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Organophosphate Pesticide-induced Cytotoxicity in Rat Peripheral Blood Lymphocytes and Ameliorating Effect of Quercetin

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Abstract– Organophosphate pesticides (OP) are the most extensively used chemicals all over the world and therefore, responsible for most of the human poisoning cases. Present study is carried out to evaluate the *in vitro* toxicity of quinalphos (QNP) and chlorpyrifos (CPF), the two commonly used OP pesticides, using rat peripheral lymphocytes, and protective effects of a natural flavanoid, quercetin. Results showed dose-dependent and exposure-time dependent cytotoxicity of both QNP and CPF, estimated by MTT assay and lactate dehydrogenase release in culture medium. The pesticides also showed dose-dependent decrease in the levels of ATP in lymphocytes. The results clearly showed compromised membrane integrity leading to cell death by lysis on exposure with QNP or CPF. The anticholinesterase activity of QNP and CPF were evident from the dose-dependent inhibition of acetylcholinesterase (AChE) activity in cultured rat lymphocytes. Quercetin, a well-known flavanoid with antioxidant activity, when given along with the QNP and CPF, resulted in decreased toxicity of both the pesticides. Quercetin co-treatment therefore, offered partial protection against OP pesticide induced toxicity.

Keywords– Quinalphos, Chlorpyrifos, Cytotoxicity, Lactate dehydrogenase release, Quercetin

I. INTRODUCTION

Organophosphate (OP) compounds are the chemicals which are most widely used as pesticides all over the world to get rid of pests that harm the agriculture, human health and the environment. Due to their high efficiency, these chemicals are extensively used by farmers for crop protection all over the world including India. Extensive use of these pesticides in various sectors has resulted in contamination of soil, water and air and deterioration of the environment [1], [2]. Non-selectivity of these chemicals together with injudicious and indiscriminate use has caused severe environmental problems and toxicity in the non-target organisms and has increased the risk of human exposure and associated health effects. The main target of OP pesticides is the enzyme, acetylcholinesterase (AChE) which hydrolyses acetylcholine (ACh) at cholinergic synapses and in neuromuscular junctions where this enzyme plays a key role in cell-to-cell transmission of nerve impulse [3]. Quinalphos (O, O-diethyl O-quinoxalin-2-yl phosphorothioate, QNP) and chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl)-phosphorothioate, CPF), are among the most widely used OP pesticides in agriculture, health and other sectors, not only in India but throughout the world. OP pesticides are involved in most of the human poisoning cases, occupational exposure and suicidal attempts, in rural India [4], [5], [6]. Besides being potent neurotoxicants, the toxicity of these OP pesticides is caused by adversely affecting the other important targets in the living organisms and disturbing the metabolic balance leading to serious consequences.

Present study is aimed to evaluate the protective effect of quercetin against CPF and QNP induced cytotoxicity in rat peripheral blood lymphocytes. The cytotoxicity of these pesticides is evaluated by MTT assay, lactate dehydrogenase leakage and alterations in the cellular levels of ATP and effect of quercetin is assessed by giving its co-treatment with pesticides.

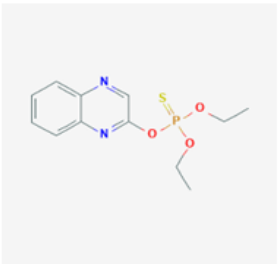
II. RELATED WORK

OP pesticides are known to produce oxidative stress. It has been previously reported that QNP and CPF exposure caused induction of oxidative stress and disturbance in redox status in different tissues of rats [7], [8], [9]. The oxidative damage of vital macromolecules viz., lipids, proteins and DNA and disturbances in enzymatic and non-enzymatic antioxidant defences have been reported on exposure with a large number of OP pesticides. OP pesticides also affect DNA and RNA synthesis, signal transduction pathways, and expression of different transcription factors due to induced oxidative stress. CPF is reported to induce oxidative stress at low concentrations, inhibit replication of cells of the nervous system, disrupt neuronal differentiation and induced generation of reactive oxygen species (ROS) in PC12 cells. Inhibition of mitochondrial activity in these cells leads to excessive ROS formation, cytochrome c release from mitochondria

