Research Article

Ameliorative Effect of Vitamin C on Alterations in Thyroid Hormones Concentrations Induced by Subchronic Coadministration of Chlorpyrifos and Lead in Wistar Rats

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The present study evaluated the ameliorative effect of vitamin C on alteration in thyroid hormones induced by low-dose subchronic coadministration of chlorpyrifos (CPF) and lead (Pb). Forty Wistar rats were divided into 4 groups of 10 animals each. Groups I and II were administered soya oil (2 mL/kg) and vitamin C (100 mg/kg), respectively. Group III was coadministered CPF (4.25 mg/kg $\sim$ 1/20th LD₅₀) and Pb (250 mg/kg $\sim$ 1/20th LD₅₀), respectively. Group IV was pretreated with vitamin C (100 mg/kg) and then coadministered with CPF (4.25 mg/kg) and Pb (250 mg/kg), 30 min later. The regimens were administered by gavage for a period of 9 weeks. The marginal decrease in serum triiodothyronine and thyroxine and the significant increase in the concentrations of thyroid stimulating hormone and malonaldehyde in the group coadministered with CPF and Pb were ameliorated by vitamin C partly due to its antioxidant properties.

1. Introduction

Man and animals are exposed to a “soup” of chemical contaminants in the environment, which directly or indirectly affect their health and well-being. Pesticides and heavy metals are the most common environmental contaminants because of their respective widespread use in agriculture and industries. Hitherto, most studies on these chemical contaminants have centered on the examination of one single agent and therefore, current understanding of the toxicity of many environmental toxicants/pollutants is based primarily on toxicity studies performed on laboratory animals exposed to a single toxic agent [1, 2]. However, the environment is heavily contaminated with many chemicals, which interact with each other in such a way that modify their toxic response in humans and animals.

Organophosphate (OP) compounds are the most widely used insecticides accounting for 50% of global insecticidal use [3] while Pb is the most widespread heavy metal contaminants with wide applications [4]. Occupational and

environmental Pb exposure continues to be among the most significant public health problems [4–7]. Due to their persistent nature in the environment and their toxicodynamics, CPF and Pb have resulted in deleterious effects in man and animals [8].

The toxicity of Pb remains a matter of public health concern [9] due to its pervasiveness in the environment and the awareness about its toxic effects [10] at exposure levels lower than what was previously considered harmful [11]. Reproductive consequences of Pb exposure are widespread [12], affecting almost all aspects of reproduction [13]. Pb induces decreased sperm count and motility and increased morphological abnormalities in animals [14, 15]. CPF is one of the most common insecticidal environmental contaminants [16, 17]. Despite restrictions placed on some of its domestic uses by United States Environmental Protection Agency (USEPA) in 2000, CPF is still widely used as residues have been detected in citrus fruits in some parts of the world [18]. Adverse reproductive outcomes have been observed following CPF poisoning [16, 17, 19–21]. The

adverse reproductive health outcomes even as a result of environmental exposure to CPF have been partly linked to hypothyroidism [17]. Furthermore, other studies have found hypothyroidism in both CPF [17, 22–24] and Pb [25–28] poisonings. Since adequate thyroidal function is essential for effective and optimal reproductive performance, therefore, measures aimed at mitigating the thyroid dysfunction instigated by exposure to low-dose environmental contaminants are pertinent.

Although the mechanisms of toxicity of the two agents differ, oxidative stress is a common feature in CPF [29–33] and Pb [34–36] poisoning. However, ascorbic acid has shown tremendous promise in mitigating toxicity evoked by CPF [31, 33, 37, 38] and Pb [28, 39]. Therefore, the present paper was aimed at evaluating the effect of low dose subchronic exposure to CPF and Pb on thyroid functions, the role of thyroidal lipoperoxidation, and ameliorative effect of vitamin C in Wistar rats.

2. Material and Methods

2.1. Experimental Animals. Forty 6-week-old adult male Wistar rats were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The rats were fed on standard rat pellets, and water was provided *ad libitum*. The experiment was performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals [40].

2.2. Chemicals. Commercial grade CPF (Termicot 20% EC, Sabero Organics, Gujarat, India) was reconstituted in soya oil to 10% solution, which was subsequently used for the experiment. Analytical grade lead acetate (Kiran Light Laboratories, Mumbai, India) used for the study was reconstituted into a 20% solution using distilled water. Commercial grade vitamin C tablets (100 mg/tablet; Emzor Pharmaceutical Ltd, Nigeria, BN: 618 N) were dissolved in distilled water to 10% solution just before daily administration.

