Research Article

METHANOL LEAF EXTRACT OF DIOSPYROS CHLOROXYLON ATTENUATES CHLORPYRIFOS-INDUCED TOXICITY IN RATS

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ABSTRACT

Background and Objective: Chlorpyrifos (CPF) is an Organophosphate insecticide commonly used for household and Agricultural purposes. Despite the several reported toxicity in humans, there has been virtually no alternative effective insecticide. Thus, the amelioration of the toxicities seems the best option in alternative medicine. This study, therefore, examine the effects of methanol extract of Diospyros chloroxylon leaf (MEDCL) on the brain and heart of rats administered with CPF.

Materials and Methods: Twenty-four rats were randomized into four groups of 6 rats each, and administered separately with distilled water (Control), CPF (5 mg/kg), MEDCL (100 mg/kg) and MEDCL (100 mg/kg) + CPF (5 mg/kg), following 7 days of acclimatization. After 4 weeks of treatments, the rats were sacrificed, and the levels of Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), reduced glutathione (GSH), Glutathione peroxidase (GPx), glutathione S-transferase (GST) and DNA fragmentation were spectrophotometrically assessed in the brain and heart, while Acetylcholinesterase (AChE) activities were assessed in the serum and brain of the rats.

Results: The results revealed that CPF significantly reduced the levels of SOD, CAT, GSH, GPx and GST, while that of MDA was elevated in brain and heart, compared with controls. The CPF significantly lowered AChE activities in serum and brain by 94% and 48% respectively, while the level of DNA fragmentation was significantly increased in the rats treated with CPF. Supplementation with MEDCL significantly ameliorated the changes in the rats.

Conclusion: From the foregoing, the suppressive potential of methanol extract of Diospyros chloroxylon leaf is marked indicated in brain and cardiac redox imbalance induced on exposure to Chlorpyrifos.

Keywords: Chlorpyrifos, Diospyros chloroxylon, Oxidative stress, Acetylcholinesterase, DNA fragmentation

INTRODUCTION

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl)-phosphorothioate] is an organophosphate pesticide used against insects, ticks and mites in protection of various crops and ornamental plants. Chlorpyrifos becomes introduced into the environment via direct usage on crops, domestic animals, lawns, homes and work environments. However, when applied, this organophosphate is majorly dispersed in the environment through volatilization. In the environment, it becomes decomposed through the sunlight, bacterial and chemical processes. Mackay et al. has observed the atmospheric formation of Chlorpyrifos oxon from Chlorpyrifos. The hepatic biotransformation of Chlorpyrifos has been reported to involve desulfuration by cytochrome P-450 dependent, to form Chlorpyrifos oxon. This oxon is rapidly metabolized to 3, 5, 6-trichloro-2-pyridinol (TCP) through the activity of Aryl-esterase. Both bioactivation and detoxification of Chlorpyrifos have been suggested to occur very rapidly, since TCP was detected as the only metabolite in the hepatic effluent under steady-state conditions. The TCP has been noted to be, several times, less toxic than either Chlorpyrifos or its oxon form.
Some earlier studies by Bakke et al. 9 and Nolan et al. 9 had indicated that the metabolism of Chlorpyrifos oxon by A-esterase could be a major detoxification pathway, since TCP or its conjugate is the major metabolite identified in rodents and humans. A kinetic analysis of the relative rates of desulfurization and detoxification of Chlorpyrifos by Chambers and Chambers 10 suggested a gender-dependence, which may explain its higher toxicity in female rats than male ones. Various mutagenicity studies using Chlorpyrifos revealed that it could cause metaphasic chromosomal aberrations in the cell culture of mouse spleen 11, sister chromatid exchange in human lymphoid cells 12 and induction of micronuclei, chromosomal lesions and DNA damage in many organisms 13–15. However, the USEPA 16 reported the non-mutagenicity of Chlorpyrifos in both bacterial and mammalian cells, with slight genetic aberrations in yeast and bacterial cells. Tumor developments in mammalian organs, such as prostate 17, breast 18, 19 and rectum 20 have been reported to be caused by Chlorpyrifos exposure.