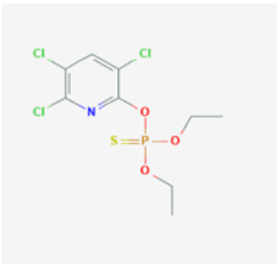
and activation of apoptotic cell death [10], [11], [12], [13]. Exposure of CPF also altered expression of proteins involved in antioxidant defence in rat tissues. *In vitro* exposure of CPF, parathion and malathion has been shown to cause oxidative DNA damage, both single and double strand breaks, in rat peripheral blood lymphocytes [14]. CPF exposure in HepG2 cells for 24 h has also showed increased Ca^{++} concentration, increased plasma membrane potential and decreased mitochondrial transmembrane potential [15]. In another study, CPF exposure to human erythrocytes induced lipid peroxidation and disturbed the activity of antioxidant enzymes, suggesting the involvement of oxidative stress in its overall toxicity [16]. Malathion induced oxidative stress and increase in the levels of malondialdehyde indicating oxidative damage to lipids has also been observed in erythrocytes given *in vitro* exposure and the oxidative stress was reduced by pre-treatment with vitamin C and E [17]. Malathion induced oxidative stress, cytotoxicity and genotoxicity has also been observed in HepG2 cells in an *in vitro* study [18] and apoptosis in N2a neuroblastoma cells [19]. Induction of apoptosis by OP compounds may be the outcome of disturbances in mitogen activated protein (MAP) kinases signaling pathways [20].

Organophosphate and carbamate (OPC) poisoning is a major global health hazard requiring immediate medical intervention. Atropine (ATR) is an essential antidote in organophosphate and carbamate poisoning, with the inclusion of cholinesterase reactivators and other anticholinergics, namely pralidoxime (PAM) and glycopyrrolate (GPR). Numerous sensors have been developed to monitor the dynamic change of water quality for ecological, early warning, and protection reasons [21].

The available literature and the previous studies in our lab have given sufficient evidences that induction of oxidative stress is a major contributor te overall toxicity of OP pesticides. Present study is therefore, undertaken to evaluate the *in vitro* cytotoxicity of CPF and QNP, and the cytoprotective effect, if any, of quercetin.



Quinalphos (O, O-diethyl O-quinoxalin-2-yl phosphorothioate, QNP)



Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl)-phosphorothioate, CPF)

Figure1. Presenting the two organophosphate pesticides namely Quinalphos and Chlorpyrifos.

III. METHODOLOGY

Chemicals and Experimental Animals

Acetylthiocholineiodide, di-sodium hydrogen phosphate, sodium dihydrogen phosphate, dithionitrobenzoic acid, sodium pyruvate, NADH, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), dimethylsulphoxide, trypan blue, sodium pyruvate and quercetin were obtained from Sigma Chemicals Inc., St. Louis, Mo, Hi-Sep was obtained from Himedia Chemical India Ltd, and ATP detection assay kit was purchased from Cayman Chemicals, Michigan, USA. Quinalphos and Chlorpyrifos, were kind gift from Devidayal (Sales), Limited, Mumbai, India.

Adult male rats of Wistar strain, used in the present study were purchased from the Animal Facilities of All India Institute of Medical Sciences, New Delhi, India.

Study Design

The study was conducted taking peripheral blood lymphocytes isolated from the blood of rats. The isolated lymphocytes were given treatment of different concentrations of QNP and CPF, separately, dissolved in DMSO for 1 h, 3 h and 6 h. In the case of quercetin co-treatment, lymphocytes were incubated with different concentrations of pesticides along with quercetin for different time periods.

Ethical clearance

The care and maintenance of animals were as per the approved guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), India. Ethical clearance for the use of animals for the present study was obtained (# IAEC/JU/44).

Isolation of lymphocytes

Peripheral blood (2-3 ml) was collected in falcon tube from the ocular vein of albino rats with the help of glass capillary and was diluted 1:1 with phosphate buffer saline (pH 7.4, containing 0.9% NaCl). Diluted blood was layered carefully over equal volume of HiSep, centrifuged at 1000 g for 10 min at 4°C, white buffy coat at the interface was aspirated, washed twice with PBS and the pellet was suspended in 1.0 ml RPMI 1640 medium.

Viability testing: Cell viability was tested by the method described by Phillips [22]. Equal volume of lymphocyte cell suspension and trypan blue solution (0.4%) were mixed, and counted in haemocytometer under light microscope. Live and dead cells were counted separately and % of live cells was calculated. The preparation having viable cell count ≥ 95% were used for further studies.