2.3. Animal Treatments. Forty adult male Wistar rats were divided into 4 groups of 10 animals per group. The rats in group I (C/oil) were administered corn oil (2 mL/kg), while those in group II (VC) were administered vitamin C (100 mg/kg). Rats in group III (CPF) were coadministered CPF 4.25 mg/kg, $\sim 1/20$ th LD₅₀ [24] and lead acetate 225 mg/kg, $\sim 1/20$ th LD₅₀ [28], respectively. Rats in group IV (VC + CPF + Pb) were pretreated with vitamin C, and then coadministered with CPF (4.25 mg/kg) and Pb (225 mg/kg), 30 min later. These regimens were administered orally by gavage once daily for a period of 9 weeks. At the end of the dosing period, the rats were sacrificed by jugular venisection after light chloroform anesthesia. Serum obtained from each blood sample was used to evaluate for the concentrations of triiodothyronine (T₃), thyroxine (T₄), and thyroid stimulating hormone (TSH), while the thyroid gland was weighed and then evaluated for the malonaldehyde (MDA) concentration as an index of lipoperoxidation.

2.4. Evaluation of Concentrations of Triiodothyronine—Thyroid Stimulating Hormone and Thyroxine. The concentrations of T₃, T₄, and TSH were assayed using enzyme-linked immunosorbent assay (ELISA) kits (Microwell TSH, T₃, T₄ kits; Synthron Bioresearch, Inc, USA).

2.5. Effect of Treatments on Thyroid Gland Lipoperoxidation. The level of thiobarbituric acid reactive substance, malonaldehyde (MDA) as an index of lipid peroxidation was evaluated in the thyroid gland using the double heating method of Draper and Hadley [41]. The principle of the method was based on spectrophotometric measurement of the color developed during reaction of thiobarbituric acid (TBA) with malonaldehyde. The thyroid glands from each animal in all the groups were weighed and then homogenized in a known volume of ice-cold phosphate buffer to obtain a 10% homogenate, which was centrifuged at 2000 g for 10 min to obtain the supernatant. The supernatant was then used to assess the level of protein and MDA in the sample. The assessment of MDA concentration in the supernatant of thyroid gland homogenate was performed thus; 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL of the thyroid gland homogenate in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling under tap water for 5 min, the mixtures were then centrifuged at 1000 g for 10 min. 2 mL of the supernatant was added to 1 mL of 6.7 g/L (0.67%) thiobarbituric acid (TBA) solution in a test tube and placed in a boiling water (100°C) bath for 15 min. The solution was cooled under tap water and the absorbance was thereafter measured at 532 nm using a UV spectrophotometer (T80+UV/VIS Spectrometer PG Instruments Ltd, UK). The concentration of MDA calculated by the absorbance coefficient of MDA-TBA complex ($1.56 \times 10^5 \text{ cm}^{-1}$) and expressed as nmol/mg of tissue protein. The protein concentration was determined using the method described by Lowry et al. [42].

2.6. Statistical Analysis. Data obtained were expressed as mean + SEM and then subjected to one-way analysis of variance followed by Tukey's post hoc test using Graphpad prism version 4.0. Values of $P < .05$ were considered significant.

3. Results

3.1. Effect of Treatments on Serum Triiodothyronine Concentration. The effect of treatments on serum T₃ level is shown in Figure 1. There was no significant difference in the serum T₃ level between the groups. However, the CPF + Pb group had the lowest T₃ concentration with its level decreasing by 14.3%, 29%, and 14.3%, respectively, relative to the S/oil, VC, and VC + CPF + Pb groups.

3.2. Effect of Treatments on Serum Thyroxine Concentration. There was no significant ($P > .05$) difference in the serum T₄ level between the groups. However, the lowest T₄ level was recorded in the CPF + Pb group as its level decreased by 0.6%, 32%, and 26% compared to S/oil, VC, and VC + CPF + Pb groups, respectively (Figure 2).

FIGURE 1: Effect of subchronic coexposure of soya oil (S/oil) and vitamin C (VC) and/or combination of chlorpyrifos (CPF) and lead (Pb) on triiodothyronine concentration in Wistar rats.

