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Oxidative stress and inflammation following sub-lethal oral exposure of cypermethrin in rats: mitigating potential of epicatechin

Olusegun Kayode Afolabi [a,*], Felix Adesola Aderibigbe [a], Dasola Teslim Folarin [a], Abimbola Arinola [a], Adedoja Dorcas Wusu [b]

[a] Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[b] Lagos State University, Ojo, Lagos, Nigeria

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ABSTRACT

Cypermethrin (CYP), a synthetic pyrethroid is a common environmental toxicant owing to its wide usage as a broad-spectrum insecticide. Its exposure to non-target organisms, including man, elicits numerous adverse effects making it a major public health issue. Epicatechin (EC) has proven anti-oxidative and anti-inflammatory properties. The present study was undertaken to evaluate the protective efficacy of epicatechin with regards to altered oxidative and inflammatory parameters subsequent to CYP treatment in rats. Animals were divided into four groups. The first group served as the control, while groups 2, 3, and 4 were orally treated with EC (30 mg kg^{-1} body weight), CYP (25 mg kg^{-1} body weight), and CYP plus EC, respectively. Oral administration of CYP for 14 days increased the levels of oxidative stress markers such as malondialdehyde, lipid hydroperoxides, and advanced oxidized protein products in the liver and kidney. These were accompanied by a decrease in glutathione and total antioxidant capacity levels. The activity of the enzyme superoxide dismutase was increased while catalase and glutathione peroxidase activities were decreased in these organs. Moreover, CYP increased plasma levels of the pro-inflammatory cytokines, interleukin-6 and tumor necrosis factor alpha. The plasma content of the nitrative nucleic acid marker, 8-nitroguanine was also markedly elevated by CYP. Administration of EC to CYP-exposed rats mitigated the induced oxidative and inflammatory effects. These data suggest that EC can attenuate the toxic effects induced by CYP exposure.

1. Introduction

Pyrethroids are synthetic pesticides derived from the natural pesticides pyrethrins that are found in chrysanthemum flowers [1]. Due to their perceived low mammalian toxicity, lower environmental persistence and selective insecticidal activity, they have gained wide acceptance of use as pesticides in agriculture, household, and animal husbandry [2]. The increased use of pyrethroids has, however, correspondingly increased the opportunity for human exposure and other non-target organisms. The widespread application of pyrethroids has led to contamination of various environmental compartments with a high possibility of bioaccumulation in food products [3]. Their lipophilic nature facilitates their bioaccumulation in membranes leading to the generation of reactive oxygen species (ROS) that elicits oxidative damage in animals [4, 5, 6].

Cypermethrin (CYP), a type II synthetic pyrethroid is one of the commonly used pyrethroids for agricultural, residential and commercial pest control applications [7, 8]. Its residues are frequently detected in the environment, food and in breast-milk [9, 10]. Humans are exposed to CYP during the application or through consumption of the pesticide-contaminated products [11]. Although reported to have low toxicity, CYP bioaccumulation and persistence in mammalian tissues are of toxicological significance [12, 13]. CYP is a hydrophobic compound that on passing through the cell membrane perturbs the structure and causes the efflux of cytoplasmic enzymes [14]. Studies have linked CYP exposure with toxic manifestation in several systems of the body, including reproductive toxicity, hepatotoxicity, neurotoxicity, immunotoxicity, genotoxicity [15, 16, 17]. CYP is reported to exert its toxic effects through the generation of radical oxygen species (ROS). In animal models, CYP induces mitochondrial dysfunction and oxidative stress evidence with elevated oxidative stress markers and reduced antioxidant activities [18]. It has also been reported to increase apoptotic index in the liver of rats but information is sparse on its inflammatory effect [19].

The use of phytochemicals in addressing human health issues is on

* Corresponding author.
E-mail address: okafolabi@lautech.edu.ng (O.K. Afolabi).

