereby, on the 28th day it was observed that the mean AChE level of the B2 treatment was the same as the untreated group (B1) in male mice which is an indicator of recovery, similar to B3 and B4 groups. Hence, the recovery effect observed only in male mice was significant in all treatments (B2, B3, and B4) including methanolic bark extract of *A. nilotica*, ascorbic acid, and normal feeding.

Depression of AChE level by chlorpyrifos (CPF) and recovery efficacy

AChE depression was observed when mice were poisoned with CPF solution made by dissolving 3ml of CPF active ingredient to 200ml of normal saline administered to the mice orally from day 0 to 14th and recovery was attempted through administering the methanolic stem bark extract of *A. nilotica* (C2), Ascorbic acid (C3) and normal feeding (C4) from day 21st to 28th (Table 4). Results show that the mean AChE level on day 0, male mice of the treatment groups C3 and C4 were decreased significantly compared to an untreated group at $P<0.05$ while no significant difference observed in female mice in both groups at $P>0.05$, but on 14th day all treated mice were very weak. AChE depression continued up to 21st day for all treatment groups, despite CPF was removed on 14th day and presence of the recovery indication on day 28th day for male mice was observed to be significant equal to an untreated group at $P<0.05$ in treatment groups C2, C3, and C4. Results indicate that there is sex variation in mean AChE recovery effects due to CPF poisoning.

Discussion

Reducing power

This study evaluated *in vitro* reducing power of crude methanolic extract from *A. nilotica*. An increase of absorbance readings have shown to be significantly in a crude methanolic extract from *A. nilotica* compared to ascorbic acid (Table 1). Increase of absorbance readings indicates increase in reducing power (Hajimahmoodi *et al,* 2008). The current results of this study indicate that the crude methanolic extract from *A. nilotica* has high reducing power compared to ascorbic acid (Figure 1) , which is similar to previous reported result (Aadil, Barapatre, Sahu, Jha, & Tiwary, 2014). Also it is reported that solvent used in extraction may influence changes of results (Kalaivani & Mathew, 2010). In this study maximum reducing power was 3.716 ± 0.045 where methanol was used while study in Pakistan by Sadiq *et al.*, (2015) reported to be 2.53 ± 0.06 where ethanol was used. These variations are based on changes in polarity.

The better results have shown at a dilution of 1:1 (S1) table 1 to be 3.716 ± 0.045 for crude methanolic extract of *A. nilotica* and 1.468 ± 0.052 for ascorbic acid and poor results have shown at dilution of 1:10 where for the crude extract was 2.895 ± 0.006 while 0.108 ± 0.006 for ascorbic acid (Figure1). This is similar to the study done by Del *et al.,* (2008) and Duganath *et al.,* (2010) in India where they reported that as a concentration of crude extract *A. nilotica* increase also absorbance reading increase and found that at 10ug/ml (absorbance 0.50), 20ug/ml (absorbance 0.65) and at 30ug/ml (absorbance was 0.8). In addition for *in vivo* study in order to attain the maximum effect of reducing the power the dilution ratio between crude extract and vehicle should be considered.

On the other hand it has been reported that geographical areas and part of the plant taken may lead to the variability of reducing power (Atif Ali, 2012; Ravikumar & Angelo, 2015).

AChE depression and recovery

OPs are reported to inhibit AChE in the blood (Chidiebere, 2012). Inhibition of AChE activity is an important indicator of OP poisoning (Malaysiana *et al.*, 2017). Different OPs exert different adverse effects by irreversible inhibition of AChE at the cholinergic synapses in the central and peripheral nervous systems (Maitra, 2018). In this current study, the poisoning and recovery effect FNT, PFP and CPF were assessed through testing AChE level of mice's blood. This evidence has been proven from result table 2 – 4 where there is a significance of

AChE depression, which is an indication of AChE inhibition due to exposure from FNT, CPF and PFP from day 0 to 14th day to all mice sexes for all treatment groups. The inhibition of AChE causes excessive accumulation of Acetyl Choline (Ach) at the synapses and neuromuscular junction, leading to overstimulation of ACh receptors (Jindal & Kaur, 2014). Recovering of AChE from OP-inhibition after being removed from OP exposure is very crucial in order to resolve the over accumulation of ACh in both control and treated group. Results of this present study shows that there is a sex variation which indicates that the female sex group is at higher risk to be affected by OPs compared to the male mice, similar statement as reported by Comfort & Re, (2017) and Ngowi *et al*., (2017).

