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ORIGINAL ARTICLE

Modulatory role of quercetin against chlorpyrifos induced blood toxicity in rats

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ABSTRACT

Chlorpyrifos (CPF) is a broad-spectrum organophosphate insecticide and has been reported to cause a number of serious deleterious effects on physiological systems. The present study was designed to evaluate the role of quercetin (QC) during CPF induced toxicity in blood cells. Female Wistar rats weighing 150–200 g were divided into four different groups viz: Normal Control, CPF treated (13.5 mg/kg body weight every alternate day), QC treated (50 mg/kg body weight/day) and combined CPF + QC treated. The effects of different treatments were studied on various hematological parameters as well as on anti-oxidant defense system. CPF treated animals showed a significant decrease in total leukocyte counts (TLC), lymphocyte counts, hemoglobin (Hb) levels and aminolevulinic acid dehydratase (ALAD) activity, which however showed appreciable improvement upon simultaneous treatment with QC. Contrarily, neutrophils counts were found to be significantly increased, which, however, were decreased upon simultaneous treatment with QC. Further, CPF exposure caused a significant increase in the levels of lipid peroxidation (LPO) and protein carbonyl content (PCC) as well as increased the activities of super oxide dismutase (SOD) and catalase (CAT) in blood, which were decreased on QC co-treatment. Moreover, CPF treatment also caused inhibition of glutathione system in blood but QC co-treatment was able to up-regulate the glutathione system. Therefore, the present study, suggests that QC unveils a protective potential in containing CPF induced blood toxicity.

KEY WORDS: quercetin; oxidative stress; blood

Introduction

A large number of organophosphates are constantly used worldwide to inhibit the growth of unwanted weeds and pests. Despite the exposure from organophosphates causes a number of health hazards, its usage across the globe is still high (Hung *et al.,* 2015). CPF is an organophosphate that is widely used as an insecticide in agriculture as well as in household sectors (Fu *et al.,* 2015). It induces toxicity in various organs of reproductive (Nishi & Hundal, 2013), cardiac (Zafiropoulos *et al.,* 2014), renal and neurological systems (Nasr *et al.,* 2015).

Although there is enough information available on detrimental effects of organophosphates on brain, only limited evidences of their effects on hematological profile are available. Blood profiling has been considered as one of the vital parameters for the detection of pathophysiological alterations during stress conditions induced by various xenobiotics/chemicals (Kolesnikova *et al.,* 2014). Few studies have shown that organophosphate insecticides can induce hematological changes in experimental animals (Sunmonu & Oloyede, 2010; Enis Yonar *et al.,* 2012). Earlier findings have suggested that CPF exposure to rats can lead to microcytic hypochromic anemia by inhibiting the process of erythropoiesis and hame synthesis (Acker *et al.,* 2012; Elsharkawy *et al.,* 2013). Reports have also indicated that CPF can alter the activities of various enzymes that are associated with anti-oxidant defense system as well as apoptotic machinery (Li *et al.,* 2015; Smida *et al.,* 2017).

On the other hand, QC is a bio-flavonoid, which is abundantly found in fruits, vegetables, tea and wine (Haleagrahara *et al.,* 2013). It is one of the most widely consumed phytochemicals that exerts protective effects against various diseased conditions including cancer, diabetes and neurodegeneration (Sharma *et al.,* 2015; Yarahmadi *et al.,* 2018). Further, QC exhibits anti-carcinogenic (Maurya & Vinayak, 2015; Kashyap *et al.,* 2016) anti-inflammatory (Heeba *et al.,* 2014; Liu *et al.,* 2018), anti-apoptotic (Yang *et al.,* 2014; Roslan *et al.,* 2017) and antiviral properties (Wu *et al.,* 2015; Rojas *et al.,* 2016). Reports have revealed that QC has the ability to regulate

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biological processes such as mitochondrial biogenesis, cell growth and cell migration (Miles *et al.,* 2014; Lan etal., 2017; Qiu *et al.,* 2018). In the light of paucity of information on hematological parameters, the present study was designed to elucidate the possible protective potential of QC during CPF induced blood toxicity in rats.

