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Mitigating Effects of Jimsonweed (*Datura Stramonium*) on Chronic Chlorpyrifos-Induced Toxicity in Wistar Rats

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Abstract

Chlorpyrifos (CFS), a widely used organophosphorus pesticides for agricultural, industrial and residential purposes, caused accidental poisoning in non-target mammals and other living organisms, including labourerers tasked with spraying economic plants. This study evaluated chronic chlorpyrifos-induced toxicity and the ameliorative potential of ethanolic leaf extract of Datura stramonium ((ELEDS) in male Wistar rats. The LD_{50} of the CFS and ELEDS were found to be 87.3 and 4260 mg/kg. bd. wt., respectively. Fifty (50) adult male Wistar rats were placed at random into five groups of 10 each and treated for 36 weeks, as follows: group I; soya bean oil (SBO) orally at 2 mL/kg. bd. wt., group II; ELEDS at 85.2 mg/kg. bd. wt. orally, group III; CFS at 3.49 mg/kg. bd. wt. orally, group IV; CFS+ELEDS, group V; CFS + atropine (AP) at 0.02 mg/kg. bd. wt. Anaemia developed in CFS-treated rats with significant ($p<0.05$) decreases in the packed cell volume (PCV) (at week 24, 28, 32 and 36), red blood cell count (RBC) (at week 20, 28, 32 and 36), haemoglobin concentration (Hb) (at week 28 and 36), and platelets (PLT) (at week 28, 32 and 36). CFS-treated rats showed brain vacuolation, degeneration, perivascular and perineuronal oedema and necrosis of the Purkinje cells. While the heart manifested myocardial hypertrophy, degeneration, mild haemorrhages, thickened inter alveolar septa, alveolar capillary congestion, haemorrhages and severe mononuclear cellular infiltration and necrosis of pneumocytes occurred in the lungs. In addition, vacuolation, sinusoidal congestion and circumferential zone of hepatocellular necrosis occurred in the hepatic tissue of CFS-treated rats. In the SBO, CFS+ELEDS, and CFS+AP-treated groups, in contrast, no significant changes were observed in all the parameters analysed. The findings are useful especially in areas where animals are prone to CFS toxicity and the occupationally induced toxicity in individuals exposed to daily low doses of such pesticides, with poor accessibility to antidotes.

Keywords: *Chlopyrifos; Anaemia; Necrosis; Respiratory Pathology; Datura Stramonium; Amelioration*

INTRODUCTION

Chlorpyrifos (CFS) was reported as one of the most commonly used organophosphorus (OP) pesticide worldwide for agricultural, industrial and domestic purposes, with hazardous and serious public health concerns [1,2,3,4]. Toxicity-induced pathologies included imbalance between free radicals and body antioxidant defense system (ADS), histopathological changes, neurotoxicity that induced neurobehavioural changes, immunological abnormalities, teratogenicity, reproductive and developmental toxicity [5,6,7] in addition to hepatotoxic and haematological deterioration [8]. Chlorpyrifos molecules spread through aerosol or surface of run off natural water bodies, and on accumulation, caused death of several different organisms including fishes in aquatic habitat [9,10,11,12,13,14]. Although indoor residential use of CFS was banned in US [15], its usage continued widely as pesticide with over 8 million pounds of CFS being utilised in agriculture annually [16,17]. Despite standardization to minimize exposure to CFS in some countries such as China and USA, its residues were frequently detected in food, grains, air and water bodies due to its wide usage in agriculture that led to bio-accumulation [18,19,20]. Deoxyribonucleic acid (DNA) instability in rat leucocytes occurred following oral doses of CFS as low as 0.01 mg/kg. bd. wt. over a 28-day study [21] though, this dose is 10-fold higher than the current acceptable operator exposure level (AOEL) and acceptable daily intake (ADI) values proposed [22]. Chlorpyrifos insecticide interfered principally with signaling from the neurotransmitter acetylcholine (ACh) when its metabolite, CFS-oxon, bound permanently with the enzyme acetylcholinesterase (AChE), preventing it from deactivating Ach in the synapse [23,24]. This leads to build-up of ACh between the neurons and a stronger, longer-lasting signal to the next neuron. Normal function can only be restored when new AChE is being synthesized. Atropine (AP), the conventional treatment of CFStoxicity is a controlled drug, hence not freely available, in addition to the side effects, characterized bydry mouth, mydriasis, rapid pulse, photophoia, dry and flushed skin, restlesess and excitement [25]. Hence, the need to widen atropine availability or develop an alternative antidote with high content of atropine and other anticholinergic contents.

Medicinal plants provided over 75% of drugs used in primary healthcare worldwide [26], includingabout one-fourth of all prescribed medicines [27]. *Datura stramonium* exhibited medicinal value with validated anticholinergic effect [28,29,30,31]. Indeed, the use of alkaloids from *Datura* spp. in many medical applications, included antidote for OP toxicity and lowering of heart rate [32,33]. In addition, there was report of terrorists using OP nerve agents [34] and ELEDS can be used as alternative antidote to increase chances of survival.

Several studies portrayed blood as the most important tissue in the body with reproducible metabolic alterations and a very diverse cellular and enzymatic constituents, thus, serving as medium for clinical investigation as well as nutritional status of animals, homeostatic responses, drug transport biotransformation and metabolic excretion, endocrine and neurohumoral transmissions, toxic assessment and toxicity management [35,36,37]. Accordingly, laboratory evaluation of haematological parameters became essential in assessing the toxicities of exogenous compounds in the body system [38,39] for its sensitivity, accuracy and reliability for research, diagnosis of diseases, prevention and treatment.