The hepatic cytochrome P-450 dependent metabolism of testosterone and estradiol was reported to be inhibited Chlorpyrifos exposure 21, 22. Furthermore, this organophosphate insecticide has been reported to cause decrease in testicular testosterone biosynthesis, and low productions of major steroidogenic acute regulatory (StAR) protein, steroidogenic enzymes and luteinizing hormone receptor stimulated cAMP as reported by Viswanath et al. 23.

Diospyros chloroxylon is a shrub plant, which belongs to the Diospyros species of the family, Ebenaceae 24. This shrub and some other members of the species have been used in orthodox medicine all over the world in treatments of several ailments and diseases 25–27. Studies have shown that the secondary metabolites, such as, alkaloids, flavonoids, tannins, triterpenoids, saponins, and phenolics present in D. chloroxylon could be responsible for its medicinal properties 28. An important triterpenoid, betulinc acid, found in Diospyros species 29, has been reported to have several biological properties 30–35. In the recent time, methanol extract of D. chloroxylon leaf has been reported potent against some environmental toxicants 36, 37. The present study hypothesized that methanol extract of Diospyros chloroxylon leaf (MEDCL) could attenuate redox-induced injuries in the brain and heart of rats administered with Chlorpyrifos.

MATERIALS AND METHODS

Duration and Place of Study

Both experimental work and data analysis were carried out between the months of February and June, 2018, in the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Chemicals

Glutathione, hydrogen peroxide, Epinephrine and 5, 5 diithiobis-(2-nitrobenzoate) (DTNB) were bought from the Sigma chemical Co. Saint Louis, MO, USA. Trichloroacetic acid, 2-thiobarbituric acid, Triton X-100 and Diphenylamine were purchased from the British Drug House (BDH) Chemical td, Poole, UK. All other reagents were of good analytical grades.

Collection and Extraction of Plant material

Diospyros chloroxylon leaves were bought in February, 2018, from a local herb seller at Sabo market in Ogbomoso, and authenticated at the Department of Biology, Botany Unit, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The leaves were washed with distilled water, air-dried and pulverized with an electrical grinder. The leaf powder was soaked in methanol for 72 hours. The extraction was repeated twice and the extract was collected, filtered with fine linen and concentrated under vacuum using rotary evaporator at 45°C. The resulting crude extract was stored under refrigeration at 4°C.

Experimental animals and design

Twenty-four (24) male Wistar rats (140.09 ± 9.61 g) were bought from the Animal house of the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan, Nigeria. The rats were later brought to the Animal house of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. They were randomized into four (4) groups (6 rats each) and acclimatized for 7 days, fed with rat pellets and drinking water (ad libitum), under 12-h light/dark cycle and temperature of 29 ± 2°C. The 4 groups of rats were separately treated as follows: distilled water (Control), CPF (5 mg/kg), methanol extract of D. chloroxylon leaf (MEDCL) (100 mg/kg), and CPF (5 mg/kg) + MEDCL (100mg/kg). CPF and MEDCL were administered twice per week and every other day, respectively for 4 weeks.

Collection of blood and organs

After 4 weeks, the rats were fasted overnight. Blood was collected from the rats into non-heparinized bottles by ocular bleeding, followed by sacrifice through cervical dislocation. Blood was centrifuged at 3000xg for 10 minutes to obtain serum. Brain and heart were excised, and blood stains were removed by rinsing in ice-cold 1.15% potassium chloride solution. Each organ was divided into 2 portions. One portion was homogenized with phosphate buffer (pH 7.4) using a Teflon homogenizer, followed by centrifugation at 10,000 x g for 10 minutes using a high speed refrigerated centrifuge to obtain homogenates for antioxidant and Acetylcholinesterase assays. The other portion of organs was kept for DNA fragmentation assay.
Biochemical Assays

**Determination of protein level**

Protein levels of brain, heart, and serum were determined using a method according to Lowry et al. using Bovine serum albumin as the standard.

**Determination of malondialdehyde level**

Malondialdehyde (MDA) levels of brain and heart were estimated using a method according to Ohkawa et al. The absorbance of the clear pink mixture was measured spectrophotometrically against a reference blank at 532 nm. The concentration MDA was calculated using a molar extinction coefficient (E) of 1.56 x 10^5 M^-1 cm^-1.

**Determination of superoxide dismutase activity**

The activities of Superoxide dismutase of brain and heart were assayed according to the method of Aebi. The increase in absorbance of the assay mixture was monitored spectrophotometrically at 480 nm for 150 seconds at an interval of 30 seconds. The specific activity of SOD was expressed in units/mg protein.