Material and methods

Chemicals

CPF was procured from Merck Ltd. (India) and was of 99.7% purity. QC was purchased from Sigma Pvt. Ltd. (USA) with 95% purity. Chemicals trichloroacetic acid, urea, thiobarbituric acid, D-aminolevulinic acid, triton X-100, cresyl violet, giemsa stain, 2,4-dinitrophenylhydrazine, Ehrlich's solution and nitro blue tetrazolium were obtained from Sigma Chemicals Company (USA). All other chemicals such as ammonia solution, 5,5'-dithiobis-(2-nitrobenzoic acid), ethanol, ethyl acetate, nitro blue tetrazolium, hydroxylamine hydrochloride methanol, tris buffer, sodium arsenite, hydrogen peroxide, 1-chloro-2,4-dinitrobenzene were procured from Merck, (India) whereas sodium chloride, nicotinamide adenine dinucleotide phosphate hydrogen, glutathione disulfide and dithioerythritol from HiMedia (India).

Experimental design

Healthy female Wistar rats in the weight range of 150–200 g were obtained from the central animal house of Panjab University, Chandigarh, India. The animals were housed in polypropylene cages with a hygienic bed of husk in a well-ventilated animal room until the end of the experimental period. The animals had free access to drinking water and standard animal feed (Ashirwad Industries, Punjab, India) throughout the treatment period. All the procedures related to animals were performed in accordance with the principles and guidelines approved by the Institutional Animal Ethics Committee.

To carry out various investigations, the animals were segregated into following four groups with 6 animals in each group. The animals in Group 1 served as normal control and were given corn oil by gavaging, which was used as a vehicle for the treatment of animals in CPF group. The animals in Group 2 were administered CPF orally every alternate day through intubation gavaging at a dose level of 13.5 mg/kg body weight in corn oil (Malhotra & Dhawan, 2014). Group 3 animals were given QC daily at a dose level of 50 mg/kg body weight in drinking water (Jahan *et al.,* 2015). The animals in group 4 were given a combined treatment of CPF and QC in a similar manner as given to Group 2 and Group 3 animals, respectively. The study was carried out for a total duration of 8 weeks.

Collection of blood samples

for the purpose of studying various hematological parameters, the blood samples were drawn after 8 weeks from all the animals belonging to different treatment groups. The samples were collected under light diethyl ether anesthesia by puncturing the ocular vein with a fine sterilized glass capillary. The blood samples were collected into the heparinized tubes and the supernatants were used as erythrocyte lysates.

Preparation of erythrocyte lysates

erythrocyte lysates were prepared by following the method of Ceballos-Picot *et al.* (1992). Lysates were obtained from the blood samples by centrifugation at 2500 rpm for 10 minutes (min) at room temperature. After centrifugation, both the plasma and buffy coats were removed. Later, the erythrocytes were washed twice with normal saline followed by removal of the supernatants. Further, water was added to the pellets (thrice its volume), which stored at –80 °C until use for future experiments. Lysed erythrocytes were prepared by freezing and thawing (two times) and cell membranes were removed by centrifugation at 3000 rpm for 20 min.

Hematological parameters

Hemoglobin estimation

Hb contents were estimated by following oxyhemoglobin method of Dacie & Lewis (1991). Fresh and non-clotted blood samples diluted in 0.004% ammonia solution (freshly prepared) and the optical densities were measured at 540 nm and Hb contents were expressed in g %.

Total Leukocytes Counts

TLC in blood samples were recorded by using the method of Dacie & Lewis (1991). Fresh blood samples were mixed with Turk's fluid to dilute the blood in the ratio 1:20 (v/v). A drop of diluted blood was immediately poured on Neubaur's chamber, which then was covered with a glass cover slip. Finally, the WBCs' were counted in the four-corner square of Neubaur's chamber.

Differential leucocytes counts (DLC)

DLC were recorded according to the method of Dacie & Lewis (1969). The blood samples were drawn from the animals of all the treatment groups by puncturing the ocular vein with a sterilized glass capillary and a drop blood was placed on a clean microscope glass slide, which was later spread by using another slide. The slides were air dried and fixed in methanol for 5–10 min. The smeared slides were stained for 30 min with giemsa stain (diluted 1:10). After staining, the excess stain was removed by running tap water followed by air-drying. Differential leucocytes were counted by using an oil immersion lens. Hundred consecutive stained white blood cells were counted starting from one direction to the other in random fashion and in all the possible planes. This process was repeated in all stained slides and percentage of neutrophils and lymphocytes were calculated.

Aminolevulinic acid dehydratase

ALAD activity was measured according to the method of Burch & Siegel (1971). In this method, incubation mixture was prepared by adding blood sample with triton X-100 reagent. Further, D-aminolevulinic acid (ALA) substrate was added to it and mixed with trichloroacetic acid (TCA). After incubation at 37 °C again, TCA reagent was added. The tubes were again centrifuged and clear supernatant

fluid was removed. Further, to aliquots, modified Ehrlich's reagent was added and absorbance was read at 555 nm.