Pesticides and quercetin treatment: Isolated lymphocytes (2 x 10^6 cells) were mixed with 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml, QNP and CPF and incubated for 1 h, 3 h and 6 h (5% CO_2, 37°C) to perform does- and time- response study. Effect of quercetin was observed by giving co-treatment (**30** µg ml^{-1}) with different doses of pesticides.

Estimations

MTT Assay

Cell viability and cytotoxicity test was performed by the method of Mosmann [22]. In a 96 well culture plate, 2 × 10^6 cells per well were taken and incubated with different concentrations of QNP and CPF, in a total volume of 200 µl for 1 h, 3 h and 6 hat 37^0 C in a CO_2 incubator at 5% CO_2. Ten µl MTT solution (5mg/ml) was added in each well and incubated for 1 h at 37^0 C in the CO_2 incubator. After incubation the MTT solution and media was decanted by inverting the microtiter plate. In control wells, equal volume of RPMI was added. The crystals of formazon formed were dissolved by adding 100 µl dimethylsulphoxide**.** Absorbance was measured in ELISA reader at 570 nm and 630 nm and the difference $A_{570} - A_{630}$ was noted and was compared with control.

Lactate dehydrogenase (LDH)

LDH was estimated in the culture supernatant obtained after centrifugation of the treated lymphocytes by the method of Martinek [24]. In a reaction mixture of 1 ml, sodium phosphate buffer (0.1 M, pH 7.5), sodium pyruvate (0.1 M), 50 µl culture supernatant were added in each tube, mixed and the reaction was started by addition of NADH (2 mg/ ml). The absorbance change at 340 nm was measured for 4 min at every 30 sec interval. LDH activity was calculated using molar extinction coefficient of NADH as 6.3 x 10^{-3} M^{-1} cm^{-1}. LDH activity is expressed as nmole NADH oxidized min^{-1} ml^{-1} culture supernatant.

ATP

ATP estimation in the cell homogenate was performed as per the manufacturer's protocol using luminescence based assay kit obtained from Cayman Chemicals. The ATP level is expressed as fmole/ 0.2 million cells.

Acetylcholinesterase (AChE)

AChE activity in lymphocytes was measured by the method of Ellman [25]. Cells were harvested by centrifugation at 5000 g for 5 min at 4°C, sodium phosphate buffer (0.1 M, pH 7.0) was added and the cells were sonicated for 30 sec. For estimation of AChE activity, 2.6 ml sodium phosphate buffer, 0.15 ml DTNB (10 mM), 0.1 ml cell homogenate, were taken in a tube and the reaction was initiated by the addition of 0.15 ml acetylthiocholine iodide (12.5 mM), mixed and absorbance change per min was recorded for 5 min at 412 nm. The activity is calculated by using molar extinction coefficient of adduct formed as 13600 M^{-1} cm^{-1}. The results are expressed as nmole adduct formed min^{-1} per million cells.

Statistical Analysis

Results are expressed as mean ± SE of four set of observations. Statistical analyses were performed using EZANOVA statistical software. All the statistical analyses were performed using one-way analysis of variance with *post hoc* Bonferroni's multiple comparison test applied across the treatment groups. Significance is based on $p < 0.05$.

IV. RESULTS AND DISCUSSION

Pesticides induced cytotoxicity in cultured rat blood lymphocytes *in vitro* and protective effect of quercetin

The MTT assay, a widely used method for screening various compounds for their cytotoxicity in *in vitro* cell culture studies is carried out to evaluate the cytotoxicity of quinalphos (QNP) and chlorpyrifos [CPF], in cultured rat peripheral blood lymphocytes. Different concentrations viz., 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP and CPF were incubated with freshly isolated rat peripheral blood lymphocytes for 1 h, 3 h and 6 h followed MTT assay. The results showed a dose-dependent decrease in reduction of MTT dye indicative of loss of viable cells at all the three time points. When the lymphocytes were given 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP exposure for 1 h, the decrease observed was 13%, 27%, 37% and 57%, respectively, while the same exposure when given for 3 h, the decrease observed was 19%, 28%, 41% and 56%; and 30%, 33%, 4% and 63%, respectively, in the samples given 6 h exposure. When the lymphocytes were incubated with 10 µg, 20 µg, 40 µg and 80 µg/ ml CPF, the decrease observed was 6%, 15%, 24% and 35% in 1h; 3%, 19%, 34% and 44% in 3 h and 17%, 27%, 53% and 60% in 6 h, respectively. Co-treatment of quercetin with all the doses of either of the pesticides, showed protection against cytotoxicity and the decrease remained 22%, 25%, 28% and 53% in lymphocytes treated with 10 µg/ ml QNP for 1 h, 13%, 30%, 37% and 53% in lymphocytes treated with 20 µg/ ml while the lymphocytes treated for 6 h showed 30%, 30%, 40% and 43% decrease, respectively, in the absorbance when compared with only QNP treated samples. Co-treatment of quercetin with different doses of CPF also showed protection and the decrease observed was nil, 12%, 15% and 29% in 1 h, 6%, 6%, 28% and 44% in 3 h and 10%, 23% 52% and 57% in 6 h exposure with 10 µg, 20 µg, 40 µg and 80 µg/ ml CPF, respectively (Table 1).