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the increase. They are shown to possess therapeutic properties, with their use in treating dysregulation in biological systems produces little or no side-effects [20, 21]. The ability of these naturally occurring compounds to modify the toxicological effects of environmental contaminants like pesticides are been given consideration. Flavonoids are a large group of natural phenolic compounds with different subclasses [22]. They are the most common and widely distributed group of plant phenolic compounds. They possess several biological and pharmacological properties that have made them useful as therapeutic agents against chemical-induced toxicity, among others. Several studies have shown flavonoids with the potential to protect against pesticide-induced toxicities [23, 24]. Epicatechin (EC) is a flavan-3-ol, a sub-class of the flavonoids found in green tea, grape, and cocoa [25]. EC has been reported to possess such pharmacological effects as anti-inflammatory, antioxidant, anti-hyperlipidemic effects while also exhibiting anticarcinogenic and cytoprotective effect [26, 27]. Moreover, epicatechin has also been reported to exert its effects through other mechanisms including gene function regulation, hormone, and immune modulation, metabolic pathway modulation [28]. Suggestions have also been made of its protective role against cancer, diabetes, and neurodegeneration [25]. In addition, EC has been found to have ameliorative properties against a number of toxic agents, such as doxorubicin, radiation, and nicotine [29, 30, 31]. The current study was, therefore, designed to evaluate the adverse effects of commercial CYP formulation exposure on oxidative and inflammatory markers in rats and the protective effect of epicatechin against these CYP-induced toxicities.

2. Materials and methods

2.1. Chemicals

A commercial cypermethrin formulation Delthrin® (10% cypermethrin per volume; Candel Company Limited, Lagos, Nigeria) was purchased and used for the study. Epicatechin, xylenol orange, paraoxon, tripyridyl-5-triazine (TPTZ), were products of Sigma-Aldrich Chemicals (Germany). ELISA kits for interleukin-6, TNF-α, and 8-nitroguanine determinations were by Abcam (UK), RayBiotech Inc, (USA), and Cell Biolabs Inc. (USA), respectively. Other chemicals and reagents were all of analytical grade.

2.2. Animals and treatment

Twenty-eight healthy male albino Wistar rats, weighing between (180–200 g; 10–12 weeks old) bred in the Animal House, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso (LAUTECH), were used in this study. The animals were housed in plastic cages and were allowed to acclimatize for a week before the commencement of the experiment. The animals were kept under controlled conditions of normal light-dark cycle (12 h light/dark) and temperature (25 ± 2 °C). All animals have access to food and water ad libitum. Experimental design and animal handling in the study followed the recommendations of the Research Ethical Committee of the Faculty of Basic Medical Sciences, LAUTECH (REC/FBMS No. 017/0451), which is in agreement with the National Institute of Health guidelines for laboratory animal care and use.

The rats were randomly divided into four groups of seven animals each. In the first group or the control group, rats received by oral gavage a volume of 1 mg L^{-1} body weight (b.w.) of corn oil only once per day. The second group (EC) orally received epicatechin at a dose of 30 mg kg^{-1} b.w., while animals in the third group (CYP) were administered a dose of 25 mg kg-1 b.w. CYP in corn oil (1/10 of the LD50). The fourth group (CYP + EC) received the same treatments as did animals in the second and third groups. The dose for epicatechin was obtained from previous studies [32, 33]. The commercial formulation of CYP was suitably diluted with corn oil to administer a constant volume of 1 mg l^{-1} for each test concentration. All treatments were carried out over a period of 14 days. At the end of the experiment, blood from rats was collected into heparinized tubes through cardiac puncture, under light ether anesthesia after an overnight fast. Liver and kidney were excised from the animals for biochemical analysis. The blood samples were centrifuged at 4000 rpm for 10 min to obtain plasma which was stored at -20 °C until analyzed.

2.3. Biochemical analysis

2.3.1. Protein estimation

Protein content was determined according to the method of Lowry et al. [34], using bovine serum albumin as standard.

2.3.2. Determination of lipid peroxidation (LPO) levels

LPO in tissues was estimated by the TBA reaction with malondialdehyde, a product of lipid peroxidation process [35]. Briefly, 10% tissue homogenate prepared in 0.15 M KCl was mixed thoroughly with a stock solution containing 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 M HCl. The mixture was heated for 15 min in a boiling water bath and then cooled. Thereafter, it was centrifuged at 1000 g for 10 min and the absorbance of the supernatant was determined at 535 nm. Using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}, the concentration of MDA was calculated and expressed as nmol per gram of wet tissue.