Lipid peroxidation

LPO was determined by following the method of Wills (1966). The supernatants were added to thiobarbituric acid (TBA) and the color was developed by boiling at 100 °C for 10 min. The samples were cooled and absorbance was read at 532 nm. The amount of malondialdehyde (MDA) formed was calculated on the basis of molar extinction coefficient of MDA-TBA chromophore (1.56×10^5 $m^{-1}cm^{-1}$) and the results were expressed as nmoles of MDA/min/mg protein.

Protein carbonyl content

PCC was estimated by following the method of Levine *et al.* (1994). Blood lysates were added with 2,4-dinitrophenylhydrazine (DNPH) prior to incubation at room temperature for 1h in the dark. Further, 20% TCA was added and the solution was allowed to rest for 15 min at 4 °C. The precipitates were centrifuged at 11,000×*g* for 5 min. The bud was washed thrice with ethanol: ethyl acetate (1:1) and finally dissolved in 6 mol/l urea. Further, the reaction mixture was incubated at 37 °C for 30 min and the absorbance was read at 366 nm. The results were expressed as nmoles of reactive carbonyls formed (C=O) /mg protein, using the molar extinction coefficient of 21,000 mol/l cm^{-1}.

Superoxide dismutase

SOD activity was estimated according to the method of Kono (1978). The reaction mixture contained sodium carbonate, nitro blue tetrazolium (NBT), Triton X-100 and hydroxylamine hydrochloride. The rate of NBT reduction in the absence of the enzyme source was recorded for about 3 min with 30 seconds (sec) intervals. The reaction was followed at 560 nm against a reference containing sodium carbonate. The percentage inhibition in the rate of NBT reduction by enzyme SOD was recorded and one international unit enzyme was expressed as inverse of the amount of protein (mg) required in inhibiting the reduction rate of NBT by 50%. The activity of enzyme was expressed as international unit/ mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%.

Catalase

CAT activity was determined by using the method of Luck (1971). In this method, 0.5 absorbance was measured by using H_2O_2 and phosphate buffer and later RBC lysates were added to it. The decrease in absorbance at 240 nm was read on spectrophotometer after every 15 sec for 3 min against a reference solution lacking hydrogen peroxide. The amount of hydrogen peroxide decomposed was calculated on the basis of molar extinction coefficient of H_2O_2 which is 0.0394 mol/l^{-1} cm^{-1} and the activity of enzyme was expressed as µmoles hydrogen peroxide decomposed/min/mg protein.

Glutathione-S-transferase (GST)

The activity of GST was estimated according to the method of Habig *et al.* (1974). The samples were mixed with reaction mixture consisting of sodium phosphate buffer and 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was initiated by the addition of 0.05 ml of GSH to reaction mixture and absorbance was read at 340 nm after 30 sec of time interval. The enzyme activity was expressed as µmoles of GSH-CDNB conjugate formed/ min/mg protein, using molar extinction coefficient of the conjugate ($\varepsilon= 9.6\times10^6$ mol/l^{-1} cm^{-1}).

Reduced glutathione (GSH)

GSH was estimated by following the method of Moron *et al. (*1979). 25% TCA was added to the sample and the contents were thoroughly mixed. Further, the precipitated proteins were separated by centrifugation at 2000 g for about 15 min. The supernatant was diluted with 2.0 mol/l sodium phosphate buffer (pH 8.0). Finally, freshly prepared 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) of 10 mmol/l was added to each sample and absorbance was read at 412 nm. The glutathione contents were expressed as µmoles of glutathione/g tissue.

Glutathione reductase (GR)

The assay of GR activity was performed according to the method described by Carlberg & Mannervirk (1985). To cuvette, phosphate buffer, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and glutathione disulfide (GSSG) were added. The reaction was initiated by the addition of sample to the cuvette and the decrease in absorbance at 340 nm was followed for 2 min. A unit of GR activity is defined as the amount of enzyme that catalyses the oxidation of 1 nm of NADPH /min/mg protein using 6.22×10^3 as the molar absorption of NADPH.

Total glutathione content (TG)

The TG content was measured by following the method of Zahler & Cleland (1968). The disulfide in the sample was mixed with dithioerythritol and the reduction was allowed to proceed for 20 min. After reduction, tris buffer, sodium arsenite and water were added. DTNB prepared in sodium acetate was then added and the absorbance was recorded for 3 min. The TG contents were expressed in terms of µmoles of GSH/mg tissue.