Pesticides induced LDH leakage in cultured rat blood lymphocytes *in vitro* and protective effect of quercetin

Lactate dehydrogenase (LDH) leakage in culture medium is a very common indicator used for measurement of loss of membrane integrity of the cultured cells in response to exposure with cytotoxic chemicals. Results showed increased LDH activity in the culture supernatant of rat peripheral blood lymphocytes cultured in RPMI medium on exposure with 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP and CPF, for 1 h, 3 h and 6 h. A dose-dependent increase in LDH activity was observed in the culture medium when the lymphocytes were exposed with different concentrations of QNP or CPF. The increase in LDH activity was 26%, 71%, 165% and 188% in the culture supernatant of lymphocytes given exposure of 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP, respectively, for 1 h while 3 h exposure with the same concentrations of QNP caused 69%, 137%, 175% and 232% increase in LDH activity when compared with respective controls. When the same doses of QNP were used for 6 h, the increase observed in LDH activity were 142%, 169%, 190% and 279% when

compared with control (Table 2). When the cultured lymphocytes were exposed with 10 µg, 20 µg, 40 µg and 80 µg/ ml CPF for 1 h, 3 h and 6 h, the increase in LDH activity was observed was 13%, 44%, 50% and 156%; 78%, 87%, 192% and 308%; and 33%, 65%, 227% and 316%, respectively, when compared with control. Co-treatment of quercetin with QNP or CPF significantly decreased LDH leakage in culture medium at all the doses. The increase in LDH activity in culture medium remained 14%, 68%, 103% and 125% in lymphocytes given exposure of 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP along with QNP for 1 h while 3 h co-treatment caused 60%, 74%, 138% and 219% and 6 h co-treatment showed 99.7%, 126%, 185% 280% increase, respectively. Co-treatment of CPF and quercetin also decreased LDH leakage at all the doses and all the three time points. The increase remained 6%, 38%, 48% and 87% in 1 h; 53%, 77%, 188% and 320% in 3 h; and 24%, 38%, 204% and 268% in 6 h, respectively (Table 2). The decrease in LDH activity on quercetin co-treatment at all the doses of QNP or CPF indicates the protection from cytotoxicity.

Pesticides induced alterations in the level of ATP in cultured rat blood lymphocytes *in vitro* and protective effect of quercetin

Intracellular levels of ATP are decreased at the time of cell death in *in vivo* or *in vitro* systems. In the present study, the results showed depletion of intracellular levels of ATP on exposure of different doses of QNP and CPF for different period of time. On 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP exposure for 1 h, the decrease observed was 3%, 8%,13% and 19%, respectively, while 3 h and 6 h exposure caused 15%, 8%, 11% and 18%; and 13%, 17%, 21% and 24%, respectively, when compared with respective control. Treatment with CPF also caused dose- as well as exposure time-dependent decrease in the levels of ATP in the cultured lymphocytes. The decrease observed was 8%, 16%, 19% and 27% in 1 h; 17%, 28%, 29% and 34% in 3 h; and 21%, 26%, 33% and 45% in 6 h when exposure of 10 µg, 20 µg, 40 µg and 80 µg/ ml CPF, respectively, was given. Quercetin co-treatment with QNP had not shown any recovery of ATP levels in lymphocytes; however, the same co-treatment with CPF showed significant elevation in ATP levels when compared with only CPF treatment group. The decrease in ATP levels remained 2%, 6%, 17% and 22% in 1 h; 9%, 15%, 27% and 31% in 3 h and 15%, 20%, 25% and 39% in 6 h CPF + quercetin treated samples (Table 3).

