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Protective Role of Quercetin on Carmustine-induced Lung Toxicity in Adult Male Albino Rats

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Abstract

Background: Carmustine (BCNU) is an alkylating agent used as an antineoplastic agent. A major problem with the clinical use of BCNU is the occurrence of pulmonary toxicity. Quercetin (QUE) is an important polyphenolic flavonoid that exhibits antioxidant, anti-inflammatory and other health-promoting effects. This study aimed to investigate the protective effect of QUE on BCNU-induced lung injury in rats using histologic and biochemical methods.

Methods: Forty adult male albino rats were divided into 4 groups: Group I served as the control group. Group II received QUE orally in a dose of 100 mg/kg per day for 7 days. Group III received a single dose (30 mg/kg) of BCNU intraperitoneally (i.p.) on the 7th day of the study. Group IV received (BCNU + QUE). On the 8th day of the experiment, lung tissues were collected for histopathological examinations. The levels of malondialdehyde (MDA), hydroxyproline (HYP), myeloperoxidase (MPO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were also determined in all dissected tissues.

Results: Pretreatment with QUE ameliorated lung morphological changes noticed in the BCNU group and the levels of MDA, HYP, and MPO were significantly decreased whereas those of GSH, GSH-Px, and SOD were significantly increased.

Conclusions: QUE provides a protective effect against BCNU-induced lung injury by reducing oxidative stress and pulmonary fibrosis.

Keywords: Antioxidants, Quercetin, Lung Injury, Carmustine

1. Introduction

Carmustine {BCNU, [1,3-bis (2-chloroethyl)-1-nitrosourea]} is a cell-cycle phase nonspecific antineoplastic agent belonging to the nitrosourea group of compounds, which exerts tumour cytotoxicity via multiple mechanisms (Schmitz *et al*. 2002) [25]. It is a potent, lipid-soluble alkylating agent used for the treatment of numerous neoplasms and is particularly effective against gliomas (Helal and Helal 2009) [10]. BCNU undergoes spontaneous nonenzymatic decomposition under physiological conditions to release reactive intermediates with alkylating activities, which are thought to be responsible for the antineoplastic and cytotoxic activities of BCNU (Tirelli *et al*. 2012) [29]. BCNU has been used for more than 40 years and has a well-defined place in different oncological treatment protocols (Schmitz *et al*. 2002) [25].

A major problem with the clinical use of BCNU is the appearance of lung toxicity (Wu *et al*. 2001) [34]. Lung damage and fibrosis have been estimated to occur in 20-30% of the patients receiving this drug (Weiss *et al*. 1981) [33]. It has been found that the administration of multiple doses of BCNU is able to induce marked lung damage in rats (Smith and Boyd 1984) [28].

Oxidative stress and inflammation have been reported to be implicated in lung toxicity after chemotherapy (Abushamaa *et al*. 2002) [1]. Previous investigators have reported BCNU as a potent inhibitor of glutathione reductase in several tissues (Wu *et al*. 2001) [34]. It has been found that glutathione depletion is associated with the augmentation of an oxidative stress-mediated pro-inflammatory state in rat alveolar epithelium (Haddad 2000) [7]. In addition, Inappropriate production of TNF was found to be strongly associated with pulmonary fibrosis (Oikonomou *et al*. 2006) [22].

Many types of antioxidant dietary supplements have been found to have health benefits. Utilization of these products leads to a reduction in various proinflammatory and/or oxidative stress biomarkers (Vouldoukis *et al*. 2004) [32]. Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against the deleterious effects of reactive oxygen species (ROS) and other free radicals (Manda and Bhatia 2003) [19]. Compounds that reduce the side effects and

trigger immunity can be extremely useful in ameliorating cancer treatment. Recently, many researchers have been concerned with several compounds of plant origin that are eligible for minimizing the harmful effects of chemotherapy on normal cells without compromising its antineoplastic activity (Pratheeshkumar and Kuttan 2010) [24].

Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family is a well-known antioxidant (Kelly 2011) [15]. It is found in fruits and vegetables such as blueberries, onions, curly kale, broccoli, and leek (Manach *et al*. 1999) [18]. Due to their structural features, flavonoids possess the promising ability to transfer electrons to free radicals, induce antioxidant enzyme activation, and suppress oxidative stress (Heim *et al*. 2002) [9]. It is well documented that QUE has broad bioactivity, such as antioxidative, hypolipidemic properties (Bischoff 2009; Boots *et al*. 2008 [4]), ROS scavenging, anti-inflammatory and anti-fibrotic properties (Hwang *et al*. 2009; Lu *et al*. 2006) [12, 16]. The antioxidant activity of QUE is primarily credited to its phenolic hydroxyl groups (Materska and Perucka 2005) [20]. The presence of these structural features in QUE enables it to act as a hydrogen donor for quenching free radicals (Heijnen *et al*. 2002; Meyers *et al*. 2008) [8, 21]. Beneficial health effects of QUE against various oxidative stress-related diseases have been documented (Flora 2009) [6].

In experimental studies performed in several models of cancer toxicity caused by anticancer agents, QUE was reported to prevent this toxicity (Jeong *et al*. 2009) [14]. Studies have demonstrated that QUE provides antioxidant restoration (Ozcan *et al*. 2005) [23], inhibits inflammatory responses, and consequently prevents oxidant-induced inflammatory cell damage by CYP toxicity (Sekeroglu *et al*. 2011) [26].

Therefore, this study aimed to evaluate the protective effects of QUE on BCNU-induced lung toxicity in rats using histologic and biochemical methods.

2. Methods

1. Animals

The present study was carried out on 40 healthy adult male albino Wistar rats weighing from 200-250 g. They were purchased from the animal house of Assiut Faculty of Medicine, Assiut University, Egypt. The rats were housed in polypropylene cages under standard lightening in a temperature-controlled room (25 ± 2◦C) and had free access to laboratory food and water throughout the experiment. They were acclimatized to their environment for at least two weeks before starting the experiment. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the *local Institutional Animal Ethical Committee* of Faculty of Medicine, Sohag university, Egypt.

2. Experimental design

After the acclimatization period, rats were weighted, randomly divided into four groups (ten rats in each) as follows:

Group I (Control group): Received ethanol 30% orally (vehicle) for 7 days.

Group II: Received QUE dissolved in ethanol 30% orally (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) in a dose of 100 mg/kg per day for 7 days (Şengül *et al*. 2017) [27].

Group III: Received a single dose (30 mg/kg) of BCNU dissolved in ethanol 30% intraperitoneally (i.p.) (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) on the 7th day of the study. (Fahmy *et al*. 2022) [17].

Group IV: Received QUE dissolved in ethanol 30% orally (100 mg/kg/day) and a single injection of i.p. BCNU (30 mg/kg) was administered on the 7th day.

The animals were weighted at the beginning and at the end of the experiment. The changes in body weight were recorded. Twenty-four hours after the last drug regimen, the rats were sacrificed by exsanguination via resection of the aorta. A median sternotomy was performed, and lungs were removed from the thoracic cavity.

3. Biochemical study

The right lungs were immediately snap frozen in liquid nitrogen and stored at -80 °C for biochemical analysis. The lung tissues were rinsed with 10% cold phosphate buffered saline (PBS) solution (PH 7.4) to remove any residual blood clot. Tissues were homogenized in PBS and centrifuged at 8000 rpm for 15 min at 4 °C to collect supernatant fluids. These supernatant fractions were used to measure the desired biochemical markers.

Analysis of tissue malondialdehyde (MDA) level as an indicator of lipid peroxidation, was performed by the spectrophotometry method (Kurokawa *et al*. 2006). This method was used to obtain a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA at 535 nm. The MDA level is expressed as nmol/g tissue protein.

Hydroxyproline (HYP) is an efficient index of collagen deposition since collagen contains significant amount of this amino acid. The hydroxyproline content was quantified as described by Terashima *et al*. (2019) [11]. The right lung was weighted and then hydrolyzed in 2 ml of 6 N HCl at 100°C for 72 h. The hydrolysate was cooled, neutralized with an equal amount of 6 N NaOH, and centrifuged at 13,000 g for12 min. The supernatant was filtered to remove debris and mixed with three times its volume of 3M NaCl. 40 µl of the supernatant were added to a microtiter plate and incubated with 25 µl of chloramine T solution at room temperature (20-22°C) for 10 min. Then, 150 µl of Ehrlich's solution was added and incubated at 65°C for 20 min. After cooling, absorbance was measured at 560 nm using a multiplate spectrometer. The hydroxyproline content was quantified using a standard curve of high-purity hydroxyproline (WakoPure Chemicals, Osaka, Japan). Results were expressed as µmol/g tissue protein.