Statistical analysis

The statistical significance of the data was determined by using one-way analysis of variance (ANOVA) and a *pos hoc* test. The results were expressed as mean ± S.D of 6 observations. The comparisons were made as follows: $^{a}p\leq0.05$, $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post hoc* analysis when values are compared with the normal control. $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post hoc* analysis when values are compared with CPF treated group.

Results

Hematological findings

The levels of Hb, TLC and lymphocytes were found to be decreased following CPF treatment; however, they were increased following QC co-supplementation (Figure 1 and Figure 2). On the contrary, neutrophils counts were found to be elevated in CPF treated animals when compared to the normal control animals. No significant changes were observed in differential leucocytes counts following

QC treatment to normal animals. However, neutrophils count showed an appreciable decrease following QC co-treatment. No significant changes were found in the number of monocytes and eosinophils in any of the treatment groups. On the contrary, ALAD activity was found to be significantly inhibited in the animals treated with CPF when compared to the normal controls; however, it was increased significantly following QC co-treatment (Figure 3).

Lipid peroxidation and protein carbonyl content

The MDA as well as PCC levels were found to be increased in erythrocyte lysates of CPF treated animals when compared to normal control group. No significant changes were observed in the levels of MDA and PCC of animals treated with QC alone. However, QC co-administration to CPF treated animals was able to decrease both the elevated lipid peroxidation and PCC levels (Figure 4 and Figure 5).

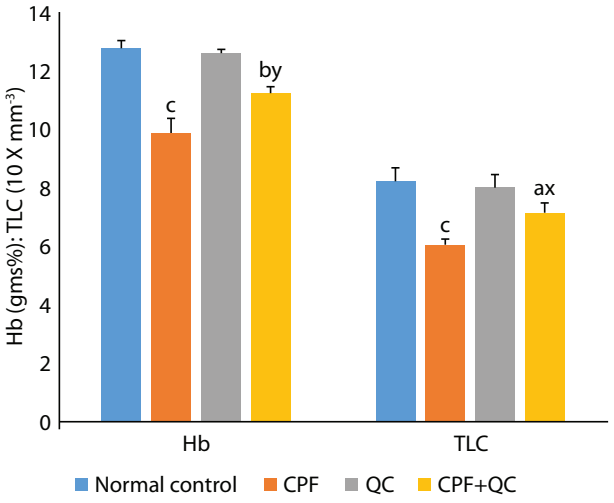

Figure 1. Effect of QC on Hb and TLC in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. [a]$p \leq 0.05$ [b]$p \leq 0.01$, [c]$p \leq 0.001$ by *post-hoc* analysis when values are compared with normal control; [x]$p \leq 0.05$, [y]$p \leq 0.01$, [z]$p \leq 0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

Superoxide dismutase and catalase

The enzyme activities of SOD and CAT were found to be increased in erythrocyte lysates following CPF treatment; however, they were found to be significantly decreased upon QC supplementation (Figure 6 and Figure 7). Moreover, no significant changes were observed in the activities of SOD and CAT when QC alone was given to animals.

Glutathione system

The enzyme activities of GST and GR as well the levels of GSH and TG were observed to be decreased in erythrocyte lysates of CPF intoxicated group animals. However, simultaneous QC supplementation to CPF treated rats was able to increase the declined levels of GSH and TG as well as enzyme activities of glutathione system (Figure 8, Figure 9, Figure 10 and Figure 11).

Discussion

The present study was designed to evaluate the effects of CPF intoxication on anti-oxidant defense system in red blood cells and the protection provided by QC, if any, upon its supplementation. Here we demonstrate that CPF treatment of animals for 8 weeks resulted in significant alterations in various hematological indices; however, they were found to be modulated upon QC co-administration to CPF treated animals.