Pesticides induced alterations in the activity of acetylcholinesterase (AChE) in cultured rat blood lymphocytes *in vitro* and protective effect of quercetin

OP compounds are irreversible inhibitors of cholinesterases including AChE and thus are neurotoxic in nature. Thus, AChE activity can be considered as marker of OP pesticide exposure. Present study has shown that both QNP and CPF inhibited AChE activity in lymphocytes cultured *in vitro* for different periods of time. A dose-dependent inhibition was observed in AChE activity on exposure with 10 µg, 20 µg, 40 µg and 80 µg/ ml QNP; the inhibition ranged from 5%- 23% in 1h; 13%-35% in 3h and 16%-37% in 6 h in rat lymphocytes. When the lymphocytes were exposed with the same concentrations of CPF, the inhibition of AChE activity ranged from 8%- 27% in 1 h; 17%- 34% in 3 h and 22%-45% in 6 h, respectively. Quercetin co-treatment with pesticides, caused marginal recovery of AChE activity and the inhibition remained 0%- 20% in 1 h, 9%- 25% in 3 h and 10%- 35% in 6 h in QNP treated group, when compared with respective control while co-treatment with CPF + quercetin caused 2%-22% inhibition in 1 h; 9%-31% in 3 h and 15%- 39% in 6 h, respectively when compared with control (Table 4).

DISCUSSION

The widespread use of pesticides in public health and agricultural programs has caused severe environmental pollution and health hazards, including cases of severe acute and chronic human poisoning. The introduction of new, more toxic and rapidly disseminating pesticides into the environment has necessitated accurate identification of their potential hazards to human health so that effective therapeutic strategies could be planned. Free radicals play an important role in the toxicity of pesticides and environmental chemicals. Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation. OP compounds are known to inhibit AChE activity in neural and non-neural tissues. Dose-dependent inhibition of AChE activity has been observed in lymphocytes exposed to QNP and CPF *in vitro* in the present study. Anticholinesterase activity of OP compounds can be taken as marker for evaluating their exposure. Results of the present study showed that in *vitro* exposure of both QNP and CPF induced cytotoxicity in rat lymphocytes. The dose-dependent decrease in reduction of MTT, indicative of loss of viable cells, has been observed with both the pesticides. The results also showed that longer duration of pesticide exposure is more toxic to the cells and cytotoxicity of both the pesticides is time-dependent. The OP pesticides induced cytotoxicity in different cell types, has been reported earlier also [26], [27]. Another important parameter used for assessment of plasma membrane integrity is the release of cytosolic enzyme LDH, in the culture medium. Present study showed the leakage of LDH in the culture medium from lymphocytes exposed to different doses of QNP or CPF *in vitro*. Both these assays indicate damage of the cell membrane in response to pesticide exposure indicating the cell-death induction. It is likely that these pesticides may induce necrosis in the exposed cells which is characterized by swelling and rupture of intracellular organelles, eventually leading to plasma membrane leakage and breakdown [28], [29], [30]. Thus, necrosis evokes inflammatory responses and is closely associated with inflammatory diseases [31]. In contrast to apoptosis, another mechanism of cell death, which is a programmed event, and the cells undergo shrinkage before death.

Results showed depletion of ATP levels on exposure with either QNP or CPF in dose-dependent manner. Decrease in ATP levels indicate disturbance in mitochondrial integrity, the main site of ATP production. ATP depletion, increase in ADP/ ATP ratio and reduced mitochondrial trans-membrane potential leading to necrotic cell death has also been shown in pheochromocytoma, PC12 cells in response to mevinphos exposure [32]. Although the preliminary study indicates cytotoxicity yet more evidences are required to confirm the mechanism of cell death as many studies reported that OP-pesticides induce apoptosis [33], [34], [35].

Many studies proved that the mechanism of the toxicity of pesticides, drugs as well as some other chemicals compounds may be attributed to their capacity to generate oxidative stress in the body. Therefore, number of studies has been carried out to evaluate the protective effect of natural antioxidants against toxicity induced by variety of chemicals and pollutants [36], [37], [38]. Quercetin, a plant flavonoid widely distributed in different fruits and vegetables, is known to possess antioxidant activity through ROS scavenging besides other pharmacological activities [39], [40], [41]. Results of the present study showed that co-treatment of quercetin with QNP and CPF reduced toxicity to a greater extent at every dose when compared with corresponding pesticide treated groups. The results clearly showed quercetin offered partial protection against OP pesticide induced cytotoxicity, reduced LDH release in culture medium, tend to restore ATP levels and reduced the level of inhibition of AChE when given co-treatment with pesticides *in vitro.* Quercetin mediated protection against DAVV-induced cell death of HCT116 cells *in vitro* [42], against mixture of OP pesticides in rats [43], and dimethoate induced oxidative stress in human peripheral blood lymphocytes [44] has been reported.