Myeloperoxidase (MPO) activity, an index of the degree of neutrophil accumulation, was measured in tissues with commercially available ELISA kit (Bioxytech MPO-EIA, USA). The absorbance was read at 405 nm using Multi-Detection Microplate Reader. Quantifications were achieved by the construction of standard curve using known concentrations of MPO. Results were expressed as ng/mg tissue protein (Guneli *et al*. 2007).

Antioxidant activity was detected by measuring reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). This is an index of oxidative stress and production of reactive oxygen species (ROS). Colorimetric assay for assessment of GSH concentration was used and the level of GSH was measured at 412 nm by spectrophotometer. Results were expressed as µmol/g tissue protein (Vardi *et al*. 2008).

The GSH-Px enzyme activity was measured in tissues with

commercially available Glutathione Peroxidase Assay Kit (ab102530; abcam, Cambridge, United Kingdom) and the level was measured at 340 nm by spectrophotometer. Results were expressed as units/g tissue protein (Shi *et al*. 2009).

Xanthine/xanthine oxidase assay was used to estimate SOD (Superoxide Dismutase Assay Kit, Item No. 706002; Cayman Chemical Company, Ann Arbor, USA) by measuring the amount of reduced nitro blue tetrazolium (NBT) with one unit of SOD, which is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. SOD was expressed as units/mg tissue protein (Shi *et al*. 2009).

4. Pulmonary edema

The lower lobes of left lungs from all animals were weighted, and then placed in a stove for 7 days at 37°C. After this period, the specimens were weighted again, and the ratio of the weight before and after drying was calculated. Lung edema was represented by an increase in this ratio (Ingelse *et al*. 2019).

5. Histological examination

The upper lobes of the left lungs were maintained inflated with trapped air by ligation of the corresponding bronchus, and fixed in 10% of neutral buffered formalin for 24 hours. Paraffin-embedded sections (4 µm thickness) were stained with hematoxylin and eosin (H&E) (Bancroft and Layton 2013), and examined under a light microscope to detect histopathological changes. Other sections were stained with Masson's trichrome stain for light microscopic evaluation of degree of fibrosis.

The degree of inflammation and destruction was scored for each group (Gokakin *et al*. 2013) (Table 1). A mean score for each of the variables was then calculated. A total histopathological score (maximum 12) was derived from the sum of the mean scores of the variables. All the samples were examined by the same pathologist to achieve correct score, and mean value of each group was used for statistical analysis.

Table 1: Scoring of inflammation and destruction

Pathological lesion	Score
Edema	1
Hyperemia	1
Thickness in interalveolar septum	2
Mononuclear cell infiltration	2
Loss of alveolar epithelium	3
Hemorrhage	3
Total	12

6. Assessment of pulmonary neutrophil sequestration

The pulmonary tissue neutrophil sequestration was determined according to the method described by Koksoy *et al*. (2001). A single pathologist blinded to all groups examined the pathological specimens. At least two different sections of each specimen were examined to determine the degree of injury. Lung neutrophil sequestration was quantified by counting alveolar septal wall neutrophils in the peripheral lung parenchyma. It was expressed as the mean number of neutrophils per 10 non-overlapping high-power fields (400×). Quantitative measurements were carried out using an image analysis system (Leica Qwin 500 C Imaging System Ltd., Cambridge, England) in Central Research Lab, Assiut Faculty of Medicine, Egypt.

7. Statistical Analysis

All values were expressed as mean ± standard deviation (SD). The data were analyzed by unpaired Student's t-test using the software Statistical Package for Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant if the probability of chance (*P* value) was < 0.05.

3. Results

None of the experimental rats died during the experiment period (7 days).