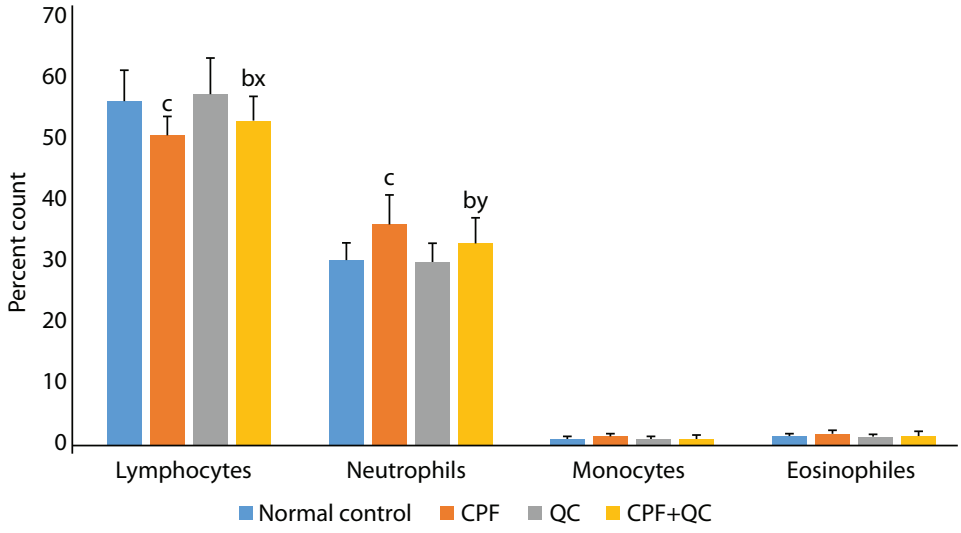

Figure 2. Effect of QC on DLC in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. [a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p \leq 0.001$ by *post-hoc* analysis when values are compared with normal control; [x]$p \leq 0.05$, [y]$p \leq 0.01$, [z]$p \leq 0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

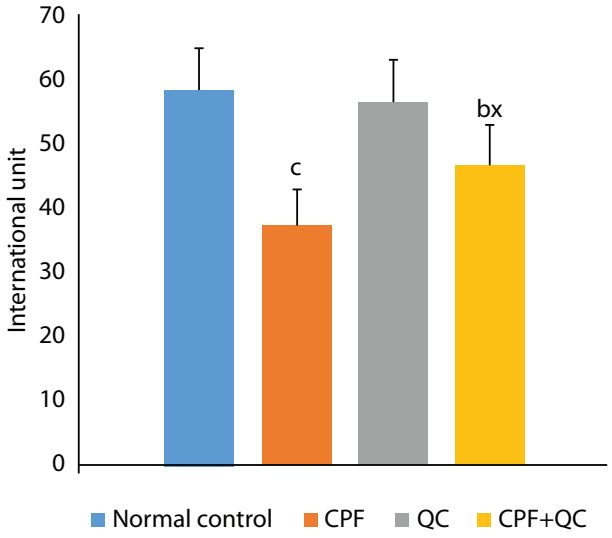

Figure 3. Effect of QC on ALAD levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

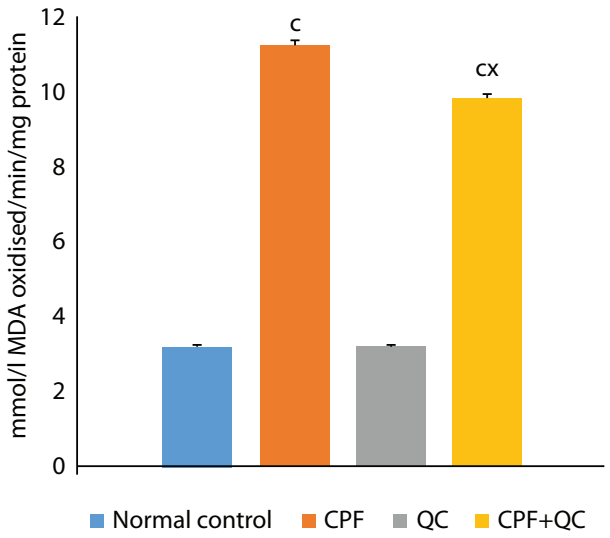

Figure 4. Effect of QC on LPO levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

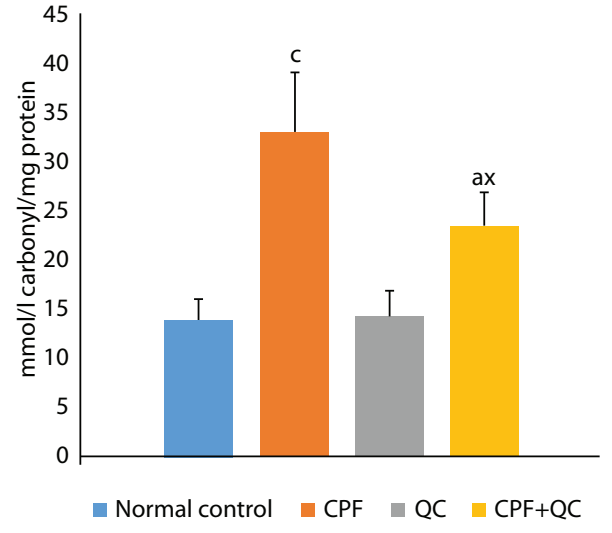

Figure 5. Effect of QC on PCC levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