Table 1. Dose- and time- dependent cytotoxic response of rat peripheral blood lymphocytes given *in vitro* exposure of QNP and CPF and effect of quercetin

		1 h		3 h		6 h	
	Conc (µg ml^{-1})	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**
1.	**Con**	0.32±0.03	0.33±0.03	0.30±0.01	0.32±0.02	0.30±0.01	0.30±0.01
2.	**QNP 10**	0.26±0.01$^{\neq}$	0.25±0.01$^{*,\neq}$	0.26±0.01**	0.26±0.06$^{\neq,a\neq}$	0.21±0.02**	0.21±0.01^{***,a***}
3.	**QNP 20**	0.22±0.08*	0.24±0.01$^{*,a\neq}$	0.23±0.03*	0.21±0.01^{**,a***}	0.20±0.02***	0.21±0.02^{**,a**}
4.	**QNP 40**	0.19±0.01**	0.23±0.02$^{*,a\neq}$	0.19±0.02***	0.19±0.03^{**,a**}	0.18±0.01***	0.18±0.01^{***,a***}
5.	**QNP 80**	0.13±0.01***	0.15±0.04^{**,a*}	0.14±0.01***	0.14±0.02^{***,a***}	0.11±0.01***	0.17±0.01^{***,a***}
6.	**Con**	0.34±0.02	0.35±0.04	0.32±0.01	0.34±0.04	0.30±0.01	0.30±0.01
7.	**CPF 10**	0.32±0.01$^{\neq}$	0.34±0.03$^{\neq,a\neq}$	0.31±0.03$^{\neq}$	0.34±0.01$^{\neq,\ a*}$	0.25±0.01*	0.27±0.03$^{\neq,a\neq}$
8.	**CPF 20**	0.29±0.01*	0.30±0.05$^{\neq,a\neq}$	0.26±0.03$^{\neq}$	0.30±0.01$^{\neq,a**}$	0.22±0.04$^{\neq}$	0.23±0.05$^{\neq,a\neq}$
9.	**CPF 40**	0.26±0.02**	0.29±0.02$^{\neq,\neq}$	0.21±0.01***	0.23±0.04$^{\neq,a\neq}$	0.14±0.01***	0.14±0.01^{***,a***}
10.	**CPF 80**	0.22±0.01***	0.24±0.01^{*,a**}	0.18±0.01***	0.18±0.01$^{**,\ a***}$	0.12±0.02***	0.13±0.01^{***,a***}

Results are expressed as ΔA of $A_{570} - A_{630}$.
Results are mean ± SE of four set of observations.
P values *<0.05, ** < 0.01, ***<0.001 and $^{\neq}$> 0.05 when compared with respective control, a when compared with quercetin untreated control.
In vitro QNP (quinalphos) or CPF (chlorpyrifos) exposure was given to the lymphocytes in the final concentration of 10 µg, 20 µg, 40 µg and 80 µg ml^{-1} of medium and co-treatment of 30 µg ml^{-1} quercetin was given along with different concentrations of pesticides.

Table 2. QNP and CPF induced lactate dehydrogenase leakage in culture medium of rat peripheral blood lymphocytes and effect of quercetin

		1 h		3 h		6 h	
	Conc (µg ml^{-1})	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**
1.	**Con**	40.47±9.82	38.68±5.95	41.66±1.19	40.33±1.60	42.32±2.62	42.66±0.82
2.	**QNP 10**	51.18±4.07$^{\neq}$	42.22±4.21$^{\neq,a\neq}$	70.24±7.87**	66.66±12.45$^{\neq,a\neq}$	102.2±6.81***	84.5±7.38^{***,a**}
3.	**QNP 20**	69.04±1.37*	67.85±14.73$^{\neq,a\neq}$	98.90±9.79***	72.61±3.00$^{***,\ a***}$	113.8±5.07***	95.50±5.66^{***,a***}
4.	**QNP 40**	107.14±26.19*	82.14±9.60^{**,a*}	114.58±10.32***	99.10±4.57$^{***,\ a***}$	122.6±5.42***	120.6±1.43^{***,a***}
5.	**QNP 80**	116.66±15.73**	91.07±15.64^{**,a**}	138.26±1.65***	132.99±2.84$^{***,\ a***}$	160.5±11.13***	160.7±1.50^{***,a***}
6.	**Con**	38.09±5.14	38.68±5.95	41.66±1.19	40.58±1.36	42.32±2.62	42.66±0.82