This study was designed to investigate the mitigating potentials of *D. stramonium*on chronic chlorpyrifos-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of Crude Extract of Datura Stramonium

Fresh *D. stramonium* leaves were harvested in October 2019 in Shanono and environs, Shanono LGA, Kano State, Nigeria. The leaves sample was taken to the herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria for authentication and voucher specimen number, ABU0108 was obtained. The dried leaves sample were prepared as described earlier [40], using 70% v/v ethanol, clarified by gravitational filtration using Whatman filter paper size 1, to produce the crude ethanolic extracts.

Ethical Clearance

Animal experimentations in this study was approved by the Ahmadu Bello University, Zaria Committee on Animal Use and Care (ABUCAUC), with Approval Protocol Number: ABUCAUC/2020/024 in conformance with the Guide and Care of the Use of Animals in Research and Teaching of the University.

Research Animals Acquisition and Management

Fifty (50) apparently healthy adult male Wistar rats weighing between 180 and 220 g were acquired from the Small Animal Experimental Station, Central Diagnostic Division, National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. They were kept in aluminium rat' cages 40×20×70 cm dimensions, with beddings made up ofwood shavings at room temperature and light/dark cycle for 12 hrs in the Laboratory animal room, Department of Veterinary Pathology, A.B.U., Zaria. The rats were dewormed with albendazole at 10 mg/kg. bd. wt. and allowed to acclimatize for 4 weeks prior to commencement of the experiment. The rats were fed standard pellet diet (Grand cereals Feeds, Jos, Nigeria) and allowed free access to clean water. The rats were identified by tail colour marks relevantto their groups.

Animal Treatments

Thirty-six weeks of chronic toxicity test was conducted as described [18,41]. Fifty Wistar rats were allocated at random into five groups of 10 male Wistar rats each. Group I (SBO) was orally administered at 2 mL/kg. bd. wt., group II (ELEDS) was administered at dose rate of 85.2 mg/kg. bd. wt. (2% of the LD_{50}). Group III was administered CFS at dose rate of 3.49 mg/kg. bd. wt. (4% of the LD_{50}) dissolved in SBO oil. Groups IV and V were pre-administered CFS (3.49 mg/kg. bd. wt.) then followed by ELEDS (85.2 mg/kg. bd. wt.) and AP (0.02 mg/kg. bd. wt)30 min later, respectively.The SBO, ELEDS and CFS were administered orally to the experimental rats by gavage using rat's stomach cannula as described earlier [42], whereas, AP was administered via intraperitoneal route (i.p). All regimens were administered once daily between 09:00 am and 10:00 am to non-fasted rats. The first day of drugs administration was considered experimental week 0.

Blood Samples Collection and Laboratory Analyses

The experimental rats were fasted for 3 hrs prior to and after blood collection. Blood samples were collected in 5 mL sterile heparin-coated Vacutainer tubes, via orbital venous plexus bleeding after a lightly chloroform-anaesthetized experimental rats at week (W) 0, W12, W16, W20, W24, W28, W32 and W36 for the haematological analyses; samples were immediately transported to laboratory under chilled condition (4-8°C) using ice-packs. The packed cell volume (PCV), red blood cells count (RBC), haemoglobin concentration (Hb), platelete count, total white blood cells count (WBC) and differential leucocyte count were determined using standard procedure [43,44].

Gross and Histopathological Evaluations

At the end of the experiment (36 weeks), animals were fasted for 24 h before euthanasia. Five (5) rats from each group were humanely euthanized via jugular venesection after light chloroform anesthesia. The brain, heart, lungs, liver, small intestine, spleen, kidney and pancreas of the animals were processed for histopathology and stained as described earlier [45]. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Japan). Four-field observations were made per slide (at 40x, 100x and 250x magnification). The severity of changes in the sections were scored "1 – 4" [46]. Representative fields were photographed at x250.

Data Analysis

Quantitative results of changes in haematolocal parameters, were analyzed by Repeated measure ANOVA using SPSS version 20 software. Differences between each group were compared using the Tukey's Post Hoc Multiple Comparison Tests. The probability value, *P<0.05* was used as the critical level of significance [47].

RESULTS

Mean Packed Cell Volume of Rats in the Treatment Groups

The mean PCV values in the treatment groups are as presented in Table 1. The mean PCV values in the treatment groups were comparable as they did not differ significantly (*P>0.05*) up to week 20.However, at weeks 24, 28, 32, and 36 the mean PCV value in the CFS-treated groups (42.20 ± 1.85, 40.80 ± 1.77, 42.00 ± 1.64 and 40.60 ± 1.63%, respectively)were significantly (*P<0.05*) lower when compared to those (53.80 ± 3.02, 54.60 ± 2.56, 53.60 ± 2.42 and 52.60 ± 2.23 %, repectively) in the control groups (SBO). At week 36, mean PCV value in the CFS-treated group was significantly (*P<0.05*) lower when compared to that (50.20 ± 1.28%) in the CFS+AP-treated group.

Table 1. Mean packed cell volume of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Packed Cell Volume (%)				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
W0	52.60 ± 1.96	49.90 ± 2.39	52.60 ± 2.11	49.60 ± 2.29	51.20 ± 1.02
W12	53.00 ± 3.02	47.00 ± 4.34	47.80 ± 0.49	47.80 ± 0.80	49.20 ± 1.53
W16	49.00 ± 0.84	47.80 ± 2.85	45.80 ± 1.24	47.20 ± 1.28	48.00 ± 1.61
W20	49.60 ± 0.93	49.60 ± 1.56	46.60 ± 1.44	47.20 ± 1.20	48.20 ± 0.58
W24	53.80 ± 3.02^{b}	51.60 ± 1.43^{ab}	42.20 ± 1.85^{a}	45.60 ± 2.01^{ab}	46.20 ± 1.96^{ab}
W28	54.60 ± 2.56^{b}	52.23 ± 2.24^{ab}	40.80 ± 1.77^{a}	44.80 ± 1.98^{ab}	48.80 ± 1.77^{ab}
W32	53.60 ± 2.42^{b}	49.80 ± 3.23^{ab}	42.00 ± 1.64^{a}	47.20 ± 1.32^{ab}	50.60 ± 1.60^{ab}
W36	52.60 ± 2.23^{b}	48.80 ± 2.69^{ab}	40.60 ± 1.63^{a}	45.20 ± 1.59^{ab}	50.20 ± 1.28^{b}