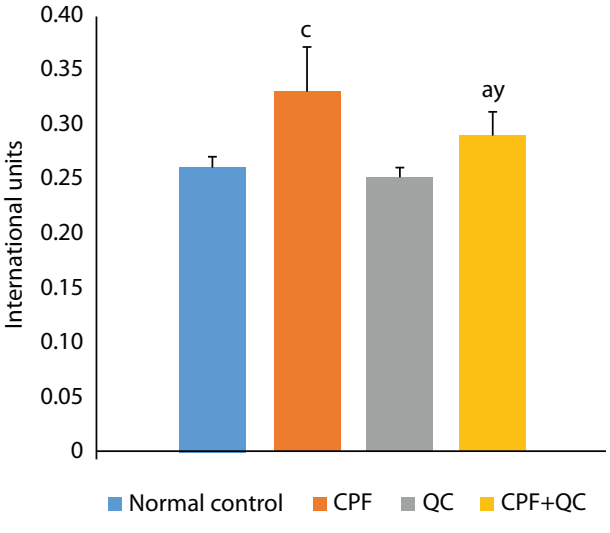

Figure 6. Effect of QC on SOD activity in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

In the present study, a significant decrease in Hb content was observed after 8 weeks of CPF treatment, which indicates CPF induced hematotoxicty in rats. Earlier studies have shown similar effects on Hb content in animals after CPF treatment (Akhtar *et al.,* 2009; Pala *et al.,* 2018). However, QC supplementation along with CPF treatment proved to be beneficial as it was able to increase the declined levels of Hb as compared to CPF treated group. QC has earlier been demonstrated to prevent the oxidation of hemoglobin molecule during oxidative stress and hence it has also prevented the CPF induced Hb oxidation in the current study (Krukoski *et al.,* 2009).

TLC and DLC were also found to be altered in CPF intoxicated animals that suggest severe disturbance in the immune system. TLC and DLC were found to be decreased after CPF exposure, which presumably is due to their decreased production from the germinal center of lymphoid organs or their increased lysis via chlorinated

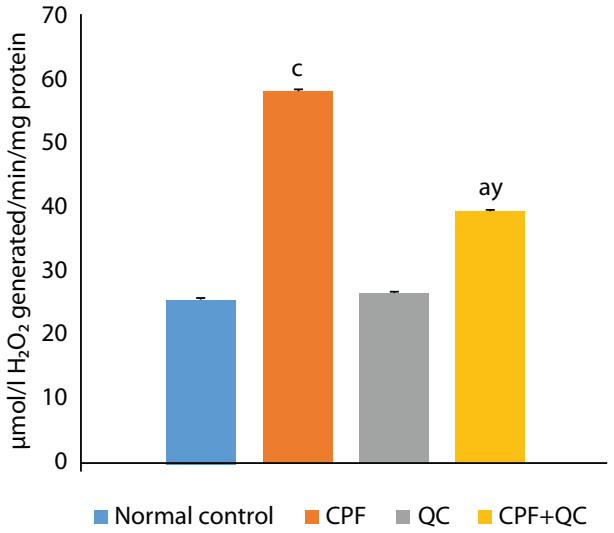

Figure 7. Effect of QC on CAT activity in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

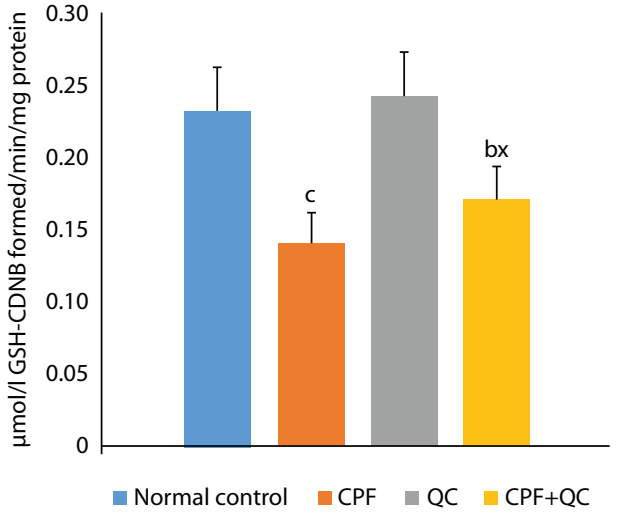

Figure 8. Effect of QC on GST activity in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