**Determination of Catalase activity**

Catalase activities of brain and heart were assayed according to the method of Aebi. The basis of the method is the ability of catalase to catalyse conversion of hydrogen peroxide in a reaction mixture. The absorbance was monitored spectrophotometrically at 240 nm for 180 seconds at an interval of 60 seconds. The activity of Catalase was expressed as units/mg protein.

**Determination of Glutathione peroxidase activity**

The activities of Glutathione peroxidase (GPx) of brain and heart were estimated using the method according to Andersen et al. This assay is based on the reaction between organic peroxide and reduced glutathione (GSH) to form disulfide glutathione (GSSG) in a reaction mixture. Glutathione reductase later catalyses the reduction of GSSG back to GSH and NADPH. The decrease in absorbance at 412 nm is directly related to the activity of GPx, which is expressed in µmol/mg protein/min.

**Determination of reduced glutathione level**

Reduced Glutathione (GSH) levels of brain and heart were estimated according to the method of Mitchell et al. The assay is based on the oxidation of reduced glutathione by DTNB, a sulphydryl reagent, to form a yellow derivative, 5'-thio-2-nitrobenzoic acid, whose absorbance is monitored at 412 nm. The GSH values were expressed as U/ mg protein.

**Determination of Glutathione-S-transferase activity**

Glutathione-S-transferase (GST) activities of brain and heart were assayed according to the method of Habig et al. The method is based on the ability of GST to catalyse the conjugation of L-glutathione and CDNB to form a conjugate, GS-DNB, with an absorbance at 340 nm. The rate of increase in absorbance at 340 nm is directly proportional to GST activity. Specific activities were expressed as µM/mg protein/min.

**Determination of Acetylcholinesterase activity**

Acetylcholinesterase (AChE) activities of serum and brain were determined using the method described by Ellman et al. with acetyltihiocholine iodide as a substrate. In this method AChE hydrolyzes acetylthiocholine iodide into thiocholine and butyric acid. The thiocholine reacts with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) to form 5'-thio-2-nitrobenzoic acid to form a yellow product, whose absorbance was measured spectrophotometrically at 412 nm.

**Determination of DNA fragmentation level**

Finally, a spectrophotometric method according to Wu et al. was used to determine the percentage fragmented DNA. Briefly, the brain and heart were homogenized in Tris-HCl-EDTA (lysis) buffer, followed by centrifugation at 27,000 x g for 10 mins. The intact DNA (pellet) was separated from the fragmented DNA (supernatant), and both the pellet and supernatant were treated with freshly prepared Diphenylamine (DPA) solution for colour production. The mixtures were incubated at 37°C for 20–24 hours. The absorbance was spectrophotometrically measured at 620 nm. The formula below was used for the calculation of percentage fragmented DNA:

\[
\text{Percentage Fragmented DNA} = \frac{\text{Absorbance of supernatant} \times 100}{\text{Absorbance of pellet} + \text{Absorbance of supernatant}}
\]

**Statistical analysis**

The values obtained were expressed as the mean ± standard deviation of six rats per group. Data were analysed with the Graph Pad Prism 6.0 package. One-way analysis of variance (ANOVA) and Tukey multiple were used for comparison of the values, taken the significance level as p = 0.05.

**RESULTS**

**Effect of MEDCL on body weight of CPF-exposed rats**

The effects of Chlorpyrifos (CPF) on the body weights of the rats are shown in Table 1. Treatment with CPF significantly (p < 0.05) lowered the body weight gain (in grammes) of the rats (10.26 ± 1.9) relative to the control (35.97 ± 2.7). In the MEDCL-supplemented rats, the weight gain was significantly increased (27.20 ± 2.6 g) in relation to the CPF-treated group.