FIGURE 2: Effect of subchronic coexposure of soya oil (S/oil) and vitamin C (VC) and/or combination of chlorpyrifos (CPF) and lead (Pb) on serum thyroxine concentration in Wistar rats.

3.3. Effect of Treatments on Serum Thyroid Stimulating Hormone Concentration. The effect of treatments on TSH concentration is shown in Figure 3. There was a significant ($P < .01$) increase in the TSH concentration in CPF + Pb group compared to S/oil group. The TSH concentration in the VC + CPF + Pb group significantly ($P < .05$) increased compared to S/oil group. There was no significant ($P > .05$) change in the TSH concentration in the VC group compared to S/oil, CPF + Pb, or VC + CPF + Pb group.

3.4. Effect of Treatments on Thyroid Malondialdehyde Concentration. The effect of treatments on thyroidal MDA concentration of various groups is shown in Figure 4. There was a significant ($P < .01$) increase in the MDA concentration in the CPF + Pb group when compared to S/oil, VC, or VC + CPF group. The MDA concentration significantly ($P < .01$) increased in the VC + CPF + Pb group compared to VC group.

3.5. Effect of Treatments on the Weight of Thyroid Gland. The effect of treatments on the weight of thyroid gland is shown in Figure 5. There was no significant change ($P > .05$) in the

FIGURE 3: Effect of subchronic coexposure of soya oil (S/oil) and vitamin C (VC) and/or combination of chlorpyrifos (CPF) and lead (Pb) on thyroid stimulating hormone concentration in Wistar rats. ^a $P < .01$ versus S/oil group, ^b $P < .05$ versus S/oil group.

FIGURE 4: Effect of subchronic coexposure of soya oil (S/oil) and vitamin C (VC) and/or combination of chlorpyrifos (CPF) and lead (Pb) on thyroid malonaldehyde concentration in Wistar rats. ^{abc} $P < .01$ versus S/oil, VC and VC + CPF + Pb groups, respectively; ^d $P < .01$ versus VC.

weight of the thyroid gland in between the groups. However, the thyroid weight was higher in the CPF group by 11.4%, 8%, and 7.2% relative to C/oil, VC, and VC + CPF groups, respectively.

4. Discussion

Low-dose subchronic coadministration of CPF and Pb mimicking environmental exposure, which did not cause any apparent systemic toxicity, has been shown to cause a nonsignificant decrease in the serum level of T_3 and T_4 in the present study. Although, the alteration in the concentration of thyroid hormone was not significant, the fact that these very low doses of CPF and Pb did cause some level of hormonal changes should not be ignored as it may indicate ongoing subclinical metabolic alterations within the system. CPF has been shown to induce hypothyroidism in ewes [22] and rats [23, 24, 43]. Zaidi et al. [44] recorded hypothyroidism in the serum of some pesticide formulators. Similarly, Meeker et al. [17] demonstrated an

FIGURE 5: Effect of subchronic coexposure of soya oil (S/oil) and vitamin C (VC), and/or combination of chlorpyrifos (CPF) and lead (Pb) on the weight of thyroid gland in Wistar rats.

inverse association between urinary concentration of CPF metabolites, 3,5,6-trichloro-2-pyridinol and T_4 level and a positive association with TSH. In the same vein, hypothyroidism has been associated with Pb [45, 46] poisoning. Perhaps the reason why the coadministration of CPF and Pb did not result in significant alteration in thyroid hormone concentrations may be due to the very low doses of the two chemicals used.

Although T_3 is a poor indicator of subclinical or overt hypothyroidism, the marginal decrease in serum T_3 level observed in the group subchronically coadministered with CPF and Pb may be due to the low T_4 level observed in the group rather than a decrease synthesis. This is because T_4 have to be converted to T_3 for the biological effect of the hormone to be manifested. In addition, the relative low T_3 level may also be due to deficiency in the synthesis of 5'-deiodinase, an enzyme responsible for the conversion of T_4 to the more metabolically active T_3 . Indeed, Pb has been shown to inhibit the activity of type-I iodothyronine 5'-monodeiodinase (5'-D) activity [45]. CPF has been suggested to differentially affect peripheral deiodination [23]. The low 5'-deiodinase activity may have been due to pathological changes in the organs responsible for its synthesis by both CPF and Pb.