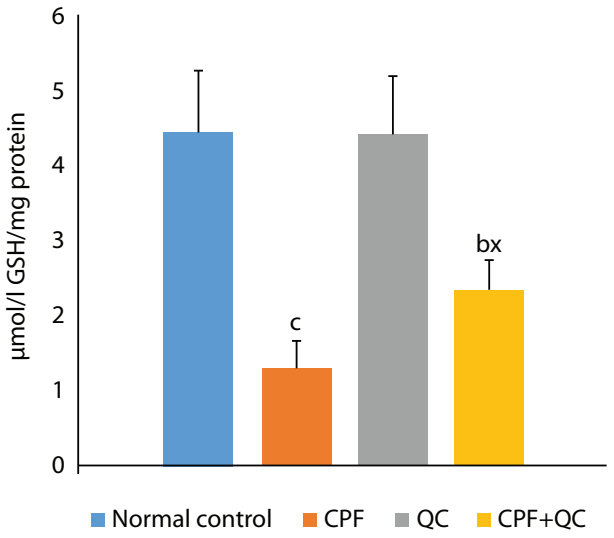

Figure 9. Effect of QC on GSH levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

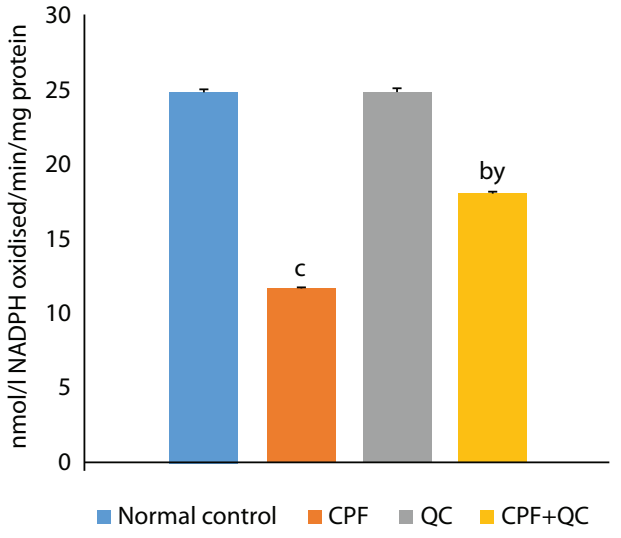

Figure 10. Effect of QC on GR levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

compounds present in the body (Goel *et al.,* 2006). On the contrary, CPF exposure caused a significant elevation in the neutrophils counts suggesting neutrophilia. The increase in neutrophils number indicates the activation of the primary defense system of the body, which caused an increased leukocyte mobilization as a consequence of CPF induced cytotoxicity and thus led to decreased leukocytes counts. QC administration to CPF treated animals significantly improved the TLC and also attenuated the disturbed DLC indices of the rats, which attributes to the immunomodulatory property of QC (Benkovic *et al.,* 2009).

During the present study, ALAD activity was also found to be significantly reduced in the CPF treated group when compared to normal control group. Earlier reports have also shown that decrease in heme synthesis and Hb content in the body can lead to altered ALAD activity (Alhawaj *et al.,* 2015; Olakkaran *et al.,* 2018). Further, QC

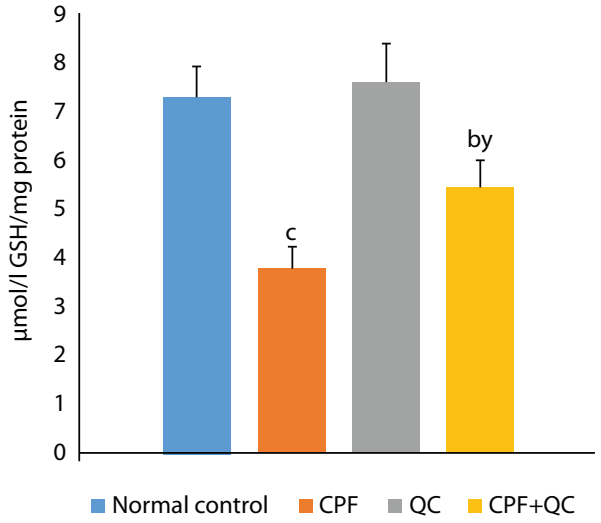

Figure 11. Effect of QC on TG levels in erythrocytes of CPF intoxicated rats. All the values are represented as mean ±SD for each treatment group; n=6 for each treatment group. $^{a}p\leq0.05$ $^{b}p\leq0.01$, $^{c}p\leq0.001$ by *post-hoc* analysis when values are compared with normal control; $^{x}p\leq0.05$, $^{y}p\leq0.01$, $^{z}p\leq0.001$ by *post-hoc* analysis when values are compared with chlorpyrifos treated group.