**Effects of MEDCL on oxidant parameters of CPF-exposed rats**

The data in Table 2 show that CPF significantly (p < 0.05) reduced the SOD activity (2.30 ± 1.3 and 1.85 ± 0.6 U/mg protein) versus control (5.14 ± 1.2 and 3.54 ± 1.0) in both brain and heart, respectively. Furthermore, the catalase activities were reduced (3.91...
modulate the activity of ChE and therefore cause the ChE activities to significantly decrease in the rats treated with CPF. Chlorpyrifos was found to be a substrate for CPF. The result in Fig. 3 shows that CPF treatment caused the ChE activities to significantly (p < 0.05) lower the GSH concentrations in both brain (7.13 ± 0.8 and 4.13 ± 0.4 U/mg protein) and heart, respectively relative to the rats administered with CPF (Fig. 1). The GSH concentration was significantly (p < 0.05) lowered by CPF administration in both brain (11.62 ± 0.3 U/mg protein) and heart (9.74 ± 10 U/mg protein) as against the control (18.77 ± 14 and 15.56 ± 12 U/mg protein), respectively. When MEDCL was administered, the levels were elevated, and found to be 17.51 ± 3.2 and 15.91 ± 1.8 U/mg protein, in brain and heart, respectively compared with the CPF group, as shown in Fig. 2.

Table 3 presents the results on GPx and GST activities during the treatments. Chlorpyrifos was observed to significantly (p < 0.05) reduce GPx in brain (6.74 ± 1.0 U/mg protein) and heart (2.13 ± 0.5 U/mg protein) relative to the respective controls (8.47 ± 2.1 and 3.48 ± 1.2 U/mg protein). However, supplementation with MEDCL significantly attenuated the CPF-induced effects in the two organs, as shown in Table 3. Furthermore, CPF significantly (p < 0.05) reduced GST in brain (2.75 ± 0.8 µM/mg protein) and heart (1.13 ± 0.2 µM/mg protein) compared with the control rats (5.04 ± 1.2 and 2.48 ± 0.9 µM/mg protein, respectively) as shown in Table 3. On supplementation with MEDCL, the activities were found to be 4.05 ± 1.4 and 2.98 ± 0.8 µM/mg protein in the respective organs compared with the group treated with CPF.

Effects of MEDCL on Acetylcholinesterase activity and DNA fragmentation in CPF-exposed rats

The result in Fig. 3 shows that CPF treatment caused the AChE activities to significantly (p < 0.05) decrease in brain (0.11 ± 0.0 U/ml) and heart (0.18 ± 0.1 U/ml), when compared with the control treatment (0.19 ± 0.1 and 0.25 ± 0.0 U/ml). When supplemented with MEDCL, the activities of AChE enzyme were observed to be 0.21 ± 0.1 U/ml (brain) and 0.24 ± 0.0 U/ml (heart) in the experimental rats. The result of the effect of CPF on the percentage of DNA fragmentation in the two organs has been presented in Fig. 4. Treatment with CPF significantly (p < 0.05) elevated the level of fragmented DNA in brain (49.51 ± 2.4 %) and heart (64.78 ± 5.3 %), compared with the controls (28.47 ± 3.1 and 32.50 ± 3.8 %, respectively). However, the CPF-induced DNA damage was significantly attenuated by MEDCL in brain (30.90 ± 3.2 %) and heart (44.20 ± 5.1 %) in the rats.

DISCUSSION

The major findings in the present study include production of oxidative stress, DNA damage and inhibition of Acetylcholinesterase activity in rats treated with Chlorpyrifos. Interestingly, these adverse changes were attenuated on treatment of the experimental rats with methanol extract of Diospyros chloroxylon leaf (MEDCL).

The rats administered with CPF showed a significant decrease in body weight gain, while on supplementation with the extract, the effect was improved comparable to the control animals. Studies conducted by Perera et al. 47 and Whyatt et al. 48 observed a correlation between CPF intoxication and reduced birth weight and body length. This reduction may be a result of loss of appetite, malabsorption and digestive disorder, as reported by Neugent et al. 49.

Oxidative stress occurs when the level of reactive oxygen species (ROS) outweighs the capacity of the cellular antioxidant defence system, causing oxidation of DNA, protein or lipid molecules. The damage, in turn, may lead to altered gene expression 50, increased cell proliferation 51, chromosomal damage, gene mutation or tumourigenesis 52, 53.