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Red Blood Cell Count of Rats in the Treatment Groups

The mean RBC values in the treatment groups were as presented in Table 2. The mean RBC values in the treatment groups were comparable and did not differ significantly (*P>0.05*) up to week 16.However, at weeks 20 and 24 the mean RBC value in the CFS-treated groups(6.92 ± 0.13, 7.02 ± 0.21 $x10^{12}$/L, respectively)were significantly (*P<0.05*) lower compared to those (8.26 ± 0.13, 8.52 ± 0.43 $x10^{12}$/L, respectively) in the control groups (SBO). At weeks 28, 32 and 36 of treatment: The mean RBC value in CFS-treated groups (6.28 ± 0.21, 6.18 ± 0.12 and 6.16 ± 0.10 $x10^{12}$/L, respectively) were significantly (*P<0.05*) lower compared to those (7.92 ± 0.14 and 7.62 ± 0.26, 7.66 ± 0.15 and 7.32 ± 0.18, and 8.00 ± 0.10 and 7.4 ± 0.16 $x10^{12}$/L, respectively) in the SBO- and CFS+AP-treated groups.

Table 2. Mean red blood cell count of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Red Blood Cell Count ($x10^{12}$/L)				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
W0	8.38 ± 0.04	8.00 ± 0.44	8.40 ± 0.56	8.30 ± 0.39	8.08 ± 0.22
W12	8.52 ± 0.36	7.92 ± 0.46	7.92 ± 0.28	7.86 ± 0.11	7.56 ± 0.25
W16	8.02 ± 0.35	7.88 ± 0.37	7.32 ± 0.24	7.70 ± 0.10	7.46 ± 0.07
W20	8.26 ± 0.13^{b}	7.70 ± 0.29^{ab}	6.92 ± 0.13^{a}	7.42 ± 0.07^{ab}	7.58 ± 0.27^{ab}
W24	7.98 ± 0.13	7.76 ± 0.37	7.02 ± 0.21	7.48 ± 0.19	7.86 ± 0.25
W28	7.92 ± 0.14^{b}	8.00 ± 0.09^{ab}	6.28 ± 0.21^{a}	7.12 ± 0.24^{abc}	7.62 ± 0.26^{b}
W32	7.66 ± 0.15^{b}	7.22 ± 0.20^{ab}	6.18 ± 0.12^{a}	7.16 ± 0.26^{ab}	7.32 ± 0.18^{b}
W36	8.00 ± 0.10^{b}	7.58 ± 0.20^{ab}	6.16 ± 0.10^{a}	7.08 ± 0.28^{ab}	7.40 ± 0.16^{b}

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Haemoglobin Concentrations of Rats in the Treatment Groups

The mean Hb values in the treatment groups were as presented in Table 3. The mean Hb values in the treatment groups were comparable as they did not differ significantly (*P>0.05*) up to week 24, and 32.However, at weeks 28 and 36 the mean Hb value in the CFS-treated groups (13.58 ± 0.58 and 14.42 ± 0.46 g/dL, respectively) were significantly (*P<0.05*) lower compared to those (16.90 ± 0.66 and 17.50 ± 0.74 g/dL, respectively) in the control groups (SBO).

Table 3. Mean haemoglobin concentration of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Haemoglobin Concentration (g/dL)				
	SBO	ELEDS	CFS	CFS+ ELEDS	CFS+AP
W0	17.50 ± 0.65	16.78 ± 1.10	17.50 ± 0.70	16.48 ± 0.76	17.08 ± 0.34
W12	17.68 ± 1.01	14.34 ± 1.25	15.90 ± 0.16	15.90 ± 0.28	16.40 ± 0.51
W16	16.32 ± 0.29	15.26 ± 0.62	15.22 ± 0.42	15.76 ± 0.43	16.00 ± 0.55
W20	17.10 ± 0.54	14.16 ± 0.65	15.50 ± 0.48	15.76 ± 0.41	16.10 ± 0.19
W24	16.84 ± 0.27	15.82 ± 0.34	14.42 ± 0.61	15.20 ± 0.67	15.40 ± 0.65
W28	16.90 ± 0.66^{b}	16.76 ± 1.74^{ab}	13.58 ± 0.58^{a}	14.92 ± 0.67^{ab}	16.26 ± 0.59^{ab}
W32	17.08 ± 0.88	14.60 ± 1.08	13.98 ± 0.55	15.76 ± 0.45	16.84 ± 0.54
W36	17.50 ± 0.74^{b}	15.26 ± 1.56^{ab}	14.42 ± 0.46^{a}	15.08 ± 0.54^{ab}	16.74 ± 0.43^{ab}

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Platelet Count of Rats in the Treatment Groups

The mean PLT values in the treatment groups were as presented in Table 4. The mean PLT values in the treatment groups were comparable and did not differ significantly (*P>0.05*) in the groups at weeks 0, 12, 16, 20 and 24.However, at weeks 28 and 32 the mean PLT value in CFS-treated groups (367.6 ± 17.71 and 347.6 ± 17.71 x10^{9}/L, respectively)was significantly (*P<0.05*) lower compared to those (460.0 ± 14.49 and 447.6 ± 12.4 x10^{9}/L; 467.6 ± 5.81 and 437.6 ± 17.71, respectively) in the SBO and CFS+AP-treated groups. At week 36 of treatment: the mean PLT value in the CFS-treated group (352.6 ± 18.54 x10^{9}/L)was significantly (*P<0.05*) lower when compared to that of (472.6 ± 12.8 x10^{9}/L) in the SBO-treated group.