1. Evaluation of body weight

Single administration of BCNU resulted in significant decrease in the body weight (202.66 g) as compared to the control group (232.41 g) possibly because of severe tissue damage caused by free radicals (P<0.05). However, no significant difference in body weight was observed between the control (232.41 g), QUE (229.12 g) and BCNU+QUE (224.51 g) groups. (Table 2)

Table 2: Body weight of rats in the different studied groups

Parameters	Group I (Control)	Group II (QUE)	Group III (BCNU)	Group IV (BCNU+QUE)
Body weight (g)	232.41±10.84	229.12±11.53	202.66±9.24[a]	224.51±12.31[b]

Data is expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at P < 0.05.
[a]p < 0.0001 compared with the control group (group I).
[b]p < 0.001 compared with the CYP group (group III).

2. Biochemical results

MDA level in the lung tissue of BCNU group increased significantly (136.44 nmol/g) (P < 0.05) when compared with control group (53.65 nmol/g). In QUE pretreated group, a significant decrease (58.66 nmol/g) in the MDA level was observed as compared with BCNU group (P < 0.05). (Table 3)

Table 3: Levels of malondialdehyde (MDA), hydroxyproline, myeloperoxidase (MPO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in the different studied groups

Parameters	Group I (Control)	Group II (QUE)	Group III (BCNU)	Group IV (BCNU+QUE)
MDA (nmol/g tissue protein)	53.65±4.22	52.41±5.81	136.44±8.77[a]	58.66±3.53[b]
HYP (µmol/g lung tissue)	0.037±0.01	0.036±0.02	0.074±0.02[a]	0.040±0.01[b]
MPO (ng/mg tissue protein)	5.78±0.43	5.48±1.23	13.81±1.08[a]	4.89±1.04[b]
GSH (µmol/g tissue protein)	6.12±0.47	6.13±0.76	2.82±1.04[a]	5.32±0.89[b]
GSH-Px (units/g tissue protein)	13.44±1.12	13.75±1.45	5.35±2.33[a]	11.91±1.57[b]
SOD (units/mg tissue protein)	4.11±0.17	4.13±0.12	2.55±0.67[a]	3.94±0.25[b]

Data is expressed as mean ± standard deviation. Results were statistically analyzed by using Student's t test at P < 0.05.
ap < 0.0001 compared with the control group (group I).
bp < 0.0001 compared with the CYP group (group III).

HYP content in the lung tissue of BCNU group increased significantly (0.074 µmol/g) ($P < 0.05$) when compared with control group (0.074 µmol/g). In QUE pretreated group, a significant decrease (0.074 µmol/g) in lung hydroxyproline content was observed as compared with BCNU group ($P < 0.05$). (Table 3)

MPO activity in the lung tissue of BCNU group was increased significantly (13.81 ng/mg) ($P < 0.05$) when compared with control group (5.78 ng/mg). However, a significant decrease (4.89 ng/mg) in the MPO activity was observed in QUE pretreated group in comparison with BCNU group ($P < 0.05$). (Table 3)

Compared with the control group, the BCNU group showed a significant decrease in the level of GSH (2.28 µmol/g), GSH-Px (5.35 units/g), and SOD (2.55(5.35 units/mg) ($P < 0.05$). However, a significant increase was determined in the level of GSH (5.32 µmol/g), GSH-Px (11.91 units/g), and SOD activity (3.94(5.35 units/mg) in QUE pretreated group as compared with BCNU group ($P < 0.05$). (Table 3)

3. Pulmonary edema

Pulmonary edema formation was assessed by wet/dry weight ratios. BCNU significantly increased (7.41) the lung tissue wet/dry weight ratios compared to control group (2.75) ($P < 0.05$). QUE pretreatment significantly decreased (3.25) the lung tissue wet/dry weight ratios compared with BCNU alone ($P < 0.05$). (Fig 1 and Table 4)