2.3.3. Determination of lipid hydroperoxides concentrations

Lipid hydroperoxides concentrations in plasma and tissue homogenates were estimated with the method of Nourooz-Zadeh et al. [36] Tissue homogenate was mixed with either 10 mM TPP in methanol or with methanol and incubated for 30 min at room temperature. FOX2 reagent (250 mM ferrous ammonium sulfate, 100 mM xylenol orange, 25 mM H_2SO_4 and 4 mM butylated hydroxytoluene in 90% methanol) was added and the mixture incubated for another 30 min. Flocculated material in the mixture was removed by centrifuging at 12,000 g for 10 min after which the absorbance was read at 560 nm.

2.3.4. Estimation of protein oxidation

The level of protein oxidation was determined spectrophotometrically in the plasma and tissue by measuring advanced oxidized protein products (AOPP) [37]. Briefly, 20% solution of the sample was prepared with phosphate buffered saline after which 1.16 M potassium iodide and acetic acid were added. The absorbance of the reaction mixture was read at 340 nm and an extinction coefficient of 26,000 M^{-1} cm^{-1} was used in calculating AOPP concentrations.

2.3.5. Determination of total antioxidant capacity (TAC)

The TAC in samples was estimated using the ferric reducing antioxidant power (FRAP) method described by Benzie and Strain [38]. The FRAP method measures the antioxidant potential through the reduction of ferric tripyridyl-5-triazine (Fe^{3+}-TPTZ) to its ferrous form (Fe^{3+}). Briefly, FRAP working reagent (300 mM acetate buffer, pH 3.6, 10 mM 2, 4,6-tripyridyl-S-triazine in 40 mM HCl and 20 mM FeCl3 in ratio 10:1:1) was pre-warmed and then mixed with 10% homogenate of the sample. The procedure was performed at 37 °C, and the absorbance was read at 593 nm. Aqueous solutions of known Fe^{2+} concentration were used for calibration.

2.3.6. Determination of reduced glutathione (GSH)

Reduced glutathione content in the organs was determined using the method of Moron et al. [39] Tissue homogenate (1 mL) was treated with 0.5 mL Ellman's reagent in 0.1% sodium citrate. After that, phosphate buffer and DTNB were added before the absorbance was read at 412 nm.

2.3.7. Determination of catalase (CAT) activity

The activity of CAT was measured by the method of Aebi [40]. The assay mixture consisting of supernatant tissue homogenate (0.1 mL), 50 mM phosphate buffer (1.9 mL) and 30 mM H_2O_2 (1 mL) was maintained

at 20 °C. The decomposition of H_2O_2 was monitored spectrophotometrically at 240 nm for 60 s. The change in absorbance was the measure of CAT activity and expressed as nmol/mg protein.

2.3.8. Determination of superoxide dismutase (SOD) activity

The method of Misra and Fridovich [41] was used in determining SOD activity in the tissues. The addition of 0.01% epinephrine (0.3 mL) to the mixture containing 2.5 mL carbonate buffer (0.05 M) and 0.2 mL sample initiated the reaction. Change in absorbance was measured at 480 nm, and the activity was expressed as unit per milligram of protein.

2.3.9. Determination of glutathione peroxidase (GPx) activity

GPx activity was determined using H_2O_2 as a substrate in the presence of reduced glutathione [42]. The reaction medium contained 0.2 mL of phosphate buffer (0.4 M), 0.1 mL of sodium azide (10 mM), 0.2mL of tissue homogenate and 0.2 mL of GSH. The reaction was initiated by adding 0.2 mM H_2O_2 to the mixture. The GSH content was quantified using Ellman's reagent. The activity was expressed as unit per milligram protein where a unit is mmol of GSH consumed per minute.

2.3.10. Determination of plasma Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)

Plasma concentrations of IL-6 and TNF-α were determined using commercial rat ELISA kits (Abcam, UK and RayBiotech Inc, USA, respectively), following the instructions in respective kit manuals. The absorbance was monitored at 450 nm on a Hawksley HA-1600 microplate reader (Hawksley, London, U.K.).

2.3.11. Evaluation of nitrative nucleic acid damage

Plasma concentration of 8-nitroguanine (8-NO_2Gua) was evaluated using the nitrosative DNA/RNA damage ELISA kit (Cell Biolabs, Inc. USA), following the procedure provided by the manufacturer.

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance, followed by Tukey's multiple comparisons test. Results were presented as mean ± standard deviation (SD) and values were considered statistically significant at $p < 0.05$. Data were analyzed using GraphPad Prism for Windows, version 6.01 (GraphPad Software, Inc., San Diego, CA, USA.).