Fig. 1. *Acacia nilotica*.

FNT have also been reported to exert their primary toxic effects through the inhibition of AChE (Malaysiana *et al*., 2017). The findings of this study have demonstrated that the 3ml of FNT can inhibition and cause depression of AChE in mice and AChE recovery can occur naturally with or without using any induced treatment.

This observation matches with that of a previous study were 2.6mg/kg bodyweight of Fenitrothion contaminated in beans used and cause slightly AChE depression and a complete AChE recovery observed at the end of the feeding period (Taylor *et al*, 2007) and study by Farghaly, (2008) reported the same effect upon 1.9 ppm of Fenitrothion, on other hand 10 mg/mL which corresponds to 10 mg/kg body weight of Fenitrothion (Sumithion 50, 500 mg/mL) cause depression of AChE within 7 days similarly to the study conducted on fish which was exposed to different levels of FNT where at 0.04 ppm FNT produced a 64% depression of AChE activity at 96 h exposure, whereas 0.02 ppm of FNT produced only a 44% reduction in AChE at 96 h and recovery occurred when submerged into fresh water (Sancho, Ferrando, & Andreu, 1997) but contrary to the Malaysiana *et al*., (2017) reported that the use of Palm oil which is a major sources of vitamin E that consist both tocopherol and tocotrienol tocotrienol-rich fraction

capable in protecting the oxidative toxic effects and AChE
recovery effect from FNT poisoning.

The inhibition of AChE by PFF produce similar effects as described in FNT. Previous studies have stipulated that antioxidants have significant protective role against OP pesticides against damage from lipid peroxidation (Morsy, 2003). Ascorbic acid (vitamin C) is amajor circulating water soluble antioxidants. The findings of this current study demonstrated that 3ml of PFP can induce inhibition of AChE and recovery of AChE can occur through usual body process without inducing neither methanolic stem bark extract of *A. nilotica* nor ascorbic acid. Similarly AChE recovery effect reported in fish exposed to OP induced the same effect despite of species variation (Oruc, 2012; Venkateswara Rao, 2006).