Table 4. Mean platelet count of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Platelet Count (10^{9}/L)				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
W0	482.6 ± 07.98	492.6 ± 15.22	465.0 ± 16.28	462.6 ± 16.85	470.0 ± 15.81
W12	455.0 ± 12.04	402.6 ± 18.80	450.0 ± 13.42	457.6 ± 18.27	455.0 ± 02.24
W16	452.6 ± 13.18	405.0 ± 12.04	425.0 ± 09.22	447.6 ± 10.19	450.0 ± 15.17
W20	467.6 ± 06.62	397.0 ± 19.40	422.6 ± 03.71	442.6 ± 14.96	455.0 ± 80.06
W24	477.6 ± 07.98	415.0 ± 22.25	422.6 ± 13.18	447.6 ± 11.99	452.6 ± 14.62
W28	460.0 ± 14.49^{b}	402.6 ± 15.29^{ab}	367.6 ± 17.71^{a}	430.0 ± 14.83^{ab}	447.6 ± 12.40^{b}
W32	467.6 ± 05.81^{b}	392.6 ± 15.93^{ab}	347.6 ± 17.14^{a}	425.0 ± 15.00^{ab}	437.6 ± 17.71^{b}
W36	472.6 ± 12.80^{b}	417.6 ± 12.80^{ab}	352.6 ± 18.54^{a}	397.6 ± 06.62^{ab}	422.6 ± 13.92^{ab}

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Total White Blood Cell Count of Rats in the Treatment Groups

The mean WBC values in the treatment groups were as presented in Table 5. The mean WBC values in the treatment groups were comparable and did not differ significantly (*P>0.05*) in the groups upto week 36.

Table 5. Mean total white blood cell count of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Total White Blood Cell Count ($x10^9$/L)				
	SBO	ELEDS	CFS	CFS+ ELEDS	CFS+AP
W0	08.48 ± 1.07	08.06 ± 0.99	09.18 ± 0.51	07.64 ± 0.96	07.52 ± 0.85
W12	09.50 ± 0.64	08.12 ± 0.35	07.24 ± 0.56	08.24 ± 0.44	09.40 ± 1.06
W16	08.72 ± 0.39	07.56 ± 0.67	07.16 ± 0.26	07.88 ± 0.83	09.00 ± 0.44
W20	08.38 ± 0.42	08.22 ± 0.22	06.78 ± 0.29	07.22 ± 0.92	08.12 ± 0.68
W24	08.22 ± 0.38	08.18 ± 0.28	06.60 ± 0.35	07.68 ± 0.67	08.02 ± 0.54
W28	09.08 ± 0.77	08.76 ± 0.44	07.18 ± 0.28	08.76 ± 0.56	08.50 ± 0.50
W32	09.60 ± 0.69	08.22 ± 0.56	06.56 ± 0.32	08.34 ± 0.64	09.48 ± 0.51
W36	08.88 ± 1.17	08.42 ± 0.18	06.04 ± 0.25	08.32 ± 0.64	08.52 ± 0.48

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Neutrophil Count of Rats in the Treatment Groups

The mean NEU values in the treatment groups were as presented in Table 6. The mean NEU values in the treatment groups were comparable and did not differ significantly (*P>0.05*) in all the groups throughout the period of the experiment.

Table 6. Mean neutrophil count of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Neutrophil Count ($x10^9$/L)				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
W0	3.03 ± 0.50	3.49 ± 0.57	3.23 ± 0.20	2.82 ± 0.43	2.86 ± 0.31
W12	3.48 ± 0.21	3.64 ± 0.19	2.65 ± 0.17	2.85 ± 0.19	3.22 ± 0.34
W16	3.54 ± 0.20	3.55 ± 0.41	2.83 ± 0.37	2.98 ± 0.39	3.59 ± 0.20
W20	3.49 ± 0.44	3.67 ± 0.62	2.77 ± 0.61	2.96 ± 0.65	3.42 ± 0.51
W24	3.02 ± 0.26	3.26 ± 0.25	2.11 ± 0.27	2.64 ± 0.19	2.87 ± 0.18
W28	3.34 ± 0.32	3.21 ± 0.24	2.33 ± 0.20	2.98 ± 0.25	3.06 ± 0.20
W32	3.28 ± 0.30	3.09 ± 0.22	2.09 ± 0.16	2.68 ± 0.16	3.10 ± 0.31
W36	3.19 ± 0.51	3.12 ± 0.19	1.88 ± 0.25	2.58 ± 0.23	2.78 ± 0.18

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Lymphocyte Count of Rats in the Treatment Groups

The mean LYM values in the treatment groups were as presented in Table 7. The LYM mean values were comparable and not significantly (*P>0.05*) different in all the treatments across the groups, throughout the period of the experiment.

Table 7. Mean lymphocyte count of rats in the different treatment groups (n = 5)

Time	Mean ± SEM Lymphocyte Count ($x10^9$/L)				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
W0	5.04 ± 0.61	4.88 ± 0.64	5.33 ± 0.43	4.86 ± 0.56	5.06 ± 0.05
W12	5.31 ± 0.38	4.30 ± 0.41	4.36 ± 0.35	4.82 ± 0.24	5.47 ± 0.77
W16	4.90 ± 0.37	4.18 ± 0.38	4.65 ± 0.18	4.81 ± 0.51	5.27 ± 0.37
W20	5.07 ± 0.37	4.15 ± 0.34	4.60 ± 0.16	4.46 ± 0.56	4.92 ± 0.51
W24	4.85 ± 0.27	4.84 ± 0.54	4.42 ± 0.17	4.82 ± 0.42	4.83 ± 0.45
W28	5.60 ± 0.55	4.99 ± 0.51	5.46 ± 0.28	5.88 ± 0.40	5.48 ± 0.40
W32	5.60 ± 0.54	4.72 ± 0.46	4.74 ± 0.30	5.56 ± 0.60	5.80 ± 0.25
W36	5.27 ± 0.72	4.91 ± 0.30	4.55 ± 0.32	5.39 ± 0.39	5.33 ± 0.36

*Repeated Measure ANOVA, SEM = Standard error of mean, n = Sample size, a different superscript (a, b) within each variable across the groups indicates (*P<0.05*) significant differences. SBO = Soya bean oil (2 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (85.20 mg/kg. bd. wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. wt.), AP = Atropine (0.02 mg/kg. bd. wt.).