3. Results

3.1. Effect on lipid peroxidation and protein oxidation indices

The changes in lipid peroxidation and protein oxidation products are shown in Table 1. The results showed that CYP exposure significantly ($p < 0.05$) elevated the levels of MDA and LOOH, both indices of lipid peroxidation. In the liver, MDA and LOOH were increased by 36% and 89% respectively, while in the kidney, the increases were 68% and 63% respectively, when compared with the control. However, co-treatment with EC caused significant reduction in liver and kidney MDA levels by 12% and 33%, respectively when compared with CYP alone. Similarly, treatment with EC significantly ($p < 0.05$) reduced the elevated LOOH levels resulting from CYP exposure by 38% and 31% in both liver and kidney, respectively. In addition, AOPP a marker of protein oxidation products was markedly increased in the CYP intoxicated group by 91% and 83% in liver and kidney, respectively. The increase in AOPP levels in these organs was abated by EC administration to 32% and 32% respectively. The liver and kidney of rats administered EC alone showed no significant change in lipid peroxidation and protein oxidation products levels in comparison to the control.

Table 1
Effects of epicatechin treatment on MDA, LOOH and AOPP levels in liver and kidney of cypermethrin intoxicated rats.

Groups	MDA (nmol/g tissue)	LOOH (nmol/g tissue)	AOPP (μmol/g tissue)
Liver			
Control	47.26 ± 2.80^a	4.35 ± 0.34^a	75.77 ± 4.02^a
EC	44.68 ± 3.48^a	4.18 ± 0.37^a	73.90 ± 3.15^a
CYP	64.30 ± 4.46^b	8.24 ± 0.65^b	144.65 ± 8.53^b
CYP + EC	56.86 ± 4.36^c	5.07 ± 0.45^c	98.31 ± 5.27^c
Kidney			
Control	25.44 ± 1.63^a	2.78 ± 0.18^a	64.52 ± 4.25^a
EC	23.51 ± 1.37^a	2.57 ± 0.21^a	62.95 ± 4.09^a
CYP	42.66 ± 2.11^b	4.52 ± 0.56^b	118.19 ± 12.98^b
CYP + EC	28.75 ± 2.24^c	3.10 ± 0.21^a	80.51 ± 6.92^c

MDA: malondialdehyde; LOOH: lipid hydroperoxide; AOPP: advanced oxidized protein product.
Data are expressed as means ±S.D. of seven animals per group. Values in the same column for a compartment, not sharing the same superscript are significantly different from each other at $p < 0.05$.

3.2. Effects on antioxidant systems

Table 2 depicts the enzymatic and non-enzymatic antioxidant status in control rats and groups exposed to CYP alone or co-administered with EC. There was no significant change in activities of SOD, CAT, and GPx in rats treated with EC alone. CYP treatment caused a significant ($p < 0.05$) increase in the activities of SOD in both the liver (70%) and kidney (75%) when compared with the control. However, co-treatment with EC restored SOD activity in these two organs back to normal in the CYP-treated rats. In contrast, CAT and GPx activities were significantly ($p < 0.05$) reduced in CYP-exposed rats. CAT hepatic and renal activities were both reduced to 55% of the control whereas GPx activities were reduced by 31% and 38% in the liver and kidney, respectively. Administration of EC during CYP intoxication stimulated the activities of these two enzymes. On one hand, CAT activities in the liver and kidney were increased by 81% and 61%, respectively while on the other hand, EC stimulated GPx activity by 25% in the kidney of CYP exposed rats but restored the hepatic activity to normalcy. The levels of GSH and TAC were significantly ($p < 0.05$) reduced in the liver and kidney of CYP treated rats when compared with the control. EC administration maintained the normalcy of hepatic GSH concentration while boosting GSH

Table 2
Effects of epicatechin treatment on enzymatic and non-enzymatic antioxidant status in liver and kidney of Cypermethrin intoxicated rats after 14 days.