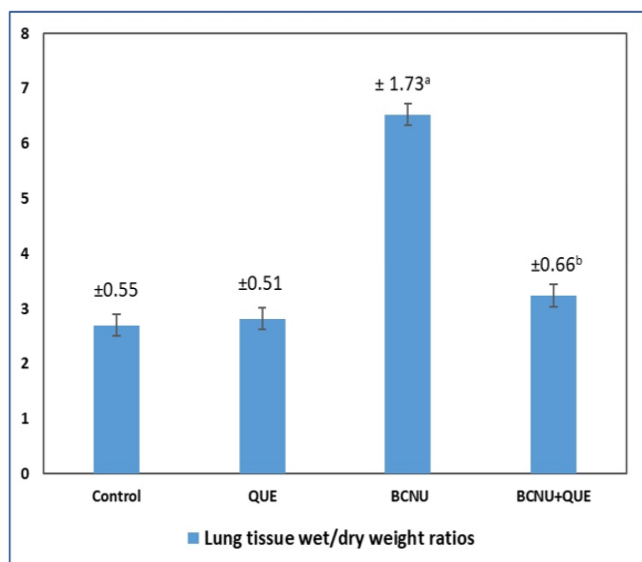

Fig 1: Lung tissue wet/dry weight ratios in the studied groups tested by using Student's t test. [a]$P < 0.05$ is significant in group III versus group I and [b]$P < 0.05$ is significant in group IV versus group III.

Table 4: The mean values of lung tissue wet/dry weight ratios and mean number of neutrophils in the different studied groups

Parameters	Group I (Control)	Group II (QUE)	Group III (BCNU)	Group IV (BCNU+QUE)
Lung tissue wet/dry Weight ratios	2.75±0.44	2.69±0.57	7.41± 1.42[a]	3.25±0.63[b]
Mean number of neutrophils	1.26±0.31	1.29±0.42	13.43± 1.55[a]	3.72±0.14[b]

Data is expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$.
[a]$p < 0.0001$ compared with the control group (group I).
[b]$p < 0.0001$ compared with the CYP group (group III).

4. Histological results

4.1 H&E-stain

Light microscopic examination of H&E-stained sections from control group revealed normal lung architecture in which the spongy structure of the lung appeared with thin inter-alveolar septa and normal clear alveoli (Fig 2a).

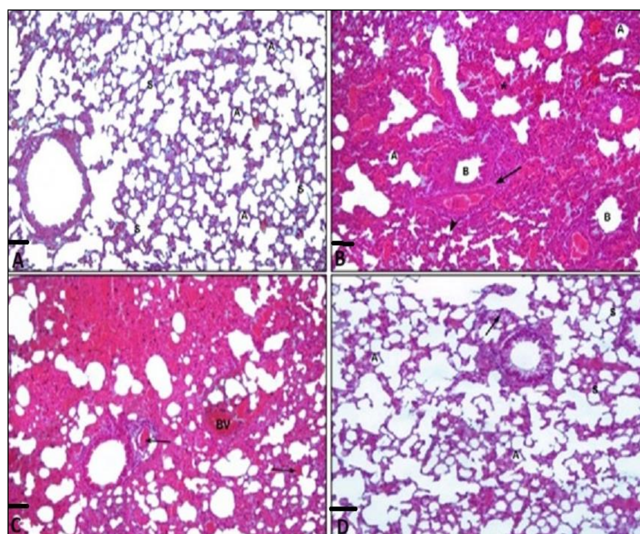

Fig 2: (A) A Photomicrograph of normal architecture of the lung in the control group showing normal clear alveoli and thin inter-alveolar septa (H&E, scale bars = 10 µm). **(B-C)** Photomicrographs of Lung of BCNU treated rats. **(B)** Loss of the normal architecture of the lung with extensive infiltration by inflammatory cells (→), Marked thickening of the inter-alveolar septa (star) and narrowed alveoli (arrow head). **(C)** Extravasation of blood in the alveolar lumen (→), and congested blood vessels (BV). (H&E, scale bars = 10 µm (B-C)). **(D)** A photomicrograph of the lung of BCNU+QUE group showing normal alveolar epithelium and marked decrease in the thickening of the inter-alveolar septa. Mild mononuclear cellular infiltration was seen (→). (H&E, scale bars = 10 µm) (A=alveoli, S= inter-alveolar septum, B=Bronchiole, BV=Blood vessel).

In BCNU group, histological changes were variable among the animals, both in pattern and severity (Table 5). H&E-stained sections revealed a marked inflammatory cellular infiltration around bronchioles, around alveoli, in perivascular spaces, and in the inter-alveolar septa. Collapsed narrowed alveoli and Thickening of the inter-alveolar septa were noticed (Fig 2b). extravasation of blood in the alveolar lumen, congested blood capillaries and interstitial hemorrhage were detected (Fig 2c).