7.	**CPF 10**	42.85±0.01$^{\neq}$	40.47±2.38$^{\neq,a\neq}$	74.17±2.19***	63.57±5.120^{**,a**}	56.19±3.37***	52.76±16.43^{*,a*}
8.	**CPF 20**	54.76±4.13*	52.37±5.50$^{\neq,a\neq}$	77.98±7.16**	73.80±3.07^{***,a***}	69.61±12.35***	58.57±31.91$^{\neq,a\neq}$
9.	**CPF 40**	57.14±5.14*	56.31±19.48$^{\neq,a\neq}$	121.43±5.67***	119.87±3.25^{***,a***}	138.57±1.37***	128.73±29.96^{***,a***}
10.	**CPF 80**	97.62±4.56***	72.02±13.93^{**,a**}	170.12±10.98***	175.00±8.33^{***,a***}	175.95±18.23***	155.59±34.60^{***,a***}

Lactate dehydrogenase activity is expressed as nmole of NADH oxidized min^{-1} ml^{-1}
Results are mean ± SE of four set of observations.
p values *<0.05, ** < 0.01, *** < 0.001 and $^{\neq}$> 0.05 when compared with respective control, a when compared with quercetin untreated control.
In vitro QNP (quinalphos) or CPF (chlorpyrifos) exposure was given to the lymphocytes in the final concentration of 10 µg, 20 µg, 40 µg and 80 µg ml^{-1} of medium and co-treatment of 30 µg ml^{-1} quercetin was given along with different concentrations of pesticides

Table 3. QNP and CPF induced changes in activity of acetylcholinesteras in rat peripheral blood lymphocytes and effect of quercetin

		1 h		**3 h**		**6 h**	
	Conc (µg ml^{-1})	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**
1.	**Con**	0.77±0.02	0.80±0.02	0.68±0.01	0.71±0.01	0.70±0.01	0.73±0.06
2.	**QNP 10**	0.73±0.05$^{\neq}$	0.73±0.08$^{\neq,a\neq}$	0.59±0.05$^{\neq,a*}$	0.62±0.02$^{*,a\neq}$	0.59±0.05$^{\neq}$	0.60±0.04$^{\neq,a*}$
3.	**QNP 20**	0.65±0.03*	0.69±0.06$^{\neq,a\neq}$	0.47±0.07^{*,a**}	0.50±0.05^{**,a*}	0.47±0.07*	0.54±0.01^{*,a***}
4.	**QNP 40**	0.58±0.03**	0.65±0.05$^{*,a\neq}$	0.49±0.02$^{*,a\neq}$	0.52±0.08$^{\neq,a\neq}$	0.49±0.02***	0.50±0.10$^{\neq,a\neq}$
5.	**QNP 80**	0.59±0.07*	0.63±0.06$^{\neq,a\neq}$	0.44±0.07^{*,a***}	0.48±0.01^{***,a**}	0.44±0.07**	0.45±0.03^{**,a***}
6.	**Con**	0.77±0.02	0.80±0.02	0.68±0.04	0.71±0.01	0.70±0.01	0.73±0.06
7.	**CPF 10**	0.66±0.01***	0.76±0.01$^{\neq,a\neq}$	0.63±0.01*	0.65±0.01$^{\neq,a*}$	0.52±0.06*	0.54±0.01^{*,a***}
8.	**CPF 20**	0.56±0.01***	0.61±0.03^{**,a**}	0.53±0.01$^{\neq}$	0.55±0.01$^{\neq,a*}$	0.42±0.01***	0.43±0.01^{**,a***}
9.	**CPF 40**	0.54±0.01***	0.50±0.03^{***,a***}	0.51±0.05**	0.51±0.01$^{\neq,a***}$	0.34±0.01***	0.38±0.03^{**,a***}
10.	**CPF 80**	0.52±0.03***	0.49±0.02^{***,a***}	0.46±0.03***	0.47±0.03$^{\neq,a***}$	0.29±0.01***	0.29±0.01^{***,a***}