Groups	SOD (U/mg protein)	CAT (nmol/mg protein)	GPx (U/mg protein)	GSH (μmol/g tissue)	TAC (μmol/g tissue)
Liver					
Control	6.82 ± 0.52^a	1.66 ± 0.13^a	83.15 ± 6.23^a	41.28 ± 3.08^a	342.45 ± 28.74^a
EC	6.13 ± 0.34^a	1.75 ± 0.15^a	81.25 ± 5.45^a	43.54 ± 3.88^a	352.15 ± 30.92^a
CYP	11.57 ± 1.02^b	0.74 ± 0.05^b	57.23 ± 9.05^b	23.84 ± 2.24^b	124.48 ± 18.24^b
CYP + EC	7.22 ± 0.53^a	1.34 ± 0.13^c	78.57 ± 6.33^a	38.21 ± 4.31^a	235.68 ± 23.14^c
Kidney					
Control	4.65 ± 0.39^a	1.37 ± 0.10^a	54.77 ± 4.04^a	27.13 ± 1.98^a	273.33 ± 19.87^a
EC	4.71 ± 0.24^a	1.45 ± 0.09^a	51.38 ± 4.51^a	28.08 ± 1.63^a	286.74 ± 23.52^a
CYP	8.12 ± 0.58^b	0.75 ± 0.08^b	33.97 ± 2.05^b	20.22 ± 1.75^b	164.64 ± 12.91^b
CYP + EC	5.12 ± 0.44^a	1.21 ± 0.08^c	42.32 ± 3.51^c	23.58 ± 2.01^c	225.13 ± 18.84^c

SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GSH: reduced glutathione; TAC: total antioxidant capacity. Data are expressed as means ±S.D. of seven animals per group. Values in the same column for a compartment, not sharing the same superscript are significantly different from each other at $p < 0.05$.

level in the kidney by 17% in CYP-treated rats. Similarly, TAC levels in the liver and kidney of CYP-exposed rats were respectively increased by 89% and 37%.

3.3. Effect on pro-inflammatory cytokines

The concentrations of IL-6 and TNF-α in the plasma of CYP-exposed and EC-administered rats are shown in Fig. 1. The pro-inflammatory cytokines levels were increased significantly ($p < 0.05$) in the CYP-exposed rats. The concentrations of IL-6 and TNF-α in the plasma were increased from 128.91 ± 9.44 pg/mL and 69.18 ± 5.45 pg/mL respectively in the control to 252.00 ± 15.76 pg/mL and 177.00 ± 8.38 pg/mL, respectively. EC co-treatment significantly reduced CYP-induced elevation of IL-6 and TNF- α by 33% and 50%, respectively ($p < 0.05$).

3.4. Assessment of nitrosative damage to nucleic acids

CYP exposure is characterized by a significant increase in plasma concentration of 8-NO_2Gua in the rats ($p < 0.05$). CYP intoxication resulted in a 205% increase in the level of 8-NO_2Gua when compared with the control. Co-treatment of CYP-exposed rats with EC significantly reduced the formation of nitrosative nucleic acids from 34.42 ± 0.87 ng/mL in CYP-intoxicated rats to 17.02 ± 1.02 ng/mL, representing a 51% reduction. Interestingly, EC alone reduced 8-NO_2Gua level in normal rat by 25% (11.28 ± 1.13 ng/mL to 8.50 ± 0.36 ng/mL) (see Fig. 2).

4. Discussion

Pesticide accumulation in tissues has been associated with increased oxidative stress and production of ROS [43, 44]. Pyrethroids induced cellular oxidative damage and accumulation of peroxidation products have been reported in literature [6, 11, 18]. These oxidative products have been extensively used as markers of oxidative stress. In the present study, CYP exposure generated oxidative stress in liver and kidney of the rat as evidenced by significant elevation of the lipid peroxidation and protein oxidation products, namely MDA, LOOH, and AOPP. The increase in the levels of MDA and LOOH in these organs is an indication of CYP-induced lipid peroxidation leading to tissue injury. CYP is metabolized by the cytochrome P450 microsomal system, with the concurrent generation of oxidative stress [45]. Due to its lipophilic nature, CYP could easily pass through the lipid bilayer and damage cell integrity [46]. Elevated AOPP concentrations in the organs are also a pointer to the degree of damage done by CYP on the tissue protein components. These results further confirmed earlier reports implying the ability of CYP to cause increased ROS generation and oxidative damage in liver and kidney of rat [47, 48]. Bhushan et al. [49] have reported histological alterations in hepatic tissues of animals exposed to a similar dose CYP. EC protects against oxidation of lipids and proteins and has been suggested to be one of the most effective antioxidants among others [50, 51]. Our data showed that oral administration of EC drastically decreased MDA, LOOH, and AOPP levels in CYP-exposed rats, to near normal levels. This is probably derived from the flavonoid's ability to modulate CYP oxidative effects through its free radical scavenging properties [52]. This is adduced to the presence of ortho-hydroxyl groups on EC which is responsible and essential for its direct detoxifying effects in its reaction with superoxide and hydrogen peroxide [28].