Table 5: Total scores of histopathological lesions in rat lungs in the different studied groups

Rats	Group III scores	Group IV scores
1	8	2
2	7	4
3	8	2
4	9	2
5	6	3
6	9	4
7	7	2
8	8	2
9	9	4
10	6	3
Mean±SD	7.7±0.62	2.8±0.24*

Data is expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$. Histopathological severity of lung injury was significantly reduced (*p < 0.0001) in BCX+CYP group (group IV) versus CYP group (group III).

Examination of QUE pretreated group revealed that most of the changes which were observed in BCNU group markedly decreased (Table 5). The lung alveoli were lined by a normal alveolar epithelium. The inter-alveolar septa were less thick than in the treated group. Mild mononuclear cellular infiltration was seen in the pulmonary interstitium. (Fig 2d).

4.2 Masson's trichrome stain

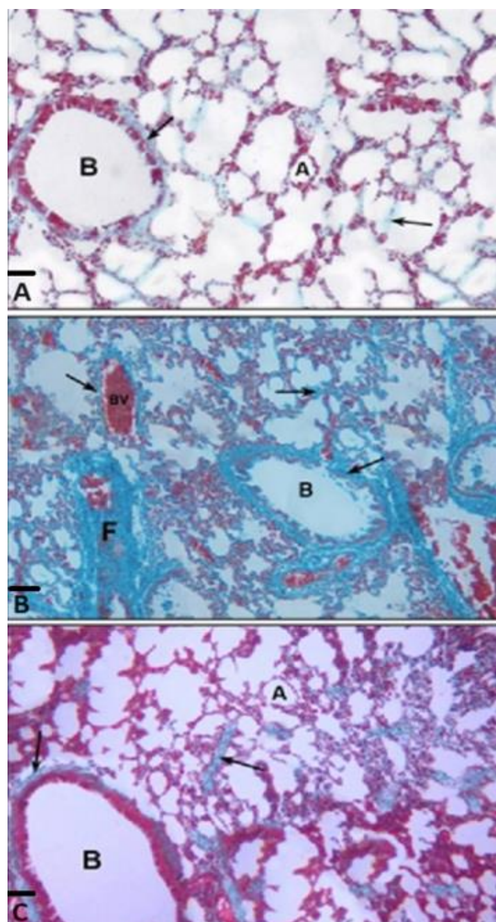

Fig 3: (A) A Photomicrograph of lung in the control group showing normal distribution of thin layer of collagen fibers in the interalveolar septa and the wall of the bronchiole (→). (Masson's trichrome, scale bars = 10 µm). **(B)** A Photomicrograph of lung in the BCNU group showing excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels and the walls of the bronchioles (→). Large fibrotic areas are seen in the parenchyma (F). (Masson's trichrome, scale bars = 10 µm). **(C)** A photomicrograph of the lung of BCNU+QUE group showing marked decrease in collagen deposition (→). (Masson's trichrome, scale bars = 10 µm) (A=alveoli, B=Bronchiole, BV=Blood vessel).

Light microscopic examination of sections from control group revealed presence of collagen fibers in the interalveolar septa and the wall of the bronchiole (Fig 3a).

The BCNU group revealed the presence of an excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels and the walls of the bronchioles. Large fibrotic areas are also seen (Fig 3b).

However, Examination of QUE pretreated group showed marked decrease in collagen deposition as compared with BCNU group (Fig 3c).

5. Pulmonary neutrophil sequestration

The neutrophil sequestration in the lung tissue was significantly higher (P = 0.0001) in BCNU group (13.43) than that in control group (1.26). However, QUE pretreatment significantly reduced the sequestration of neutrophils in lungs (3.72) (P = 0.0001). (Fig 4 and Table 4)