The data from this study showed that activities of SOD and catalase were significantly lowered in both brain and heart on exposure of the rats to CPF. Malondialdehyde (MDA) is a reactive aldehyde formed as a result of the interaction between ROS and polyunsaturated fatty acids 54. The elevated level of MDA indicates an induction of lipid peroxidation, hence oxidative damage in the organs of the CPF-treated animals. The toxicant also significantly lowered the levels of GSH, GPx and GST in the two organs of the animals compared with the controls. In several tissues, including brain, liver and testis, CPF has been reported to induce lipid peroxidation in experimental animals 55, 56 and decrease in catalase, SOD, GPx and GST 57-60. According to Bebe and Panemangalore 61, low doses of CPF were demonstrated to cause decrease in GSH and increase in GPx, although the level of GSH was found to increase during treatment with higher CPF doses.

Catalase is an endogenous antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen 62, while GPx are a group of selenoproteins involved in protecting cells from oxidative damage caused by hydrogen peroxide and organic peroxides 63. Reduced glutathione (GSH), on the other hand, is a substrate needed by glutathione peroxidase (GPx) during detoxification, by being oxidized to GSH disulfide (GSSG). The GSSG is converted back to GSH by hydrogen peroxide and organic peroxides 63. Reduced glutathione (GSH), on the other hand, is a substrate needed by glutathione peroxidase (GPx) during detoxification, by being oxidized to GSH disulfide (GSSG). The GSSG is converted back to GSH by hydrogen peroxide and organic peroxides 63.
in level of GSH has been associated with low availability of cysteine molecules required for GSH production, which in turn has resulted from defect in transulfuration characteristic of liver damage.\(^\text{64}\) Glutathione-S-transferase (GST) is a major enzyme involved in detoxification via phase II reaction. The enzyme is responsible for the conjugation of several exogenous and endogenous reactive electrophiles with GSH.\(^\text{65}\) Inhibition of GST activity has been suggested by Binkova et al.\(^\text{66}\) as a mechanism of potentiating the toxicity of environmental chemicals, resulting in increased interactions between them (or their metabolites) and cellular macromolecules. In rats and mouse, CPF is metabolized in liver, through cytochrome-p450-dependent pathway, to form CPF-oxon\(^\text{67, 68}\) which is a more toxic metabolite. The observed reduction in the activities of the antioxidant enzymes in this investigation may indicate the inhibitory influence of CPF-oxon, which has therefore enhanced accumulation of ROS, leading to oxidative imbalance in the two organs of the rats. This finding is supported by a study carried out by Ventura et al.\(^\text{19}\), who noted that Chlorpyrifos induced elevation in the level of ROS in certain breast cancer cell lines. Furthermore, CPF aws shown to cause oxidative stress in testes of rats, leading to reductions in sperm count, gonadotropin levels, systemic testosterone and activities of enzymes needed for sperm production.\(^\text{69}\) In the group of animals supplemented with MEDCL, the activities of the antioxidant enzymes were significantly improved, while MDA level was reduced relative to the toxicant group. In a recent study, betulinic acid, a triterpenoid reported to be present in Diospyros species,\(^\text{20}\) has been demonstrated to improve SOD, Catalase, GPx and GST activities, and MDA level in experimental rats treated with an environmental toxicant.\(^\text{21}\) This thus suggests the potential of the Diospyros chloroxylon leaf extract used in this study to improve the redox status of the rats.

The present study also examined the effects of CPF on the Acetylcholinesterase (AChE) activities in brain and serum of the rats. Significantly decreased activities of the enzyme were observed in rats treated with CPF relative to control rats. In several studies, CPF treatment was shown to cause neurological effects such as, sensory loss, memory impairment and depression in AChE activities in many animal species.\(^\text{70-72}\) In a study by Cutler et al.\(^\text{73}\), it has been shown that CPF itself is not a potent AChE inhibitor, but its metabolite, CPF-oxon. This metabolite phosphorylates the hydroxyl group of the serine amino acid residue at the active site of AChE enzyme, through a nucleophilic interaction, leading to acetylcholine persistence, neurotransmission disruption and death.\(^\text{74}\) Studies have revealed that CPF could potentially inhibit neural formation and cholinergic projections in the early developmental stages of the central nervous system.\(^\text{75}\) This resulted in reduced neural connections, cell signalling capacity, and eventual deficiency in cholinergic synaptic response with behavioural problems in adolescence and adulthood.\(^\text{76}\) However, CPF has been demonstrated to preferentially exert toxicity in brain glial cells, which are formed later than the neuronal cells.\(^\text{77, 78}\) Furthermore, this toxicant exerted adverse effects on the differentiation, proliferation and activity of glial cells. The toxicant also affects the neuronal metabolism and targeting within the CNS in rats, during prenatal and postnatal exposures.\(^\text{19, 79}\) In addition, Sandoval et al.\(^\text{80}\) have reported that CPF could induce generation of astroglial cells, using human neural stem cells (hNSCs) line, without affecting neuron formation. However, this study has demonstrated that supplementation with MEDCL against CPF treatment improved the activity of AChE enzyme in both brain and serum of the rats, indicating the potential of the extract in improving cholinergic transmission in the rats.