Mean Microscopic Changes Scored for Some Organs in Rats

The mean microscopic changes scored for the brain, cardiac, pulmonary, hepatic and renal tissues were significantly (*P<0.05*) higher in the CFS-treated groups with mean values of 2.80 ± 0.25, 2.40 ± 0.16, 2.40 ± 0.55, 2.50 ± 0.54 and 2.90 ± 0.38, respectively compared to the SBO- treated groups with mean values of 0.07 ± 0.07, 00.00 ± 0.00, 0.00 ± 0.00, 0.00 ± 0.00and0.22 ± 0.14, respectively. Whereas, the mean microscopic changes scored for the intestinal, splenic and pancreatic tissues did not vary significantly (*P>0.05*) among the treatment groups (Table 8).

Table 8. Mean microscopic scores of changes in some vital organs of the Wistar rats (n = 3).

Organs	Mean score ± SEM				
	SBO	ELEDS	CFS	CFS+ELEDS	CFS+AP
Brain	0.07 ± 0.07^{a}	1.10 ± 0.48^{ab}	2.80 ± 0.25^{b}	1.10 ± 0.17^{ab}	0.40 ± 0.07^{ab}
Heart	0.00 ± 0.00^{a}	0.50 ± 0.72^{ab}	2.40 ± 0.16^{b}	0.72 ± 0.18^{ab}	0.22 ± 0.07^{ab}
Lungs	0.17 ± 0.07^{a}	1.01 ± 0.62^{ab}	2.40 ± 0.55^{b}	0.67 ± 0.29^{ab}	0.28 ± 0.18^{ab}
Liver	0.00 ± 0.00^{a}	0.18 ± 0.04^{a}	2.50 ± 0.54^{b}	0.89 ± 0.11^{ab}	0.50 ± 0.08^{ab}
Intestine	0.25 ± 0.25	0.10 ± 0.05	0.67 ± 0.30	0.25 ± 0.16	0.33 ± 0.24
Spleen	0.11 ± 0.11	0.22 ± 0.10	0.89 ± 0.59	0.11 ± 0.11	0.00 ± 0.00
Pancreas	0.11 ± 0.11	0.13 ± 0.08	1.70 ± 0.69	0.44 ± 0.11	0.44 ± 0.29
Kidneys	0.22 ± 0.14^{a}	0.90 ± 0.27^{ab}	2.90 ± 0.38^{b}	1.10 ± 0.25^{ab}	1.00 ± 0.19^{ab}

*Kruskal Wallis test, a different superscript (a, b) within each variable across the groups indicates significant differences (*P<0.05*), SEM = Standard error of mean, n = Sample size, DW = Distilled water (10 mL/kg. bd. Wt.), ELEDS = Ethanolic leaf extract of *Datura stramonium* (170.40 mg/kg. bd. Wt.), PS =Physostigmine (0.02 mg/kg. bd. Wt.), SBO = Soya bean oil (2 mL/kg. bd. Wt.), CFS = Chlorpyrifos (3.49 mg/kg. bd. Wt.)

Brain

Brain tissues in the SBO- (Plate I A_1 and A_2), ELEDS- (Plate I B_1 and B_2) and CFS+AP-treated groups were normal. There were, however, shrunken dark neurons with eosinophilic cytoplasm and hyperchromatic nuclei, capillary congestion, neuropil vacuolation, and neuronal and Purkinje cells necrosis in CFS-treated rats (Plate I C_1 and C_2), while capillary congestion (Plate ID_1) occurred in cerebrum in CFS+ELEDS-treated rats.

