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Protective Effect of Quercetin on Carmustine: Induced Lung Toxicity in Adult Male Albino Rats

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Abstract
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. Forty adult male albino rats were divided into 4 groups: Group I served as the control group. Group received QUE orally in a dose of 100 mg/kg per day for 7 days. GroupI received a single dose (30 mg/kg) of BCNU intraperitoneally (i.p.) on the 7th day of the study. Group V 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. 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. QUE provides a protective effect against BCNU-induced lung injury by reducing oxidative stress and pulmonary fibrosis.

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)[1]. 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)[2]. 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)[3]. BCNU has been used for more than 40 years and has a well-defined place in different oncological treatment protocols (Schmitz *et al.* 2002)[1]. A major problem with the clinical use of BCNU is the appearance of lung toxicity (Wu *et al.* 2001)[4]. Lung damage and fibrosis have been estimated to occur in 20-30% of the patients receiving this drug (Weiss *et al*. 1981). 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)[5].

Oxidative stress and inflammation have been reported to be implicated in lung toxicity after chemotherapy (Abushamaa *et al*. 2002)[7]. Previous investigators have reported BCNU as a potent inhibitor of glutathione reductase in several tissues (Wu *et al*. 2001)[7]. 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)[8]. In addition, Inappropriate production of TNF was found to be strongly associated with pulmonary fibrosis (Oikonomou *et al*. 2006)[9].

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)[10]. 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)[11]. 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)[12].

Quercetin (3,3',4',5,7-pentahydroxyflavone), a member of the flavonoid family is a well known antioxidant (Kelly 2011)[13]. It is found in fruits and vegetables such as blueberries, onions, curly kale, broccoli and leek (Manach *et al*. 1999)[14]. Due to their structural features, flavonoid possess the promising ability to transfer electrons to free radicals, induce antioxidant enzyme activation and suppress oxidative stress (Heim *et al*. 2002)[15]. It is well documented that QUE has broad bioactivity, such as antioxidative, hypolipidemic properties (Bischoff 2009, Boots *et al*. 2008)[16,17], ROS scavenging, anti-inflammatory and anti-fibrotic properties (Hwang *et al*. 2009, Lu *et al*. 2006)[18]. The antioxidant activity of QUE is primarily credited to its phenolic hydroxyl groups (Materska and Perucka 2005)[19]. The presence of these structural features in QUE enables it to act as a hydrogen donor for quenching free radicals (Heijnen *et al*. 2002[20] Meyers *et al*. 2008)[21]. Beneficial health effects of QUE against various oxidative stress-related diseases have been documented (Flora 2009)[22].

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)[23]. Studies have demonstrated that QUE provides antioxidant restoration (Ozcan *et al*. 2005)[24], inhibits inflammatory responses and consequently prevents oxidant-induced inflammatory cell damage by CYP toxicity (Sekeroglu *et al*. 2011)[25].

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

MATERIALS AND METHODS

Animals: The present study was carried out on 40 healthy adult male albino 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.

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 (Sengül *et al*. 2017).

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).

Group IV: Received QUE dissolved in normal saline orally (100 mg/kg/day) and a single injection of i.p. BCNU (13.3 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.

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 per oxidation, was performed by the spectrophotometer method. 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)[26]. 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 micro titer 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, absorbency was measured at 560 nm using a multi plate spectrometer. The hydroxyproline content was quantified using a standard curve of high-purity hydroxyproline (Wako Pure 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 Micro plate Reader. Quantifications were achieved by the construction of standard curve using known concentrations of MPO. Results were expressed as ng/mg tissue protein

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.

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.

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 de ned as the amount of protein that inhibits the rate of NBT reduction by 50%. SOD was expressed as units/mg tissue protein.

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)[27].

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 haematoxylon and eosin (H and E) 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 (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.

Assessment of Pulmonary Neutrophil Sequestration: The pulmonary tissue neutrophil sequestration was determined according to the method described by A 2001 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.