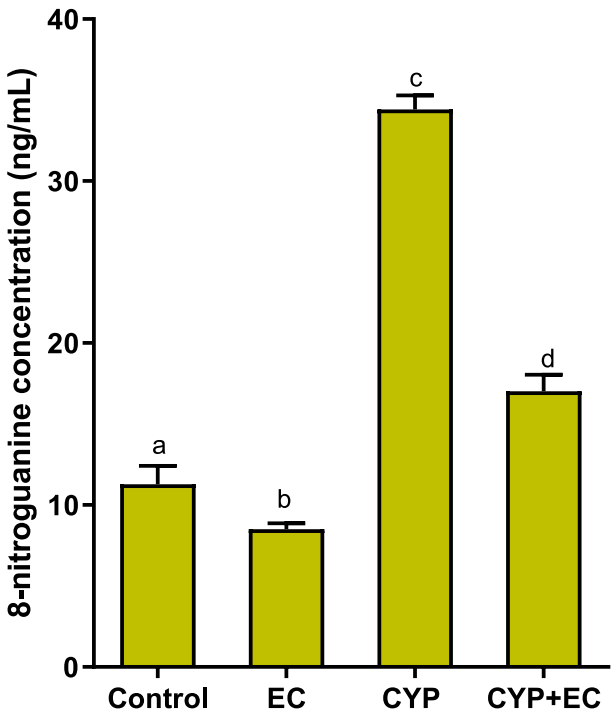

Fig. 2. Effect of epicatechin on plasma 8-nitroguanine level in cypermethrin intoxicated rats. Data represent the mean ± SD of seven rats in each group. Bars not bearing same superscript are significantly different from each other at $p < 0.05$.

Exposure to pesticides is usually characterized by impairment of the

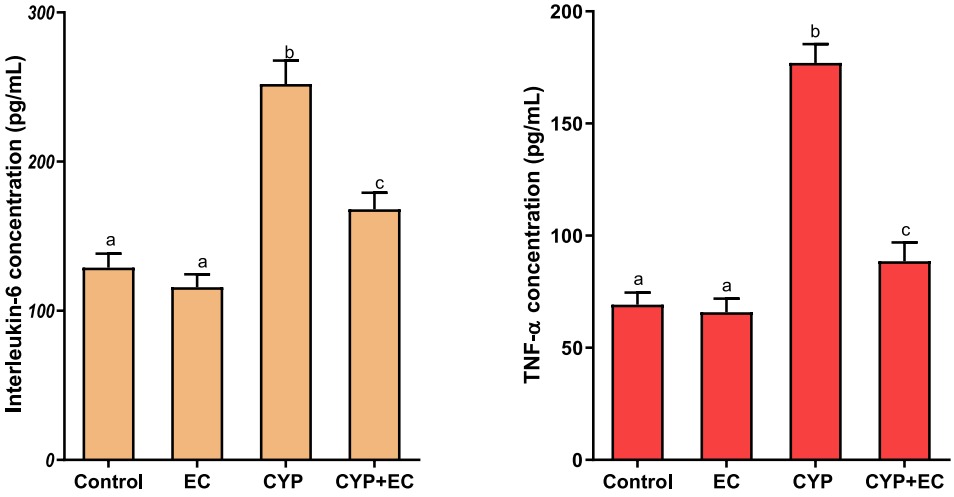

Fig. 1. Effect of epicatechin on plasma IL-6 and TNF- α levels in cypermethrin intoxicated rats. Data represent the mean ± SD of seven rats in each group. Bars not bearing same superscript are significantly different from each other at $p < 0.05$.