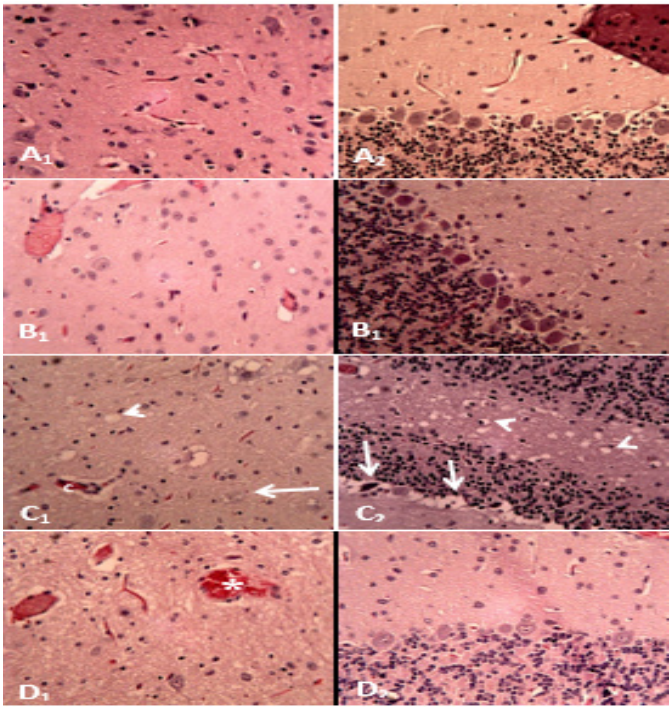

Plate I. Photomicrographs of the brains; A_1 and A_2, Cerebral and cerebellar cortexes, respectively of rat dosed with SBO at 2 mL/kg. bd. wt., showing normal cerebral and cerebellartissues. B_1 and B_2, Cerebral and cerebellar cortexes, respectively of rat dosed with ELEDS at 85.2 mg/kg. bd. wt., normal cerebral and cerebellar tissues.C_1, Cerebral cortex of rat dosed with CFS at 3.49 mg/kg. bd. wt. showing capillary congestion (*), neuronal necrosis (arrow) and vacuolation (arrow heads). C_2, Cerebella cortexes of rat dosed with CFS at 3.49 mg/kg. bd. wt. showing severe necrotic Purkinje cells (arrows) and well delineated neuronal and neuropil vacuolation (arrow head). D_1, Cerebral cortex of rat dosed with CFS+ELEDS showing congestion (c). D_2, Cerebellar cortex of rat dosed with CFS+ELEDS showing near normal cerebellar tissue. H&E stain x250

Heart

Photomicogrphs of the cardiac tissues in SBO (Plate IIA)- and CFS+AP-treated groups rats were normal, whereas, CFS-treated group showed myocardial degeneration and mild vacuolation (Plate IIC). CFS+ELEDS -treated rats showed mild congestion (Plate IID).

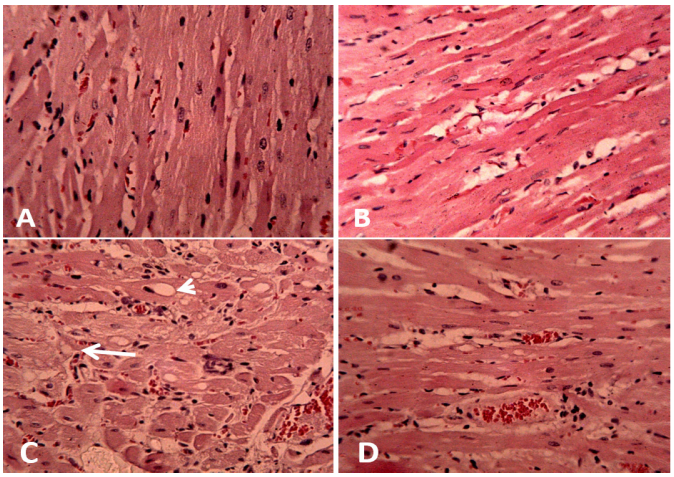

Plate II. Photomicrograph of the hearts of rats; A, Dosed with SBO at 2 mL/kg. bd. wt., showing normal cardiac tissue. B, Dosed with ELEDS at 85.2 mg/kg. bd. wt. showing normal cardiac tissue. C, Dosed with CFS at 3.49 mg/kg. bd. wt. showing myocardial degeneration (arrow) and mild vacuolation (arrow head). D, Dosed with CFS+ELEDS, showing mild congestion. H&E stain x250

Lungs

Most of the lungs in the SBO (Plate IIIA)-, CFS+ELEDS (Plate IIID)- and CFS+AP-treated rats were normal or near normal. On the other hand, most of the photomicrographs of lungs in the CFS- (Plate IIIC) treated group had pulmonary vein congeation, severe thickened interalveolar septae due to diffused interstitial mononuclear cellular infiltration, macrophages and fibrosis.

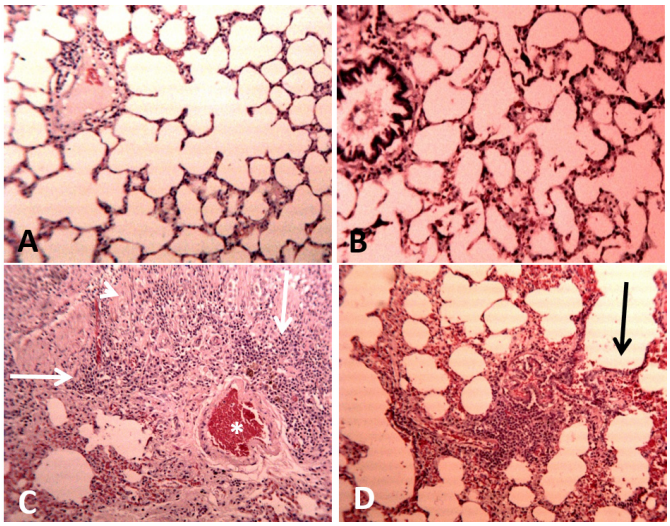

Plate III. Photomicrographs of the lungs of rats; A, Dosed with SBO at 2 mL/kg. bd. Wt., showing normal pulmonary tissue. B, Dosed with ELEDS at 85.2 mg/kg. bd. wt. showing normal pulmonary tissue. C, Dosed with CFS at 3.49 mg/kg. bd. wt. showing pulmonary vein congeation (*), severe thickened interalveolar septae due to diffused interstitial mononuclear cellular infiltration (arrows) and fibrosis (arrow head). D, Dosed with CFS+ELEDS showing mild thickened interalveolar septae (arrow). H&E stain x250

Liver

All the photomicrographs of the liver in the SBO- (Plate IVA) and CFS+AP- treated groups were normal to near normal. There were, however, severe architectural disarray, necrosis and vacuolation in CFS-treated rats (Plate IVC), while some CFS+ELEDS-treated rats showed focal portal lymphocytic infiltration (Plate IVD).