The level of DNA fragmentation caused by CPF in brain and heart of the rats was investigated according to Wu et al.\(^\text{46}\). Treatment with CPF significantly increased DNA fragmentation compared with the controls. Interestingly, the ability of MEDCL to lower the amount of fragmented DNA against CPF treatment was observed. Various forms of genotoxic effect of CPF have been reported, including bone marrow micronuclei induction and DNA hypomethylation in mouse.\(^\text{15}\) Chromosomal alterations, chromosomal breaks and sister chromatid exchange in humans,\(^\text{81}\), and micronuclei induction and chromosomal lesions in erythrocytes.\(^\text{82}\) The observed reduction in the level of DNA fragmentation by MEDCL shows that the extract could protect the organs from the DNA damaging effect of CPF. The overall findings have implied the potential of methanol extract of Diospyros chloroxylon leaf in ameliorating the toxic effects of chlorpyrifos in experimental rats. The study thus clearly shows the possible application of MEDCL in the discovery of drugs against toxicity induced by environmental agents. It could therefore be recommended that Pharmaceutical Scientists could isolate, and work on, the active ingredients of Diospyros chloroxylon leaf, for the purpose of making a drug potent against oxidative and degenerative diseases. However, this study has some limitations in that only methanol was used for extraction, and the various compounds in the MEDCL were not elucidated.

**CONCLUSION**

The data from this study have shown that Chlorpyrifos (CPF) induced significant oxidative, neurotoxic and genotoxic injuries in brain and heart of rats. However, MEDCL ameliorated the CPF-induced redox imbalance in the two organs of rats. Furthermore, the extract of Diospyros chloroxylon leaf exerted attenuation against the neurotoxic and DNA-damaging effects induced by chlorpyrifos in the experimental rats.

**Significance Statement**

This study reported the potential of Diospyros chloroxylon leaf extract, which could be beneficial for
treatments of oxidative and degenerative diseases induced by chlorypyrifs. This study will assist researchers towards the use of Diospyros chloroxylon leaf for drug discovery, which has not been previously explored. As a result, a new theory on treatments of oxidative and degenerative diseases, using Diospyros chloroxylon leaf may be put forth, in addition to the existing data on medicinal values of plants

LIST OF ABBREVIATIONS
Chlorypyrifs (CPF)
Methanol extract of Diospyros chloroxylon leaf (MEDCL)
Superoxide dismutase (SOD)
Catalase (CAT)
Malondialdehyde (MDA)
Reduced glutathione (GSH)
Glutathione peroxidase (GPx)
Glutathione S-transferase (GST)
Acetylcholinesterase (AChE)
Reactive oxygen species (ROS).

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE
This study was carried out in accordance with ethical laws on animal handling.

FUNDING
We did not receive any grant for the conduct of the study.

AVAILABILITY OF DATA AND MATERIALS
The dataset supporting the conclusions of this article are included as tables and figures in the within the article.

AUTHORS' CONTRIBUTIONS
AGE conceived, designed and supervised the study. AOT co-supervised the study and read the final manuscript. AOO, AOE and BW provided the materials used in the study, collated literatures and performed the experimental procedures. EBO did the statistical analyses and drafted the manuscript. All authors financed of the study. All authors read and approved the final manuscript with the order of author’s names.

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CONSENT FOR PUBLICATION
All the authors gave their consents for the publication of the study.

COMPETING INTERESTS
There was no competing interest among the authors.