antioxidant defense system as well as the depletion of non-enzymatic antioxidants [44]. In our study, GSH and the antioxidant potential were markedly reduced in the rats on exposure to CYP. This is in consonance with previous studies showing the ability of CYP to disrupt antioxidant system [53, 54]. GSH can act as a non-enzymatic antioxidant either by direct interaction of its thiol group with ROS or by serving as a co-substrate for biochemical conjugation of xenobiotics [55, 56]. The antioxidant capacity is a reflection of the relative cumulative capacity of the total antioxidant system in the tissue. Together with the elevated oxidative stress markers, the reduced GSH and TAC levels, therefore, indicated an imbalance in the non-enzymatic system of the organs. Also, the decrease in the non-enzymatic antioxidant contents increased the susceptibility of the organs to damage by free radical species. However, co-treatment of CYP to EC improved GSH and TAC contents in the rats. Our data confirm the protective effect of EC against oxidative stress by increasing total antioxidant capacity and GSH levels in intoxicated animals [57, 58]. EC also protect antioxidant defenses through the inhibition of enzymes involved in ROS generation like microsomal monooxygenase, mitochondrial succinoxidase and NADH oxidase [59].

Antioxidant enzymes are part of the cellular defense mechanisms that limit the negative impact of oxidant molecules on tissues and protect against oxidative cell injury by scavenging free radicals. Their ability to eliminate reactive oxygen species could be hampered by deviation in physiological concentrations with the consequence of increased oxidative damage to cellular lipids, proteins, and DNA [60, 61]. The present study showed an increase in SOD activity and a decrease in CAT activity in liver and kidney of CYP exposed rats. The increase in SOD activity induced by CYP in this study is at variance with the reports of Ikpeme et al. [62] and Soliman et al. [48] Adjuncts in commercial formulations have been suggested to modify the overall effect of pesticides [63]. The commercial formulation of CYP used in our study in contrast with the active ingredient in other studies could have contributed to the differences observed. In addition, the duration and routes of exposure employed could also play a part in the differences in results observed. SOD, the first enzyme in the antioxidant defense converts superoxide radicals to form H_2O_2 to prevent the deleterious effects of oxygen radicals in the tissues. The increase in SOD activity implies a compensatory adaptive response to the increased presence of free radicals in the liver and kidney of the CYP-treated rats, in order to mitigate the toxic effects of the ROS generated by the pyrethroid [64]. CAT, a ubiquitous enzyme found in both soluble and membrane-bound forms catalyzes the decomposition of H_2O_2 to H_2O [65]. However, the membrane-bound CAT with a higher peroxidase activity is prone to oxidative damage resulting from free radical attack on cellular membrane. The attack invariably leads to a reduction in membrane fluidity and inactivation of membrane-bound enzymes [66, 67]. The subsequent increase in H_2O_2 from the dismutase activity of SOD on the free radicals in the organs could thus be partly responsible for the inhibited CAT activity observed in these organs. The reduced CAT activity implies the conversion of more H_2O_2 to the hydroxyl radical through Fenton-mediated reaction [68]. GPx activity in liver and kidney was also inhibited by CYP exposure. This is consistent with other reports on CYP effect on GPx activity, though contrary to the report of Kutluyer et al., who reported an increase in the enzyme activity in the spermatozoa of rainbow trout [69, 70]. GPx is a peroxidase involved in the GSH-dependent detoxification of hydroperoxides in cellular systems. The reduction in GPx activity observed in the study may be attributed to GSH depletion due to its utilization as an antioxidant in terminating free radical reaction in the tissues. This has also been suggested in other studies that have associated reduction in GSH with a subsequent reduction in the activity of GSH-dependent enzymes in pyrethroid-exposed animals [13, 47, 71, 72, 73]. However, co-administration of CYP with EC resulted in a reversal of the effects of the pyrethroid either totally or to near control level. This may be ascribed to EC's potency as an antioxidant, directly scavenging free radicals and also modulating the activities of the antioxidant enzymes [28].