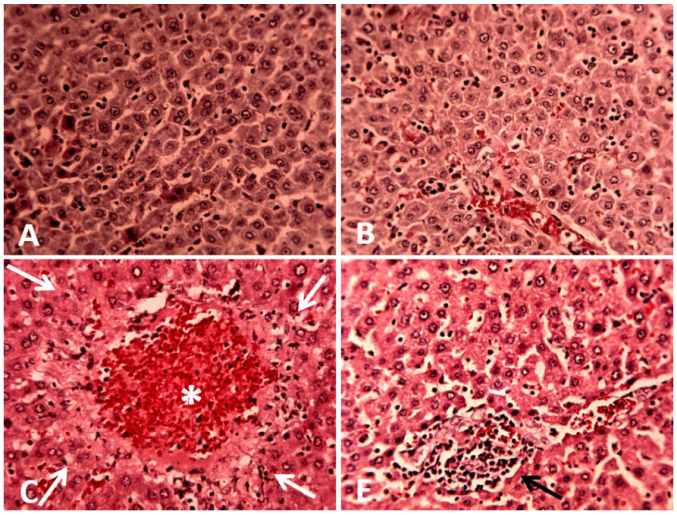

Plate IV. Photomicrograph of the liver of rats; A, Dosed with SBO at 2 mL/kg. bd. Wt., showing near normal hepatic tissue. B, Dosed with ELEDS at 85.2 mg/kg. bd. wt. showing normal hepatic tissue. C, Dosed with CFS at 3.49 mg/kg. bd. wt. showing centrilobular necrosis (arrows) and central vein congestion (*). D, Dosed with CFS+ELEDS, showing focal monouclear cellular infiltration (arrow). H&E stain x250

Kidney

Generalised coagulative tubular necrosis, capillary congestion (Plate VC), severe hydropic changes and inter tubular diffused localised interstitial mononuclear cellular infiltration were observed in the CFS-treated rats, whereas, focal interstitial mononuclear infiltration (Plates VD) were recorded in CFS+ELEDS-treated group. Pancreatic tissue in the CFS-treated rats showed generalisedacinic vacuolation and degeneration (Plate VC).

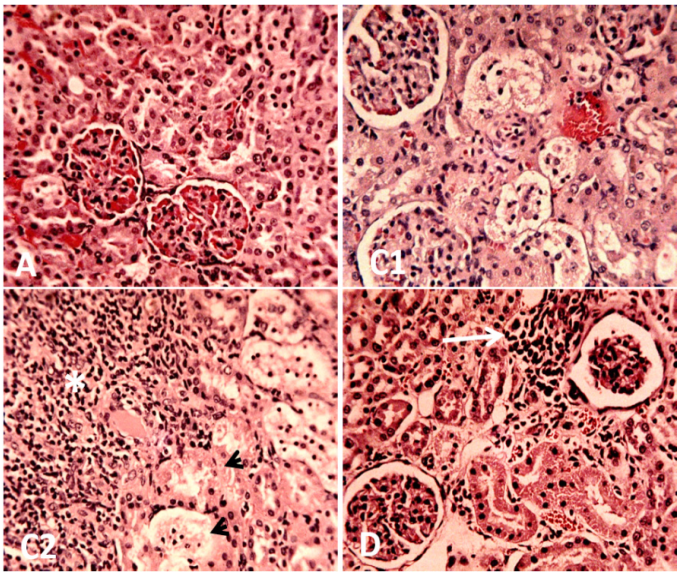

Plate V. Photomicrograph of the kidney of rat; A, Dosed with SBO at 2 mL/kg. bd. Wt., showing normal hepatic tissue. B, with ELEDS at 85.5 mg/kg. bd. wt. showing normal renal tissue. C, Dosed with CFS at 3.49 mg/kg. bd. wt. showing generalised coagulative tubular necrosis (arrow head) and inter tubular diffused localised interstitial mononuclear cellular infiltration (*). D, Dosed with CFS+ELEDS, showing focal interstitial infiltration of lymphocytes and plasma cell (arrow). H&E stain x250

Pancreas

The pancreatic tissues in SBO-, ELEDS- and CFS+ELEDS-treated rats as shown on plates VIA, VIB and VID, respectively were normal, while, CFS-treated rats showed generalized acinic vacuolation and degeneration (Plate CVI).

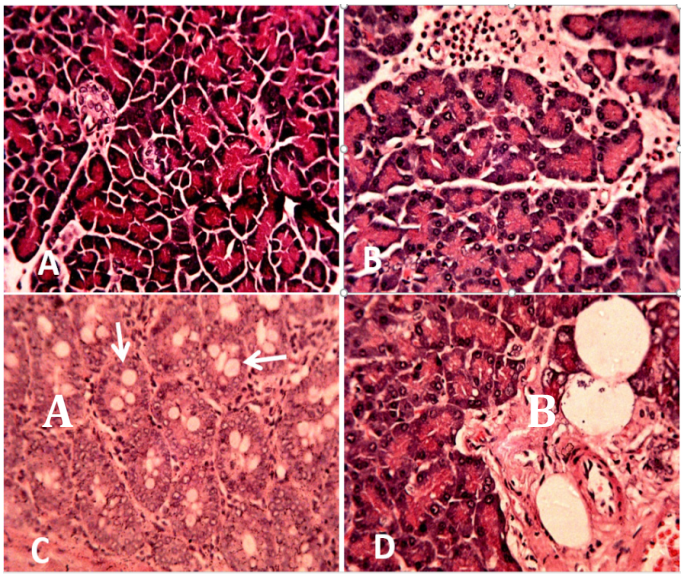

Plate VI. Photomicrograph of the pancreas of rats; A, Dosed with SBO at 2 mL/kg. bd. Wt., showing normal pancreatic tissue. B, Dosed with ELEDS at 85.2 mg/kg. bd. Wt. showing normal pancreatic tissue. C, Dosed with CFS at 3.49 mg/kg. bd. Wt. showing generalisedacinic vacuolation (arrow) and degeneration (arrows). D, CFS+ELEDS, showing normal myocardial tissue. H&E stain x250

DISCUSSION

In present study, chronic CFS toxicity induced an anaemia, as evidenced by the decrease in PCV, RBC and Hb in the CFS-treated groups, and agrees with ealier reports [36,47,49]. The anaemia in CFS toxicity was linked to its ability to decrease serum Fe concentration thereby interfering with synthesis of Hb [50]. The decrease RBC in the CFS-treated rats may also be as a result of hepatocellular toxicity, evidenced microscopically in the hepatic tissue. Hepatic injury may consequently suppress some serum protein synthesis which may lead to deficient RBC synthesis in the bone marrow. The anaemia invariably affects nutrients and oxygen transport to the tissues, consequently affecting cellular respiration [50,51].