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Table 1. Effect of methanol extract of MEDCL on body weights of rats treated with Chlorpyrifos

| Treatments    | Final weight (g) | Initial weight (g) | Weight Gain (g) |
|---------------|------------------|--------------------|-----------------|
| Control       | 150.59 ± 3.9     | 114.62 ± 6.8       | 35.97 ± 2.7     |
| CPF           | 132.56 ± 3.6     | 128.30 ± 4.1       | 10.26 ± 1.9     |
| MEDCL         | 140.10 ± 5.4     | 108.40 ± 3.6       | 31.70 ± 4.7     |
| CPF + MEDCL   | 147.17 ± 2.2     | 119.97 ± 4.2       | 27.20 ± 2.6     |

Data expressed in mean ± SD, n = 6, a statistically lower than control (p < 0.05), b statistically higher than CPF (p < 0.05), CPF-Chlorpyrifos, MEDCL- Methanol extract of Diospyros chloroxylon leaf

Table 2. Effects of MEDCL on Superoxide dismutase and Catalase activities in brain and heart of rats treated with Chlorpyrifos

| Treatments | SOD (U/mg protein) | Catalase (U/mg protein) |
|------------|--------------------|------------------------|
|            | Brain              | Heart                  | Brain           | Heart            |
| Control    | 5.14 ± 1.2         | 3.54 ± 1.0             | 6.88 ± 1.5      | 3.93 ± 0.4      |
| CPF        | 2.30 ± 1.3         | 1.85 ± 0.6             | 3.91 ± 1.2      | 3.38 ± 1.0      |
| MEDCL      | 4.51 ± 1.8         | 4.10 ± 1.6             | 7.46 ± 0.9      | 3.56 ± 0.7      |
| CPF + MEDCL| 4.68 ± 1.6         | 3.14 ± 0.6             | 7.13 ± 0.8      | 4.13 ± 0.4      |

Data expressed in mean ± SD, n = 6, a statistically lower than control, b statistically higher than CPF (p < 0.05), CPF-Chlorpyrifos, MEDCL- Methanol extract of Diospyros chloroxylon leaf

Figure 1. Effect of MEDCL on malondialdehyde level in brain and heart of rats treated with Chlorpyrifos

Data expressed in mean ± SD, n = 6, a statistically higher than control, b statistically lower than CPF (p < 0.05), MEDCL-Methanol extract of Diospyros chloroxylon leaf
Data expressed in mean ± SD, n = 6, a statistically lower than control, b statistically higher than CPF (p < 0.05), MEDCL - Methanol extract of Diospyros chloroxylon leaf

Table 3. Effects of MEDCL on Glutathione peroxidase (GPx) and Glutathione S-transferase (GST) activities in brain and heart of rats treated with Chlorpyrifos

| Treatments       | GPx (µM/mg protein/min) | GST (µM/mg protein/min) |
|------------------|-------------------------|-------------------------|
|                  | Brain                   | Heart                   | Brain                   | Heart                   |
| Control          | 8.47 ± 2.1              | 3.48 ± 1.2              | 5.04 ± 1.2              | 2.48 ± 0.9              |
| CPF              | 6.74 ± 1.0<sup>a</sup>  | 2.13 ± 0.5<sup>a</sup>  | 2.75 ± 0.8<sup>a</sup>  | 1.13 ± 0.2<sup>a</sup>  |
| MEDCL            | 9.16 ± 1.4              | 4.71 ± 1.6              | 4.87 ± 1.3              | 2.64 ± 0.7              |
| CPF + MEDCL      | 7.78 ± 2.1<sup>b</sup>  | 4.28 ± 0.7<sup>b</sup>  | 4.05 ± 1.4<sup>b</sup>  | 2.98 ± 0.8<sup>b</sup>  |

Data expressed in mean ± SD, n = 6, <sup>a</sup> statistically lower than control, <sup>b</sup> statistically higher than CPF (p < 0.05), MEDCL - Methanol extract of Diospyros chloroxylon leaf
Figure 3. Effect of MEDCL on Acetylcholinesterase (AChE) activity in brain and heart of rats treated with Chlorpyrifos

Data expressed in mean ± SD, n = 6, a statistically lower than control, b statistically higher than CPF (p < 0.05), MEDCL- Methanol extract of Diospyros chloroxylon leaf

Figure 4. Effect of MEDCL on DNA fragmentation in brain and heart of rats treated with Chlorpyrifos

Data expressed in mean ± SD, n = 6, a statistically higher than control, b statistically lower than CPF (p < 0.05), MEDCL- Methanol extract of Diospyros chloroxylon leaf