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.

RESULTS AND DISCUSSIONS

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

Evaluation of Body Weight: Single administration of BCNU resulted in significant decrease in the body weight as compared to the control group 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, QUE and BCNU+QUE groups. (Table 2)

Biochemical Results: MDA level in the lung tissue of BCNU group increased significantly (P<0.05) when compared with control group. In QUE pretreated group, a significant decrease in the MDA level was observed as compared with BCNU group (P<0.05). (Table 3)

HYP content in the lung tissue of BCNU group increased significantly (P<0.05) when compared with control group. In QUE pretreated group, a significant decrease 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 (P<0.05) when compared with control group. However, a significant decrease 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, GSH-Px and SOD (P<0.05). However, a significant increase was determined in the level of GSH, GSH-Px, and SOD activity in QUE pretreated group as compared with BCNU group (P<0.05). (Table 3)

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

Histological Results:

H and E-stain: Light microscopic examination of H and 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).

In BCNU group, histological changes were variable among the animals, both in pattern and severity) Table 5(. H and 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).

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).

Masson's Trichrome Stain: 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).

Pulmonary Neutrophil Sequestration: The neutrophil sequestration in the lung tissue was significantly higher (P = 0.0001) in BCNU group than that in control group. However, QUE pretreatment significantly reduced the

List of Abbreviations

BCNU	Carmustine
QUE	Quercetin

Declarations

Table 1. Scoring of inflammation and destruction.

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

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± 9.24a	224.51±12.31b

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<0.001 compared with the CYP group (group III).

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±8.77a	58.66±3.53b
HYP (µmol/g lung tissue)	0.037±0.01	0.036±0.02	0.074±0.02a	0.040±0.01b
MPO (ng/mg tissue protein)	5.78±0.43	5.48±1.23	13.81±1.08a	4.89±1.04b
GSH (µmol/g tissue protein)	6.12±0.47	6.13±0.76	2.82±1.04a	5.32±0.89b
GSH-Px (units/g tissue protein)	13.44±1.12	13.75±1.45	5.35±2.33a	11.91±1.57b
SOD (units/mg tissue protein)	4.11±0.17	4.13±0.12	2.55±0.67a	3.94±0.25b

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).

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± 1.42a	3.25±0.63b
Mean number of neutrophils	1.26±0.31	1.29±0.42	13.43± 1.55a	3.72±0.14b

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).

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).

sequestration of neutrophils in lungs (P = 0.0001). (Fig. 4 and Table 4)

Carmustine (BCNU) is an important chemotherapeutic drug for treating brain tumors, lymphoma and multiple myeloma (Inanc *et al*. 2022)[27]. Lung damage, including fibrosis, is a major problem with the clinical use of BCNU (Wu *et al*. 2001)[21].

Experimental studies have reported that QUE is an effective antioxidant and anti-inflammatory. (Sengul *et al*. 2017)[28].

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)[29].

In the present study, we found a signi cant 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 signi cantly decreased. This result was supported by El-Sayed *et al*. (2011)[30] 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)[30]. Helal and Helal (2009)[2] 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 signi cantly increased levels of SOD, GSH and GSH-Px and signi cantly decreased MDA and MPO levels when compared with BCNU-treated group. These findings were in agreement with Alzohairy *et al*. (2021)[31] 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)[26]. Sengul *et al*. (2017)[28] 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)[29] observed that QUE treatment significantly lowered the bleomycin-induced lipid per oxidation 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)[26] 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)[29] 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)[2] 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)[31] who denoted that pretreatment with QUE showed a normal lung tissue architecture with mild inflammatory cell infiltrate. Tripathi *et al*. (2019)[26] 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)[28]. 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)[29].

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)[2] 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)[31] who reported that QUE-treated rats showed normal collagen distribution. Verma *et al*. (2013)[29] 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)[29] 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.