co-supplementation to CPF treated animals was able to elevate the declined ALAD activity, which could be a consequence of improved Hb levels and thereby upregulated the declined ALAD activity. Studies have also revealed the potential of QC in regulating various hematological parameters that get altered during chemically induced oxidative stress conditions (Mishra & Flora, 2008; Bhatt & Flora, 2009).

In the current study, CPF treatment also caused a marked increase in MDA levels in erythrocyte lysates, which indicates enhanced lipid peroxidation. CPF exposure resulted in oxidative degradation of polyunsaturated fatty acids and thereby generated a large number of free radicals, which eventually are responsible for causing alterations in the membrane structure and function. Increased LPO and erythrocyte fragility have also been observed by various researchers after CPF exposure to animals (Ambali *et al.,* 2010; Nishi & Hundal, 2013). Further, PCC content was also found to be elevated in blood of CPF treated animals, which suggests the oxidation of proteins. This elevation is due to CPF induced increase in production of free radicals in the body, which led to alteration in the activities of various cellular proteins that are important for retaining the structural and functional integrity of erythrocytes' membranes (Ambali *et al.,* 2010). These findings clearly depict that CPF treatment led to oxidation of both the lipids as well as proteins and thus is responsible for oxidative injury in RBCs via free radicals. However, QC administration to CPF treated animals decreased the previously raised levels of MDA and PCC, which attribute to the scavenging activity of QC. Thus, QC appears to have attenuated the free radical generation process and has prevented the oxidation of membrane lipids as well as proteins. Some authors have shown similar behavior of QC during chemically induced oxidative stress in different organs (Selvakumar *et al.,* 2012; Periasamy *et al.,* 2016).

Further, CPF treatment for 8 weeks resulted in increased activities of SOD and CAT that demonstrate the physiological response of the animals in mitigating the free radicals, induced oxidative stress. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, whereas CAT converts hydrogen peroxide into water molecules. CPF exposure understandably has generated a considerable number of free radicals and in order to thwart the toxicity induced by free these radicals, the body has stimulated the activities of SOD and CAT. Previous studies have also revealed that CPF exposure is associated with the elevation of SOD and CAT activities (Kalender *et al.,* 2012; Kumar *et al.,* 2014). However, QC co-treatment to CPF treated animals was able to decrease the SOD and CAT activities. This ameliorative effect is understandably due to free radical scavenging activity of QC, which led to reduction in the load of free radicals in the cellular system and eventually has normalized the activities of antioxidants. It has been shown that QC due to its high diffusion capacity into the membranes has been considered as a strong scavenger of free radicals (Kalender *et al.,* 2012; Mohammadi *et al.,* 2014; Calabro *et al.,* 20CPF intoxication for 8 weeks suppressed the glutathione system of the body, as the activities of GSH, TG, GR and GST were found to be decreased in the erythrocyte lysates. The reduced activities are the consequence of oxidative modifications of proteins, which led to the decreased antioxidant versus oxidant ratio. These findings suggest that in order to detoxify CPF induced free radicals, a substantial amount of glutathione would have been consumed by the glutathione-related enzymes. Further, elevated LPO levels are also concomitant with our depleted GSH findings, which clearly reflect the moderating effect of GSH towards LPO induced free peroxides. Moreover, declined TG levels and GR activity also support the combating effect to contain reactive oxygen species induced by CPF exposure. Further, declined GST activity can be attributed to oxidation of SH sites or its high participation in the detoxification of CPF induced reactive species via conjugating with glutathione, which eventually decreased the GST content in the cellular system. Recently, some reports have also revealed a similar depletion of glutathione system in different organs after CPF exposure (Mosbah *et al.,* 2016; Nasr *et al.,* 2016). QC co-administration to CPF treated animals was able to ameliorate the altered glutathione system and that can be attributed to its free radical quencher capacity. The results of the present study therefore clearly demonstrate that QC treatment could up-regulate the declined glutathione system by reducing the free radical burden in the cell and thereby enhanced the antioxidant potential of the cell.

Conclusion

The present study suggests that QC mitigates CPF induced hematological alterations and therefore could be

considered as a potential candidate for alleviating CPF induced toxicity in blood.

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