Acetylcholinesterase activity is expressed as nmole of adduct formed min^{-1} $million^{-1}$ cells
Results are mean ± SE of four set of observations.
p values *<0.05, ** < 0.01, *** < 0.001 and $^{\neq}$> 0.05 when compared with respective control, a when compared with quercetin untreated control.
In vitro QNP (quinalphos) or CPF (chlorpyrifos) exposure was given to the lymphocytes in the final concentration of 10 µg, 20 µg, 40 µg and 80 µg ml^{-1} of medium and co-treatment of 30 µg ml^{-1} quercetin was given along with different concentrations of pesticides.

Table 4. QNP and CPF induced changes in the levels of ATP in rat peripheral blood lymphocytes and effect of quercetin

		1 h		**3 h**		**6 h**	
	Conc (µg ml^{-1})	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**	**Pesticide**	**Pesticide+ Quercetin**
1.	**Con**	263.62±7.61	271.98±8.64	272.43±10.45	257.04±7.66	279.48±2.22	281.30±1.03
2.	**QNP 10**	256.04±9.52$^{\neq}$	270.81±9.00$^{\neq,a\neq}$	231.02±4.65**	245.50±6.83$^{\neq,a\neq}$	241.90±1.02***	255.26±6.32^{**,a**}
3.	**QNP 20**	243.59±5.48$^{\neq}$	215.51±5.36$^{**,a\neq}$	250.38±3.80$^{\neq}$	241.84±9.07$^{\neq,\neq}$	231.34±2.12***	225.52±0.35^{***,a***}
4.	**QNP40**	230.51±3.90**	255.74±7.51$^{\neq,a**}$	243.80±9.82$^{\neq}$	220.94±3.07^{**,a**}	220.39±3.63***	220.84±12.14^{**,a**}
5.	**QNP 80**	214.78±4.73**	217.44±2.41$^{***,a\neq}$	224.02±4.40**	177.67±3.80^{***,a***}	211.32±7.87***	211.52±7.54^{***,a***}
6.	**Con**	263.62±3.51	271.98±8.64	278.96±9.49	281.47±4.28	272.43±10.45	257.04 ±16.83
7.	**CPF 10**	243.77±7.81$^{\neq}$	259.07±13.36$^{\neq,a\neq}$	232.17±7.66**	253.27±2.15^{***,a*}	213.56±3.17**	232.35±15.13$^{\neq,a\neq}$
8.	**CPF 20**	221.27±7.50**	246.81±10.38$^{\neq,a\neq}$	218.47±13.82**	236.91±12.08^{**a*}	202.09±1.99***	217.53±7.24$^{\neq,a**}$
9.	**CPF 40**	213.20±4.86***	219.55±8.96$^{**,a\neq}$	197.77±15.68**	204.49±2.15^{***,a***}	183.08±8.58***	203.93±18.41$^{\neq,a*}$
10.	**CPF 80**	192.20±9.39***	205.60±15.41^{**,a**}	184.21±6.26***	191.66±2.96^{***,a***}	149.36±21.77**	165.14±3.42^{**,a***}

ATP levels are expressed as fmole 0.2 million cells
Results are mean ± SE of four set of observations.
p values *<0.05, ** < 0.01, *** < 0.001 and $^{\neq}$> 0.05 when compared with respective control, a when compared with quercetin untreated control.
In vitro QNP (quinalphos) or CPF (chlorpyrifos) exposure was given to the lymphocytes in the final concentration of 10 µg, 20 µg, 40 µg and 80 µg ml^{-1} of medium and co-treatment of 30 µg ml^{-1} quercetin was given along with different concentrations of pesticides.

V. CONCLUSION AND FUTURE SCOPE

It can be concluded from the present study that both QNP and CPF are cytotoxic to the rat peripheral blood lymphocytes and that QNP is more toxic than CPF. It is observed that quercetin, the common antioxidant flavonoid, present in fruits and vegetables partially protects lymphocytes from QNP or CPF-induced cell death. In relation to the public health, it may be recommended to consume food rich in antioxidants including flavonoid, quercetin since there is always a possibility of pesticide contamination in the food and water which we consume, besides being other health benefits of this flavanoid.

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