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Genoprotective Effects of Quercetin against Genotoxic Damage in Peripheral Blood Cells of Healthy Individuals and Tuberculosis Patients

Abdulmwli M[1], Dandah MO[1], Mehwish F[1], Gazala L[1], Mojgan N[1], Jacobs B[3], Isreb M[2], Anderson D[1], Habas K[1#] and Gopalan RC[2*#]

[1]School of Chemistry and Biosciences, University of Bradford, UK
[2]School of Pharmacy and Medical Sciences, University of Bradford, UK
[3]Bradford Teaching Hospitals, BRI, UK

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***Corresponding authors:** Rajendran Gopalan, School of Pharmacy and Medical Sciences, Faculty of Life Sciences, University of Bradford, Bradford, Richmond Road, West Yorkshire, BD7 1DP, UK, Email: r.c.gopalan@bradford.ac.uk

Khaled Habas, School of Chemistry and Biosciences, Faculty of Life Sciences, University of Bradford, Bradford, Richmond Road, West Yorkshire, BD7 1DP, UK; Email: K.habas@bradford.ac.uk

[#]These two authors contributed equally to this work

Abstract

Tuberculosis (TB) is a bacterial infection caused by the bacterium Mycobacterium tuberculosis (Mtb) and has long been one of the leading causes of death worldwide. Flavonoids are among the most thoroughly researched natural compounds found in plants. They have been widely recognized for their diverse medicinal properties, including antimicrobial, antioxidant, anti-inflammatory and anticancer. Flavonoids have antioxidant properties and have been shown to exert anti-pathogenic potential, but few studies have examined their effects on Mycobacterium tuberculosis (Mtb) infection. The aim of the current study was to investigate in vitro the protective effect of the flavonoid, quercetin on hydrogen peroxide (H_2O_2) -induced oxidative DNA damage, using the Comet assay and the micronucleus (MN) test in peripheral lymphocytes from tuberculosis patients (TB) and compared with lymphocytes from healthy individuals. Quercetin significantly reduced DNA damage and reduction in the frequency of micronuclei in TB patient compared with healthy individuals. These findings show that the nano-quercetin has genoprotective and enzyme modulating effects and highlight its potential as a possible therapeutic supplement for TB patients.

Keywords: DNA Damage; Lymphocytes; Oxidative Stress; Quercetin Nanoparticles

Abbreviations

TB: Tuberculosis Patients; MTB: Mycobacterium Tuberculosis; ROS: Reactive Oxygen Species.

Introduction

Tuberculosis (TB) is an infectious disease caused by bacilli bacteria named Mycobacterium tuberculosis (Mtb).

Though treatable, approximately 1.5 million people died worldwide due to TB infection in 2020. Current treatment of TB requires lengthy treatment regimens with undesirable side effects. Tuberculosis is among the most important inflammatory condions affecting the lungs and has a more overwhelming role in the emergence of cancer. Moreover, chronic inflammation due to TB can also produce genetic mutation and alternations [1].

The Mycobacterium is known to stimulate production of free radicals in the host by triggering numerous defence mechanisms that result in oxidative stress [2]. The lung has developed various biological mechanisms, including oxidative stress responses, to reduce TB infection. M. tuberculosis infection triggers the generation of reactive oxygen species (ROS) intermediates from the host phagocytic cells that can cause DNA damage [3]. ROS can oxidize a series of cellular targets, including lipids and proteins. These targets activate a series of biological processes, such as DNA damage response and inhibition of apoptosis, leading to tissue damage [4]. Oxidative stress triggers the synthesis of mediators of pulmonary inflammation in lung tissues and stimulates carcinogenic mechanisms [5].

The synergistic effects of breathable particles contribute to oxidative stress, as these particles possess high carcinogenic potential and can increase the production of pulmonary inflammatory mediators, leading to oxidative damage across key cellular components such as membrane lipids, proteins, and DNA [6]. Genomic instability, characterized by a heightened frequency of genetic alterations in cells, is a crucial factor in mutagenesis and the genetic changes linked to carcinogenesis. One method to assess genomic instability is by measuring the frequency of chromosomal alterations induced by mutagens. The micronucleus (MN) in dividing cells forms when chromosome breakage occurs due to unrepaired or improperly repaired DNA lesions. Additionally, chromosomal missegregation resulting from mitotic errors can also lead to the formation of an MN [7].