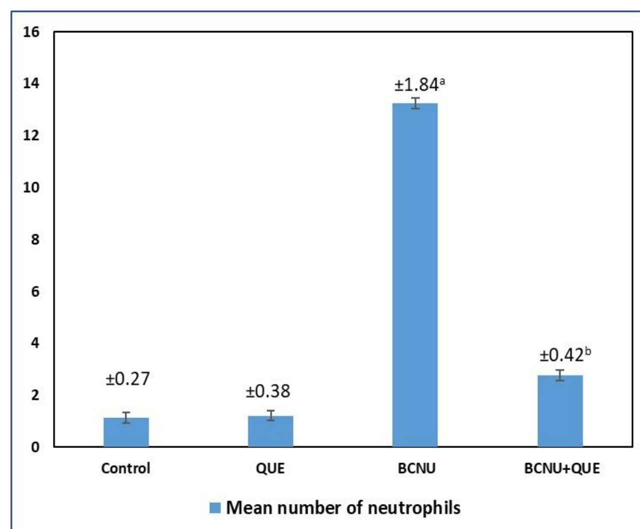

Fig 4: Mean number of neutrophils per 10 high-power fields in the studied groups tested by using Student's t test. $^{a}P < 0.05$ is significant in group III versus group I and $^{b}P < 0.05$ is significant in group IV versus group III.

4. Discussion

Carmustine (BCNU) is an important chemotherapeutic drug for treating brain tumors, lymphoma, and multiple myeloma (Inanc *et al*. 2022) [13]. Lung damage, including fibrosis, is a major problem with the clinical use of BCNU (Wu *et al*. 2001) [34]. Experimental studies have reported that QUE is an effective antioxidant and anti-inflammatory. (Sengul *et al*. 2017) [27].

The present study investigated the influence of QUE on BCNU-induced lung toxicity and evaluated its antioxidant and anti-inflammatory effects.

The administration of BCNU resulted in a significant decrease in body weight possibly because of severe tissue damage caused by free radicals. However, in the combined BCNU and QUE group, Body weight was near to that in the control group. This was supported by a previous study showing that the body weight of QUE-treated rats remained comparable to the control group rats throughout the period of experiment (Verma *et al*. 2013) [31].

In the present study, we found a significant increase in the MDA, hydroxyproline, and MPO levels in the BCNU-treated group as compared with the normal group. On the other hand, the levels of antioxidant enzymes namely GSH, GSH-Px, and SOD were significantly decreased. This result was supported by El-Sayed *et al*. (2011) who revealed that injection of rats with BCNU in a single dose of 30 mg/kg, i.p., significantly increased bone marrow content of MDA, but decreased the bone marrow activities of SOD, GPx and CAT as well as the glutathione bone marrow content. Another study revealed that BCNU reduced the activity of the antioxidant enzymes SOD, CAT, and GPx. It has been

reported that GPx is capable of reducing free hydrogen peroxide, while CAT and SOD provide the first line defense against oxygen radical toxicity (El-Sayed *et al.* 2010) [5]. Helal and Helal (2009) [10] reported that BCNU inhibits the antioxidant enzyme glutathione reductase (GR) massively. As one of the most effective and abundant ROS-scavenging systems, glutathione plays a critical role in the maintenance of the redox balance in all cells. Therefore, inhibition of GR by BCNU leads to the accumulation of reactive oxygen species.

Treatment with QUE in combination with BCNU significantly increased levels of SOD, GSH, and GSH-Px and significantly decreased MDA and MPO levels when compared with BCNU-treated group. These findings were in agreement with Alzohairy *et al.* (2021) [2] who found that pre-treatment with QUE at 50 mg/kg significantly increased the level of antioxidant enzymes, including CAT, GST, and GSH, and significantly decreased MDA levels in rat lung after Benzopyrene treatment. Another study declared that QUE (50 mg/Kg BW) supplementation 1h prior to hypoxia exposure has significantly reduced MDA levels and significantly elevated the level of GSH, GPx, and SOD compared to hypoxia control (Tripathi *et al.* 2019) [30]. Sengul *et al.* (2017) [27] found that With QUE treatment, oxidative-stress-mediated lung tissue damage induced by CYP was prevented. The administration of QUE significantly prevented the increase in MDA levels and induced a significant increase in the activities of SOD and GSH in experimental rats. Verma *et al.* (2013) [31] observed that QUE treatment significantly lowered the bleomycin-induced lipid peroxidation in the lung of rats as evidenced by the near-normal MDA levels. An apparent reduction in lung hydroxyproline content was also observed. Moreover, it was observed that QUE was effective in restoring the altered activity of the antioxidant enzymes (SOD and CAT).