CONCLUSIONS

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.

Ethics Approval and Consent to Participate: The manuscript doesn't involve human participants, human data or human tissue.

Consent for Publication: The manuscript doesn't contain any individual person's data in any form.

Availability of Data and Material: All data needed is discussed in details in the manuscript.

Competing Interests: The author declares that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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REFERENCES

1. Schmitz, N., B. Pfistner, M. Sextro, M. Sieber and A.M. Carella *et al.,* 2002. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive hodgkin's disease: A randomised trial. Lancet., 359: 2065-2071.
2. Helal, G.K. and O.K. Helal, 2008. Metallothionein attenuates carmustine-induced oxidative stress and protects against pulmonary fibrosis in rats. Arch. Toxicol., 83: 87-94.
3. Tirelli, U., M. Berretta, M. Spina, M. Michieli and R. Lazzarini, 2012. Oncologic drug shortages also in Italy. Eur. Rev. Med. Pharmacol. Sci., 16: 138-139.
4. Wu, M., M.R. Kelley, W.K. Hansen and W.J. Martin, 2001. Reduction of BCNU toxicity to lung cells by high-level expression of O(6)-methylguanine-DNA methyltransferase. Am. J. Physiol. Lung Cell. Mol. Physiol., 280: 755-761.
5. Weiss, R.B., D.S. Poster and J.S. Penta, 1981. The nitrosoureas and pulmonary toxicity. Cancer Treat. Rev., 8: 111-125.
6. Smith, A.C. and M.R. Boyd, 1984. Preferential effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on pulmonary glutathione reductase and glutathione/glutathione disulfide ratios: possible implications for lung toxicity. J. Pharmacol. Exp. Ther., 229: 658-663.
7. Abushamaa, A.M., T.A. Sporn and R.J. Folz, 2002. Oxidative stress and inflammation contribute to lung toxicity after a common breast cancer chemotherapy regimen. Am. J. Physiol. Lung Cell. Mol. Physiol., 283: 336-345.
8. Haddad, J.J., 2000. Glutathione depletion is associated with augmenting a proinflammatory signal: Evidence for an antioxidant/pro-oxidant mechanism regulating cytokines in the alveolar epithelium. Cytokines, Cell. Mol. Ther., 6: 177-187.
9. Oikonomou, N., V. Harokopos, J. Zalevsky, C. Valavanis and A. Kotanidou *et al.,* 2006. Soluble tnf mediates the transition from pulmonary inflammation to fibrosis. PLoS ONE, Vol. 1 .10.1371/journal. pone.0000108.