Flavonoid is antioxidants substances that may protect cells from the damage caused by unstable molecules such as free radicals. The function of flavonoids as an antimicrobial potentiator has been related to the regulation of the activities of different proteins and molecular processes. Quercetin is antibacterial against a wide range of bacterial strains, particularly those affecting the respiratory systems [8].

In the present study we investigated the genoprotective effect of the nanoform of antioxidant compound quercetin, its abilities to regulate the development of inflammation and activate apoptosis to speed up the recovery among TB patients, using the ex vivo model system. Quercetin reduced DNA damage and reduction in the frequency of micronuclei in TB patient compared with healthy individuals. Thus, our data indicates that nano-quercetin could be developed further as a potential anti- cancer and significant biological activity against inflammatory disorders such as TB.

Materials and Methods

Ethical Approval

Ethical approval was obtained from Leeds East Research Ethics Committee (REC number: 12/YH/0464), the University of Bradford Research Ethics Sub-Committee on Research in Human Subjects (Ref: 0405/8) and the Research Support and Governance office, Bradford Teaching Hospitals, NHS Foundation (Ref: RE DA 1202). All peripheral blood samples were collected after informed consent from TB patients and healthy individuals.

Preparation of Quercetin Bulk and Nano Particles

Quercetin with CAS number 117-39-5 (>98% purity) was purchased from Fisher Scientific, UK. The preparation of a nanoform of quercetin involved dissolving bulk quercetin in ethanol to prepare a concentration of 1mg/ml and 5ml from this preparation was injected through one side of the channel of Microfluidic reactors (Y shape) which was used to prepare a nano form of quercetin. The other side of Microfluidic reactors was used to inject 20 ml of water at the rate of 15 ml/h and 60 ml/h respectively. The production was collected in a beaker of 50ml capacity containing 0.5 % w/w hydroxypropyl methylcellulose (HPMC) and poly vinylpyrrolidone (PVP) K-30 (0.5% w/w) and 1% sodium dodecyl sulphate (SDS). The Malvern nano sizer DLS was used to measure the particle size. The suspensions were also sonicated for 10 min before each use to avoid sedimentation and control aggregation. The concentrations of quercetin in both forms used in this study were selected from the previous study [9].

Determination of Cytotoxicity by MTT Assay

The cytotoxicity of chemicals was evaluated using MTT (3-(4, 5-dimethyl thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) method. Isolated lymphocytes were seeded at a density of 10,000 cells/well in a 96-well plate and incubated at 37°C and preincubated for 24 h. The quercetin nano and bulk form at the final working concentrations of 10, 25, 100µM was added to test wells. Cell free media containing complete RPMI were also run in parallel to test and control groups. 10 µl MTT was added to each well, and plates were further incubated at 37°C for 4 h. Finally, 200 µl of DMSO were added into each well. Absorbance was then measured at 550 nm. The cell survival percentages were calculated

from absorbance of drug concentrations divided by negative control absorbance and multiplied by100. Viability was also measured by the trypan blue exclusion test indicating intact cell membranes.

Comet Assay

Isolated lymphocytes were treated with nano and bulk quercetin (10, 25, 100µM) in combination with H_2O_2 (60µM) for 30 minutes at 37°C. The cell suspension was centrifuged at 3000rpm (1000g). The supernatant was discarded, and the cell pellets were subjected to the Comet assay as previously described [10].

Micronucleus Assay

Fresh blood samples from healthy individuals and TB patient groups were suspended in RPMI 1640 medium containing 1% of penicillin-streptomycin and 15% Foetal bovine serum (FBS), 25mM HEPES, L-Glutamine and phytohaemagglutinin (PHA) in 25 cm^3 culture flasks at 37 °C and 5% CO_2 in a humidified incubator for 24h.

After 24 h, the lymphocytes from healthy individuals and TB patients were treated with different concentrations of nano and bulk quercetin (10, 25, 100µM). 50µl of mitomycin C (0.4µM) was added as the positive control. The CBMN assay was performed using cytochalasin B (Cyt-B) as described elsewhere [9].

Statistical Analysis

Each experiment was performed at least three times, the results were presented as mean ± standard error of the mean (SEM). The results were analyzed by one-way and two-way analysis of variance (ANOVA) using Graph Pad prism 7 software and value of $p \leq 0.05$ was considered significantly different.

Results

Cytotoxicity of nano and bulk quercetin forms at different concentrations (10 µM, 25 µM, 100 µM) was measured using the MTT assay. The average of three experiments showed non- significant level of cytotoxicity which was less than 6% compared to the untraded cells. The viability of lymphocytes from healthy individuals and TB patients treated with different treatments was confirmed higher than 80% after 24 h treatment (Figure 1).