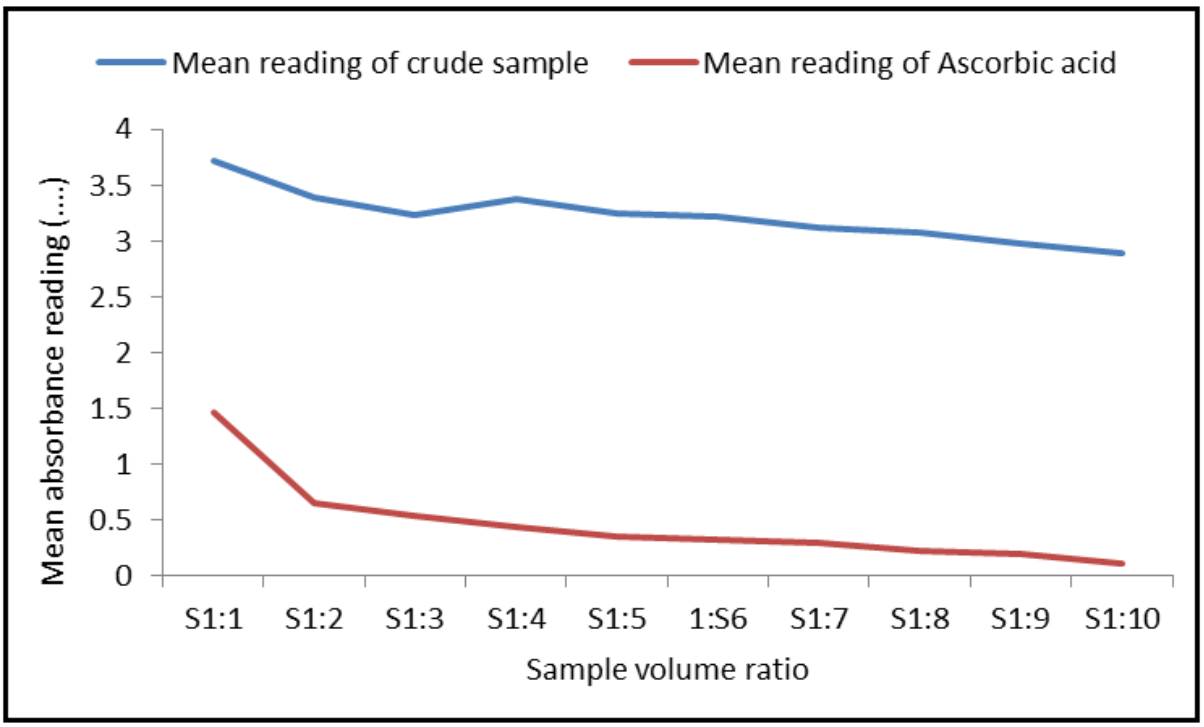

Fig. 2. Comparison of absorbance readings between crude methanolic extract of *A. nilotica* stem bark and ascorbic acid.

CPF is a known to be AChE inhibitor (Altuntas *et al.*, 2003). It may induce oxidative stress and inhibit antioxidantive and physiological activities (Mevlüt, 2013) through its non-systemic pathway to AChE inhibition at the end prevent breakdown of the neurotransmitter-acetylcholine (ACh) which lead to accumulation of ACh in the synaptic cleft and causes overstimulation of the neuronal cells, which leads to neurotoxicity and death (Akefe, 2017).

In the current study, AChE depression induced by CPF poisoning from day 0 to 14th (Table 4) was significantly, this is due to the ability of inhibiting AChE activities that lasts several weeks (Ambali *et al.*, 2012; Mevlüt, 2013). AChE recovery from CPF inhibition from this study demonstrated to be recovered and matches with untreated group on 28th day only on males in all recovery treatments of methanolic stem bark extract of *A. nilotica*, Ascorbic acid and normal feeding (Table 4) which gave a clue that at a low dose of exposure, recovery of AChE to normal level can be induced by the body itself through the normal body physiology of enzyme regeneration and other proteins through signaling system and the feedback loop.

These results are contrary to the study reported by Ambali *et al.*, (2012) where the use of vitamin E was proven to have improve restoration of AChE level due to its antioxidant efficiency. On other hand Verma *et*

al., (2007) reported that the use of combination of vitamins A, E and C give better results of AChE recovery from CPF inhibition. It has been reported that AChE recovery by induced antioxidant extract is based on its.

Hvl'ntioxidant property of containing polyphenol compounds like flavonoids, alpha-tocopherol and carotenoids which suggest having redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet -+however, in t///his study further studies of searching more plant enriched antioxidants are recommended.

Conclusion

A.nilotica has been reported to be used on scavenging ROS based on its antioxidant activity against different diseases based on ethno-botanical information, in this present study the use of methanolic stem barks from *A. nilotica* proven evidence of increasing reducing power at high concentration and nontoxic effects in implies that the plant is safe for utilization for rural communities for medicinal purpose where conventional drugs are unaffordable due to high costs.

This is a first study to assess and report the antioxidant activity of stem bark methanolic extracts of *A. nilotica* in controlling OP pesticide toxicity in mice, hence assessment is also warranted, despite OPs reported to inhibit AChE and cause AChE depression, recovery of AChE though the use of antioxidants from *A. nilotica* extract is not promising, however, at the end of the experiment male mice were safer that female mice.

This sex variation indicates that the female sex group is uniquely susceptible to pesticides compared to the male because of their physiological characteristics, lifestyle, and behavior.

Since only methanol is used in this study, other solvents are encouraged to be employed. In this study, crude extract was used, Isolation of active compounds is necessary. Also, more antioxidants enriched plants must be searched and assessed

Conflict of interests

The authors have not declared any conflict of interests.

Acknowledgements

Authors are grateful to African development bank (afdb) for financial support through the Nelson Mandela African Institution of Sciences and Technology (NM-AIST) and Mr. Emanuel Mboya for plant identification and confirmation.

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