The present study showed that BCNU-treated rats induced pulmonary edema as indicated by a significant increase in the lung tissue wet/dry weight ratios compared with the control group. On the other hand, QUE pretreatment prevented the occurrence of pulmonary edema as indicated by significantly decreased lung tissue wet /dry weight ratios compared with the BCNU-treated group. Tripathi *et al.* (2019) [30] observed that prophylactic administration of QUE (50 mg/Kg BW) to rats 1h prior to hypoxia exposure showed a significant decrease in the edema index (W/D ratio). Verma *et al.* (2013) [31] found that the percent relative lung weight in QUE-treated rats was relatively normal compared to the bleomycin-treated animals.

Histological examination of H&E-stained sections of BCNU-treated rats revealed a marked inflammatory cellular infiltration around bronchioles, around alveoli, in perivascular spaces, and in the inter-alveolar septa. Narrowing of alveoli and Thickening of the inter-alveolar septa were seen. Extravasation of blood in the alveolar lumen, and congested blood capillaries were also detected. The findings of the present study were consistent with Helal and Helal (2009) [10] who observed that sections from the lung of rats injected with BCNU show widespread diffusion of inflammatory reactions throughout the lung with marked mononuclear cell infiltration, vascular congestion, and numerous alveoli with collapsed alveolar walls forming dilated spaces.

Marked histological amelioration was observed in the lung tissue of rats treated with a combination of BCNU and QUE. The lung alveoli were lined by a normal alveolar epithelium and the inter-alveolar septa were less thick than in the BCNU-treated group. Mild inflammatory cellular infiltration was seen. These findings were supported by Alzohairy *et al.* (2021) [2] who denoted that pretreatment with QUE showed a normal lung tissue architecture with mild inflammatory cell infiltrate. Tripathi *et al.* (2019) [30] found that the lung sections of the animals fed with QUE prior to hypoxia demonstrated normal alveoli, reduced infiltration of inflammatory cytokines, and disappearance of RBCs in alveolar spaces. Another study showed that QUE treatment resulted in minimal lung damage and no significant inflammatory infiltration in the lung of CYP-treated rats. Normal alveolar septa, normal alveolar vascular permeability, and reduced polymorphonuclear cell infiltration were seen in lung tissues after treatment with QUE (Sengul *et al.* 2017) [27]. A third study observed that QUE has an ameliorative effect on the inflammatory lesions developed by bleomycin treatment. Pulmonary changes in animals treated with QUE showed mild to moderate degree of thickening of inter-alveolar septa with few inflammatory cell infiltrates. Emphysematous changes and alveolar hemorrhages were remarkably reduced in the QUE-treated group of animals (Verma *et al.* 2013) [31].

Histological examination of Masson's trichrome-stained sections of BCNU-treated rats revealed the presence of an excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels, and the walls of the bronchioles. Large fibrotic areas were also seen within the lung parenchyma. The findings of the present study were supported by Helal and Helal (2009) [10] who declared that fibrous tissue (collagen) deposition in the lungs of rats treated with BCNU is obviously increased in peribronchial, perivascular, and interalveolar septa. However, Examination of QUE pretreated group showed a marked decrease in collagen deposition as compared with the BCNU group. These results were in agreement with Alzohairy *et al.* (2021) [2] who reported that QUE-treated rats showed normal collagen distribution. Verma *et al.* (2013) [31] showed that collagen accumulation was remarkably decreased in rats from the QUE-treated group.

The present study showed that BNCU-treated rats induced significant neutrophil sequestration in the lung tissue compared with the control group. However, QUE pretreatment significantly reduced the sequestration of neutrophils in the lungs. The presence of increased numbers of activated neutrophils may be the cause of induced pulmonary injury and pulmonary edema via the excessive elaboration of inflammatory cytokines, proteolytic enzymes, and oxygen radicals. Verma *et al.* (2013) [31] observed that QUE treatment for 20 days significantly reduced the bleomycin-induced hike in neutrophils. Moreover, total leukocyte count in rats treated with QUE remained similar to that observed in control rats at the end of the experimental regimen.

In conclusion, usage of QUE showed promising results, and exerted a protective effect against BCNU-induced lung toxicity in rats by reducing oxidative stress and histopathological changes. Therefore, QUE may be a useful therapeutic agent during treatment with BCNU after validation of the study results in human studies.

Table 6: List of Abbreviations

BCNU	Carmustine
QUE	Quercetin

5. References

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