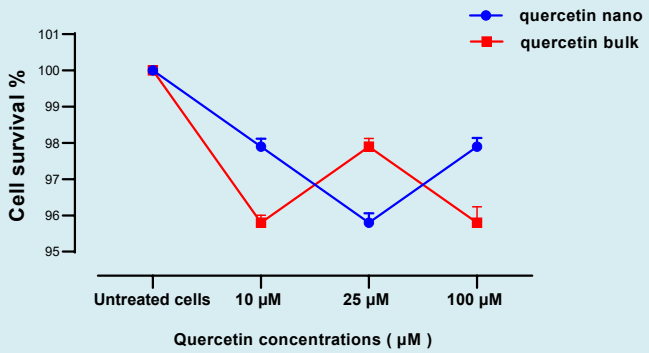

Figure 1: Graph showing the means of cell survival using MTT test of lymphocytes collected from three different healthy individuals treated with quercetin at different concentrations (donors=3).

Effect of Nano and Bulk Quercetin Forms on H_2O_2-Induced DNA Damage in Lymphocytes from Healthy Vs TB Patients Using the Comet Assay

The effect of nano and bulk quercetin forms on isolated lymphocytes from healthy individuals and TB patients was examined using the Comet assay. Results from Olive tail moment (OTM) and % tail DNA showed a significant decrease in DNA damage comparable to the positive control 60 µM of H_2O_2 (**p<0.01) and (***p<0.001) respectively. There is a non- significant effect of three concentrations of nano and bulk quercetin forms in lymphocytes from healthy individuals and TB patients (Figure 2A & 2B).

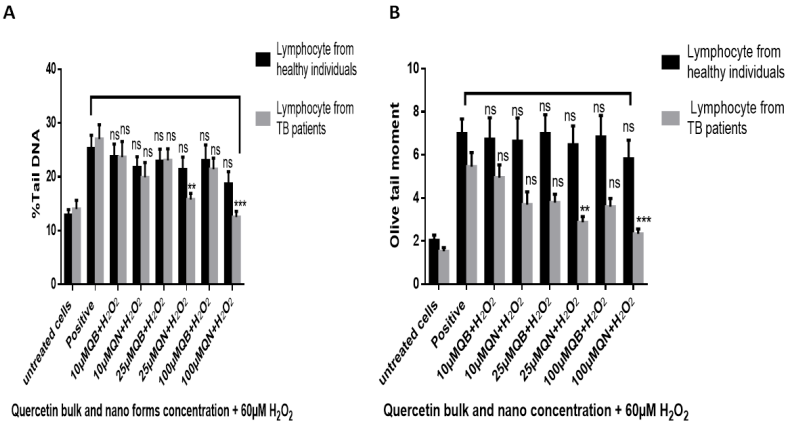

Figure 2A & 2B: DNA damage measured as OTM and % Tail DNA showing the effect of quercetin nano and bulk in lymphocytes DNA from healthy individuals and TB patients in the presence of 60 µM of H_2O_2. ns = not significant, **($p \leq 0.027$), *** ($p \leq 0.001$), (n= 10).

Cytokinesis-Block Micronucleus (Cbmn) Assay

We next examined the effect of nano and bulk quercetin on MNi levels using the cytokinesis-blocked assay in lymphocytes from health individuals and TB patients. Both forms of quercetin have showed no-significant effect in the number of MNi in binucleated cells of healthy individuals when compared to untreated cells. The positive control groups, 0.4 µM mitomycin (MMC) and bleomycin (0.75µ/ml) have induced a significant increase in the number of MNi in BiNC in the healthy. However, in combination of both form of quercetin at 25 and 100µM showed a significant reduction in the number of bleomycin induced MNi in BiNC of healthy individuals compared to 0.75µ/ml of bleomycin ***P ≤ 0.001 (Table 1). Table 2 illustrates the comparative genoprotective effect of both forms of quercetin in bleomycin treated lymphocytes from TB patients. Overall, the number of MNi in BiNC from TB patients was significant decrease at 10 µM *P ≤ 0.05. Further decreased was shown when treated with concentrations at 25 and 100µM ***P ≤ 0.001. There are numerous cytological scoring parameters, including biomarkers of cell mitotic division such as mononucleated cells (MoNC), binucleated cells (BiNC), and multinucleated cells (MultiNC). From these parameters, the NDI was calculated for healthy individuals and TB patients. The mean values for the NDI for all treatments were within the normal range (Tables 1 and 2).