10. Vouldoukis, I., D. Lacan, C. Kamate, P. Coste and A. Calenda *et al.,* 2004. Antioxidant and anti-inflammatory properties of a cucumis melo LC. extract rich in superoxide dismutase activity. J. Ethnopharmacol., 94: 67-75.
11. Manda, K. and A.L. Bhatia, 2003. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. Cell Biol. Toxicol., 19: 367-372.
12. Pratheeshkumar, P. and G. Kuttan, 2010. Ameliorative action of vernonia cinerea L. on cyclophosphamide-induced imm-unosuppression and oxidative stress in mice. Inflammopharmacology, 18: 197-207.
13. Kelly, G.S., 2011. Quercetin. Monograph. Altern. Med. Rev., 16: 172-194.
14. Manach, C., O. Texier, C. Morand, V. Crespy and F. Regerat *et al.,* 1999. Comparison of the bioavailability of quercetin and catechin in rats. Free Radic. Biol. Med., 27: 1259-1266.
15. Heim, K.E., A.R. Tagliaferro and D.J. Bobilya, 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem., 13: 572-584.
16. Bischoff, S.C., 2008. Quercetin: Potentials in the prevention and therapy of disease. Curr. Opin. Clin. Nutr. Metab. Care, 11: 733-740.
17. Boots, A.W., G.R.M.M. Haenen and A. Bast, 2008. Health effects of quercetin: From antioxidant to nutraceutical. Eur. J. Pharmacol., 585: 325-337.
18. Hwang, M.K., N.R. Song, N.J. Kang, K.W. Lee and H.J. Lee, 2009. Activation of phosphatidylinositol 3-kinase is required for tumor necrosis factor-a-induced up regulation of matrix metalloproteinase-9: Its direct inhibition by quercetin. Int. J. Biochem. Cell Biol., 41: 1592-1600.
19. Lu, J., Y.L. Zheng, L. Luo, D.M. Wu, D.X. Sun and Y.J. Feng, 2006. Quercetin reverses D-galactose induced neurotoxicity in mouse brain. Behav. Brain Res., 171: 251-260.
20. Materska, M. and I. Perucka, 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (Capsicum annuum L.). J. Agric. Food Chem., 53: 1750-1756.
21. Heijnen, C.G.M., G.R.M.M. Haenen, R.M. Oostveen, E.M. Stalpers and A. Bast, 2002. Protection of flavonoids against lipid per oxidation: The structure activity relationship revisited. Free Radi. Res., 36: 575-581.
22. Meyers, K.J., J.L. Rudolf and A.E. Mitchell, 2008. Influence of dietary quercetin on glutathione redox status in mice. J. Agric. Food Chem., 56: 830-836.
23. Flora, S.J.S., 2009. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxid. Med. Cell. Longev., 2: 191-206.
24. Jeong, J., J.Y. An, Y.T. Kwon, J.G. Rhee and Y.J. Lee, 2008. Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. J. Cell. Biochem., 106: 73-82.
25. Ozcan, A., A. Korkmaz, S. Oter and O. Coskun, 2005. Contribution of flavonoid antioxidants to the preventive effect of mesna in cyclophosphamide-induced cystitis in rats. Arch. Toxicol., 79: 461-465.
26. Sekeroglu, V., B. Aydin and Z.A. Sekeroglu, 2011. Viscum album L. extract and quercetin reduce cyclophosphamide-induced cardiotoxicity, urotoxicity and genotoxicity in mice. Asian Pac. J. Cancer Prev., 12: 2925-2931.
27. Sengül, E., V. Gelen, S. Gedikli, S. Ozkanlar and C. Gur et al., 2017. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. Biomed. Pharmacother., 92: 303-307.
28. Terashima, H., M. Aonuma, H. Tsuchida, K. Sugimoto and M. Yokoyama *et al.,* 2019. Attenuation of pulmonary fibrosis in type I collagen-targeted reporter mice with alk-5 inhibitors. Pulm. Pharmacol. Ther., 54: 31-38.
29. Fahmy, M.A., A.A. Farghaly, E.E. Hassan, Z.M. Hassan and H.I. Abd-Alla, 2022. Protective role of codiaeum variegatum against genotoxicity induced by carmustine in somatic and germ cells of male mice. Mol. Biol. Rep., 49: 9543-9553.
30. Inanc, M.E., S. Gungor, D. Yeni, F. Avdatek and V. Ipek *et al.,* 2022. Protective role of the dried white mulberry extract on the reproductive damage and fertility in rats treated with carmustine. Food Chem. Toxicol., Vol. 163 .10.1016/j.fct.2022.112979.
31. Tripathi, A., B. Kumar and S.S.K. Sagi, 2019. Prophylactic efficacy of quercetin in ameliorating the hypoxia induced vascular leakage in lungs of rats. PLoS One, Vol. 14 .10.1371/journal. pone.0219075.
32. Verma, R., L. Kushwah, D. Gohel, M. Patel and T. Marvania *et al.,* 2013. Evaluating the ameliorative potential of quercetin against the bleomycin-induced pulmonary fibrosis in wistar rats. Pulm. Med., Vol. 2013 .10.1155/2013/921724.