The decrease in PLT counts in the CFS-treated group further indicates the toxic effects of CFS on blood components thereby producing thrombocytopenia and the latter could lead to improper clotting process, though, not seen at this stage of this study. However, an experimental study implicated the decrease in the PLT in the development of some severe haematological disorders like leukemia [37]. However, in contrast, an increase in PLT count in an experimental CFS toxicity was attributed to hepatic damage and nephrotic syndrome [8,52]. The improvement in PLT count in the CFS+ELEDS-treated group shows the ability of the ELEDS to possibly protect the PLT from oxidative damage by reducing the formation of lipid peroxides within the PLT membranes. This improvement of cellular integrity and / or reduction of cellular destruction by ELEDS may also be partly due to its antioxidant and ability to inhibit cholinergic effect caused by CFS toxicity. The thrombocytopenia observed in the CFS-treated rats as stated above, may be due to oxidative damage to the PLT membranes resulting in the formation of lipid peroxides within the PLT membranes thereby provoking membrane lysis. It was observed that the PLT membrane is highly vulnerable to oxidative stress than the erythrocyte membrane [53].

The observed total leucocytes though not sigificant in the CFS-treated group may have resulted from oxidative damage to the leucocytes. The neutropenia observed in CFS-treated group in this study disagrees with others [50,51] who observed lymphopenia and increased NEU-LYM ratio (NLR) (also indicates relative neutrophilia) in a chronic CFS exposure in an experimental study with Wistar rats. Furthermore, this disagrees with others [54,55] who observed elevated WBC count in chronic CFS toxicity study. These differences in the WBC may be attributed to differences in the stages of the experiments. The lack of signficant changes in CFS+ELEDS-treated group depicts the ability of ELEDS at 85.4 mg/kg. bd. wt. to improve the WBC and the circulating NEU in chronic CFS toxicity. This may be due to the antioxidant properties that chalet and reduce the level of stress imposed on the animal by CFS, thereby enabling it to protect leucocytes from destruction or an elevation in its production. The elevation of LYM in the CFS-treated rats at the later stages of this study may be due to an effort by the rats to immunologically respond to the assaults, as described earlier [56].

Microscopic lesions widely used as biomarkers in various toxicological studies including pesticides toxicities [57,58] as applied in the current study revealed that the neuronal damages observed in this study agrees with several previous studies which had implicated CFS to induce variable degree of neuronal damage [59,60,61]. Daily exposures of low CFS sufficiently caused decreased brain AChE activity [62] and probably led to lipid peroxidation and oxidative neurodegenerative change [63,64], hence, the observed

lesions in the brain at the current study. The microscopic lesions observed in the heart of CFS-treated group is in line with the previous report [65]. In addition, degeneration and disorganization in myocardial fibres, cytoplasmic vacuolization in cardiomyocytes, myocardial connective tissue oedema were detected following oral administration of CFS in Wistar rats [66,67]. These changes may be due to an elevation in reactive oxygen species (ROS) production in the myocardial tissues. The pulmonary lesions observed in CFS-treated group in the current study agree with others in a chronic OP toxicity [68]in addition to several studies conducted with CFS and other OP insecticides that demonstrated similar histopathological changes in rat liver such as necrosis, vacuolization and dilatation of sinusoids [69,70,71]. These changes may be as a result of an increase ROS in liver, reported to be extremely susceptible to ROS or free radicals and oxidative damage [72], although other multiple mechanisms caused hepatic dysfunction by CFS [73]. The renal lesions associated with CFS toxicity [74] gives the credence to the finding of the renal lesions in the current study which partly contributed to the anaemic condition probably due to deficient erythropoietin production as earlier reported [75] and / or anisocytosis or a reflection of destruction and shrinkage of RBC and or lack of available Hb needed to complete the process of haemopoeisis [50,51,76]. It is very interesting to observe in the present study that the value of CFS+ELEDS-treated group comparable with control (SBO) suggests the ability of ELEDS to mitigate the anaemia induced by CFS exposure. However, the comparatively milder histopathological changes recorded in the CFS+ELED-treated groups are suggestive of amelioration of ELEDS (85.2 mg/kg. bd. wt.) against CFS toxicity.

The overall protective effects exhibited by ELEDS against the CFS toxicity may be attributed to tropane alkaloids content of *D. stramonium* such as atropine, scopolamine and hyoscyamine being antagonistic to CFS toxicity perhaps through blockage of the muscarinic receptors, thereby depriving the accumulated ACh at the synapse from binding with its receptor there by preventing over stimulation of cholinergic effects manifested by CFS toxicity. Several studies revealed that the diverse phytochemicals in *D. stramonium* were responsible for its medicinal and toxic properties [77,78]. The beneficial effect occurs when taken in correct doses [78]. Hence, the use of this plant for treatment needs great cautions due to high vulnerability of toxicity.

CONCLUSION

The present study has revealed that CFS at low concentrations (3.45 mg/kg. bd. wt.) produced alterations in the haematolocal parameters, and microscopic changes in the brain, heart, lungs, liver, kidney and pancreatic tissue. Administration ELEDS at 85.2 mg/kg. bd. wt. mitigated deleterious changesinduced by CFS, suggestive of its poteNtial role in mitigating the cholinergic effects caused by CFS toxicity.Further studies are in progress to isolate, identify, quantify, characterize and elucidate on the structures of the bioactive principles and mechanism of action and determine their potential for pharmaceutical utilization.

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