Subject	Untreated Cells	PC	Bleomycin	10µMQB	10µMQN	25µMQB	25µMQN	100µMQB	100µMQN
Mon%	50.5	59	53	55	58.5	57	55	55.5	55.3
Bio %	44	34.2	44	39	36.5	45	41	40.9	41.3
Multi %	3.8	4.8	7.3	4.8	3.7	3.1	3.3	3.2	3.1
NID	1.5	1.5	1.5	1.5	1.4	1.47	1.49	1.47	1.48
Mni P Value	1	15	15.5	9.5	2	5	2	2	1
	***	ns	--	ns	***	***	***	***	***
Bio-Buds	6	10	8.5	9.5	11	11.5	8	4.5	2
Bio-NPBS	1	4	3.5	6	1	4	1	1.5	1
Total cytogenetic damage	8	29	27.5	24	14	20.5	11	8	4

Table 1: The mean of different cytological parameters from the micronucleus assay for lymphocytes from healthy individuals treated with bulk and nano forms of quercetin represented by Mon-nucleated%, Bi-nucleated %, nuclear division index, MNi micronucleus cells, Bi-nucleated Buds, Bi-NPBs and total cytogenetics damage. The results expressed over 500 cells each slide scored, ns = not significant, *** ($p \leq 0.001$), (n=3).

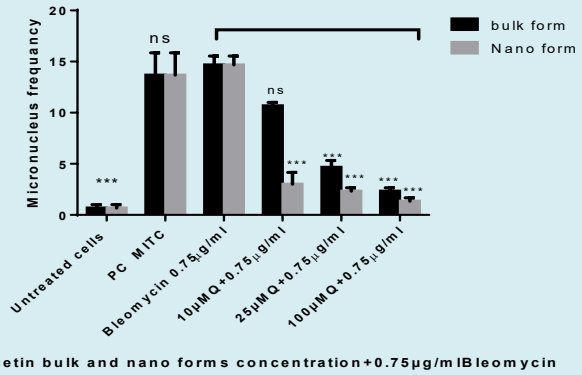

Figure 3A: A comparison between MN frequencies in lymphocytes collected from healthy individuals treated with quercetin bulk and nano forms with standard errors and significance value (n=3). ns = not significant, *** ($p \leq 0.001$).

Subject	Untreated cells	PC	Bleomycin	10µMQB	10µMQN	25µMQB	25µMQN	100µMQB	100µMQN
Mon%	53.8	53	52.2	60.2	61	64	59.6	57.5	56
Bio %	43.5	41	44.2	38.4	35.8	37.8	40.1	36.7	41
Multi %	2.7.00	6	3.6.00	1.4	3.2	1	1	6.5	3
NID	1.47	1.48	1.48	1.39	1.48	1.38	1.4	1.4	1.43
MNi P value	1.3	14	14.3	11.6	11	11.3	3.3	10.6	1.3
	***	ns	--	*	**	**	***	**	***
Bio-Buds	5.3	7	12	12.6	6.3	10.6	9	7.6	4.3
Bio-NPBS	4.3	4.3	5	4.6	4	3.6	4	4	2.6
Total cytogenetic damage	10.6	25.3	31.3	27.5	17.6	23.5	16.3	17.9	8.2

Table 2: The mean of different of MN frequencies in lymphocytes collected from patient with TB treated with the bulk and nano forms of quercetin represented by Mono-nucleated%, Bi-nucleated %, nuclear division index, MNi micronucleus cells, Bi-nucleated Buds, Bi-NPBs and total cytogenetics damage. The results expressed over 500 cells for each slide scored ns = not significant, *($p \leq 0.0283$), **($p \leq 0.027$), ***($p \leq 0.001$). (n=3).