Proinflammatory cytokines are low molecular weight proteins secreted mainly by the activation of macrophages and lymphocytes [74]. Environmental contaminants, such as CYP can induce the secretion of these cytokines [75, 76]. Dysregulation in the pathways leading to their production has been associated with pathological conditions such as inflammatory conditions and cancer [77]. Oxidative stress which can be triggered by toxicants is known to induce inflammatory responses via activation of redox-sensitive transcription factors, such as NF-κB [78]. In our study, these transcription factors could have been activated by the increased oxidative stress induced by CYP, producing the elevated IL-6 and TNF-α levels observed. This is consistent with the results of Solimon et al. [48] where CYP induced the expression of the proinflammatory cytokines IL-6 and TNF- α in rats. The increase observed in IL-6 and TNF-α levels in CYP-exposed rats thus implies the induction of inflammation by CYP exposure. Furthermore, the present investigation revealed that EC demonstrated the ability to attenuate CYP-induced inflammation as treatment with EC drastically reduced plasma IL-6 and TNF- α levels in the CYP-exposed rats. This is in consonance with other reports of EC effectively attenuating chemically-induced inflammation in rats [28]. According to Yang et al. [79], EC is able to mitigate the generation of inflammatory mediators like IL-6 and TNF- α through its inactivation of NF-κB, MAPKs, and JAK2/STAT 3 pathways. Furthermore, in the process of inflammation, endothelium-derived mediators and complement factors stimulate leucocyte adhesion with the release of inflammatory mediators. Flavonoids are known to repress this leucocyte adhesion process thereby, preventing the release of oxidants and inflammatory mediators, and the consequent injury to tissues [80]. The ability of EC to suppress leucocyte adhesion may therefore, play additional role in its anti-inflammatory effect observed in this study. In addition, ingested EC undergoes chemical modifications and these products contribute significantly to the biological effect alongside the native compound. Specifically, EC enzymatic oxidation products provide strong inhibitory effects on macrophage migration inhibitory factor (MMIF), a key molecule in the promotion and maintenance of inflammatory response [81].

During chronic inflammation, cellular constituents such as nucleic acids, proteins, and lipids are damaged by ROS and reactive nitrogen species (RNS). This is because, under inflammatory conditions, the RNS, including nitric oxide generated via the expression of inducible nitric oxide synthase (iNOS) reacts with superoxide to form highly reactive peroxynitrite ($ONOO^-$). Interaction of $ONOO^-$with guanine produces nitrative and oxidative DNA lesions, such as 8-nitroguanine and 8-oxo-deoxyguanosine, respectively [82]. In this study, plasma 8-NO_2Gua level was found to be markedly elevated in CYP-exposed rats, which further corroborates the genotoxic and mutagenic potential of CYP [12, 83]. 8-Nitroguanine, a mutagenic DNA lesion formed under chronic inflammation conditions is found to accumulate during the carcinogenic process in cancer-prone inflammatory diseases and can thus, be used as a surrogate in evaluating inflammation in a system. The accumulation of such DNA damage may lead to mutagenesis [82]. Findings have suggested that 8-NO_2Gua formation occurred to a greater extent in cancerous tissues than in non-cancerous tissues [84]. The high level of 8-NO_2Gua in this study hence implies the mutagenic property of CYP. Previous studies have already linked pesticide exposure, including cypermethrin with cancer [85, 86]. Similarly, Dikic et al. [87] in their study have reported hepatic DNA breakage in mice treated with CYP at a sub-lethal dose as used in our study. Treatment with EC however, drastically reduced the formation of 8-NO_2Gua in the rats, reflecting the ability of the flavonoid in preventing nitrosative damage to nucleic acids, especially as EC is an effective inhibitor of iNOS [29]. Furthermore, EC along with other flavonoids induce protective enzymes that inactivate electrophiles, free radicals, and reactive oxygen species, thereby inhibiting mutagenicity [88].

Even though the organ toxicity of CYP at the dose investigated has been established in other studies, and our study provided data on the oxidative and inflammatory responses elicited by exposure to CYP, we acknowledge our inability to include histological analyses as a limitation

of this study. Also, other markers of nitrosative stress apart from 8-NO_2-Gua were not determined. We therefore, could not conclusively assign a role for nitrosative stress in CYP induced toxicity.

In conclusion, the findings of the present study demonstrated that sub-lethal oral administration of CYP is able to produce oxidative stress, disrupt the antioxidant defense system and promote inflammation in rats. The inflammation and oxidative stress induced may also instigate damage to genomic nucleic acids. However, EC administration demonstrated attenuation of all parameters associated with these CYP-induced toxicities, probably due to its free radical scavenging and anti-inflammatory properties. EC could thus, be a good candidate in the therapy of CYP-induced toxicity, although further studies are required to understand its precise mechanisms of action and potential use as a treatment against pesticide-induced toxicities.

Declarations

Author contribution statement

Olusegun Kayode Afolabi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Felix Adesola Aderibigbe, Dasola Toyosi Folarin, Abimbola Arinola: Performed the experiments.

Adedoja Dorcas Wusu: Analyzed and interpreted the data.

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The authors declare no conflict of interest.

Additional information

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