The marginal decrease in the level of T_4 observed in the group coadministered with CPF and Pb may be due to some level of damage to the thyroid acinar probably due to oxidative stress induction by both CPF [23, 24, 43, 44] and Pb [47, 48]. Although, the present study did not evaluate histological changes in the thyroid glands, Pb on its own has been shown to cause hypothyroidism either by inhibiting iodine uptake [25, 26] and to cause functional impairment of the pituitary—thyroid axis [27].

Some level of improvement in the concentration of T_3 and T_4 in group pretreated with vitamin C probably underscores the role of oxidative stress in thyroid dysfunction evoked by coadministration of CPF and Pb as indicated by low lipoperoxidative changes in the VC + CPF + Pb group. The vitamin C having protected the thyroid acinar

from oxidative damage may have aided in restoring thyroid hormones' synthetic function. Apart from its antioxidant properties, some other nonantioxidant activity of vitamin C may have complemented the restoration of thyroidal function. For example, vitamin C has been demonstrated to aid the synthesis of paraoxonase, an important esterase that aids in the detoxification of OPs [49].

The significant increase in the TSH concentration in the group coadministered with CPF and Pb may be due to the attempt by the body to stimulate the thyroid gland to increase the synthesis and elaboration of T_4 in order to compensate for the apparent deficit in the system. CPF-induced increase in TSH concentration has been recorded in humans [17, 44] and laboratory animal models [23, 24]. The low T_4 concentration in the CPF + Pb group may have stimulated the hypothalamic neurons to secrete thyrotrophin releasing hormone (TRH) leading to increased stimulation of TSH synthesis [50]. The apparent normalization of the TSH concentration in group pretreated with vitamin C correlates positively with the marginal improvement in T_4 and T_3 concentration in the group.

The study revealed a significant increase in the MDA concentration in the thyroid gland of CPF + Pb group as compared to other groups. This increased MDA concentration is indicative of oxidative damage to the thyroid gland. This may be attributed to the high metabolic rate, high level of free radicals accumulation, and low level of endogenous antioxidant in the thyroid gland. Oxidative stress induction is one of the molecular mechanism of CPF [31–33] and Pb [28, 34] poisoning. Oxidative stress, characterized by an elevation in the steady-state concentration of reactive oxygen species (ROS), has been implicated in a wide range of biological and pathological conditions [51]. Thyroid hormones are associated with the oxidative and antioxidant status of the organism [52]. Because of its role in oxidative metabolism, increase concentration of ROS is formed in the thyroid glands. However, the combination of CPF and Pb was shown to have exacerbated the ROS induction perhaps due to their direct interaction with the thyroid acinar. Lipid peroxidation inactivates cell constituents by oxidation or causes oxidative stress by undergoing radical chain reaction, ultimately leading to loss of membrane integrity [53]. Similarly, the increased MDA concentration may have been exacerbated due to impairment of antioxidant enzymes that have been reported in hypothyroidism [54]. Pb on its own induces lipoperoxidation by inhibiting the activity of δ -aminolevulinic acid dehydrase leading to accumulation of its substrate δ -aminolevulinic acid, which rapidly oxidize to generate free radicals as superoxide ion, hydroxyl radical, and hydrogen peroxide [7].

Pretreatment with vitamin C has been shown by the present study to ameliorate the lipoperoxidative changes induced by subchronic administration of coadministration of CPF and Pb. This was apparently due to antioxidant property of the vitamin. The antioxidant properties of vitamin C have been demonstrated in numerous studies involving CPF-induced lipoperoxidation [31, 33, 37, 38].

The weight of the thyroid was marginally higher in the CPF + Pb group than any of the other three groups. The

reason for the relatively higher weight in the CPF + Pb group may be related to the significantly higher TSH concentration, which may have partly stimulated the proliferation of the thyroid follicular cell [55], apparently to compensate for the marginal decrease in thyroid hormones. Although, the weight of the thyroid gland was marginally higher in the group pretreated with vitamin C than those of C/oil and VC groups, the fact that it was lower compared to CPF + Pb group demonstrates apparent restoration of the weight of the thyroid gland by vitamin C.

5. Conclusion

Subchronic coadministration of CPF and Pb has been shown by the present study to marginally decrease thyroid hormones concentration, increase TSH concentration and thyroid lipoperoxidation, and marginally increase the weight of the thyroid gland. The alterations in these thyroid gland parameters were ameliorated by pretreatment with vitamin C apparently due to its antioxidant properties.

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