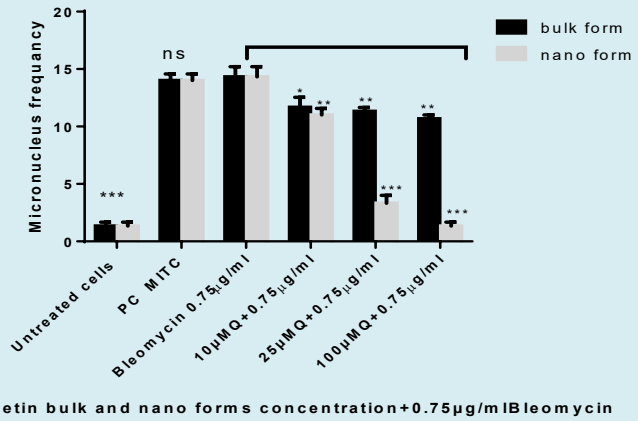

Figure 3B: Comparison between MN frequencies in lymphocytes collected from TB patient treated with quercetin bulk and nano form in the presence of 0.75µg/ml of bleomycin including standard errors and significance, ns = not significant, *($p \leq 0.0283$), **($p \leq 0.027$), ***significance ($p \leq 0.001$), (n=3).

Discussion

Tuberculosis is a chronic intracellular bacterial infection that expected to induce DNA damage in the host through the production of microorganism endotoxins exotoxins, which act as chemical mutagens and produce chromosomal damage in affected cells [11]. During tuberculosis infection, the bacteria are exposed to ROS and reactive nitrogen intermediates generated by the host's immune response, which can lead to DNA damage [12]. The genoprotective effects of quercetin have partly been studied. It has been shown to reduce H_2O_2-induced toxicity in human lymphocytes from patients with inflammatory bowel disease (IBD) [13]. However, information regarding the mechanism of action and the beneficial effect of quercetin remains limited. The effects of quercetin, both in nano and bulk forms, on H_2O_2-induced DNA damage were investigated in human lymphocytes from healthy individuals and tuberculosis (TB) patients.

The Comet assay results showed that quercetin effectively protected against DNA damage in TB human lymphocytes exposed to H_2O_2. Moreover, the highest protective effect was found at a concentration of 100µM of nano-quercetin ***P≤0.001, followed by 25 µM **P ≤ 0.01 as shown H_2O_2 (Figures 2 A and B). This implies that the quercetin prevents H2O2-induced mitochondrial dysfunction by reducing disruptions in mitochondrial membrane permeability transition and suppressing the increased expression of apoptotic proteins, including the inhibition of caspase-3 activity [14,15]. The micronucleus assay was used to investigate the effects of nano and bulk quercetin on chromosomal damage in lymphocytes exposed to bleomycin form both healthy individuals and TB patients. Our data showed a reduction in micronucleus (MNi) frequency in lymphocytes from both groups when treated with nano or bulk quercetin compared to the untreated control cells (NC). Notably, nano quercetin demonstrated a significantly greater reduction in MNi frequency than the bulk form, as shown in Tables 1 & 2, and Figures 3A & 3B.

The key finding of this study is that the nano form of quercetin exhibited higher reduction in DNA damage compared to the bulk form. Nano-quercetin showed better protective effects than its larger particle counterpart, even when used at the same concentrations. This suggests that once a chemical changes from its bulk form to nanoform, the surface area significantly increases, enhancing its immunogenicity, drug delivery efficiency, and solubility. The improved contact between nanoparticles (NPs) and surrounding materials boosts reactivity, contributing to these enhanced properties [16].

Chromosomal aberrations, including chromosome breaks, chromatid breaks, and indicate the genotoxic effects of H_2O_2. A study by Mondal, Pal, and Dey reported the induction of chromosomal aberrations and DNA damage in human peripheral blood lymphocytes as a result of H_2O_2 exposure [17].

In our study, quercetin pre-treatment attenuated H_2O_2-induced DNA damage, demonstrating its anticlastogenic potential. The anticlastogenic activity of quercetin may be attributed to the hydroxyl group at the third position of quercetin, which likely reacts with free radicals, preventing their attack on DNA, and its ability to bind with DNA, thereby blocking free radicals from interacting with and damaging the DNA [17].

Flavonoids are comprehensively studied natural products derived from plants and described for various medicinal properties such as antioxidant antimicrobial and anti-inflammatory [18]. Flavonoids have also been found be to prevent infection via suppressing the growth of the pathogenic microorganism, including drug-resistant strains of Mtb [19].

Conclusion

The ability of flavonoids to reduce oxidative stress and inflammation appear promising, however, findings of this study propose that quercetin nanoparticles produced a significant reduction in DNA damage and displayed a great decrease in MNi frequency, resulting from oxidative stress induced by bleomycin in human lymphocytes of TB patients. The results of this study suggest that a small concentrations of quercetin nano form would have a potential therapeutic role as a protective compound among patients with TB and may enable us to reduce the adverse effects of the current therapies through dose reduction.

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