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# The Role of Cellular Proliferation and Apoptosis in Acute Lung Injury: An Experimental Study

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## Abstract

**Introduction:** Acute Lung Injury (ALI) is a widespread damage to cells and structures of the alveolar capillary membrane that occurs within hours to days of a predisposing insult. A comprehensive understanding of the mechanisms of apoptosis in the initial injury and repair of lung epithelial and endothelial cells and other key cells involved in ALI is lacking. Further information is required regarding cytokine-signaling pathways, patterns of gene and protein expression. The present study attempts to correlate various parameters of lung damage and expression of certain proteins that regulate the cell cycle in an experimental model of ALI.

**Material and methods:** ALI was induced in five groups of mice via inhalation of paraquat. The animals of each group were sacrificed on days 1,2,3,4 and 6, respectively and their lungs were examined for the following parametres: focal thickening of alveolar membranes, capillary congestion, pulmonary oedema, intra-alveolar haemorrhage, interstitial neutrophil infiltration and intra-alveolar neutrophil infiltration scored from 0 to 3 according to the severity of the pathological changes. Specimens were also examined with immunoassay for the quantitative expression (percentile) of Ki-67, TUNEL staining (Todd-mediated dUTP nick end- labeling), p21, p16, p27, Cyclin B, Cyclin D1 and Cyclin E. A control group of animals that were not exposed to paraquat was also studied.

**Results:** All variables were significantly altered in all the study groups as compared with the control group suggesting the deleterious effect of the paraquat inhalation. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes. Multiple comparisons showed significantly higher mean differences in TUNEL staining and in the expression of ki-67, cyclins and cyclin-dependent kinase inhibitors (CDKIs) particularly after 2-3 days from the paraquat inhalation.

**Conclusion:** These findings implicate that lung tissue damage is characterized by enhanced cellular proliferation and apoptosis both being regulated by complex interactions of cyclins and CDKIs.

**Keywords:** Acute lung injury; Apoptosis; Paraquat

## Introduction

Acute lung injury (ALI) may be either a consequence of direct causes such as pneumonia, aspiration, pulmonary contusion, fat or air embolism and inhalation injury, or indirect causes such as sepsis, burn, severe trauma, cardiopulmonary bypass, drug overdose and acute pancreatitis [1]. In ALI, widespread damage to cells and structures of the alveolar capillary membrane occurs within hours to days of a predisposing insult. A comprehensive understanding of the mechanisms of apoptosis in the initial injury and repair of lung epithelial and endothelial cells and other key cells involved in ALI is lacking. Further information is required regarding cytokine-signaling pathways, patterns of gene and protein expression, and functional responses in mesenchymal cells that lead to deregulated matrix remodeling and relentless fibrosis [2].

The present study attempts to correlate various parameters of lung damage and expression of certain proteins that regulate the cell cycle in an experimental model of ALI.

## Materials and Methods

The experimental protocol was approved by the bioethics committee of the University of Athens. ALI was induced in five groups of mice via inhalation of paraquat [3]. Each group was consisted of 8 animals. Twenty microlitres of PQ solution was applied through the nares. The animals of each group were sacrificed on days 1,2,3,4 and 6, respectively and their lungs were examined for the following parametres: focal thickening of alveolar membranes, capillary congestion, pulmonary oedema, intra-alveolar haemorrhage, interstitial neutrophil infiltration and intra-alveolar neutrophil infiltration scored from 0 to 3 according to the severity of the pathological changes. Specimens (i.e. lung tissue) were also examined with immunohistochemistry for the quantitative expression (percentile) of Ki-67, TUNEL staining (Todd-mediated dUTP nick endlabeling), p21, p16, p27, Cyclin B, Cyclin D1 and Cyclin E. A control group of eight animals that were not exposed to paraquat was also studied. Normality test of Kolmogorov-Smirnov and equal variances test of Levene indicated that the variables did not meet the parametric requirements and therefore comparisons were made with the non-parametric Kruskal-Wallis test. Furthermore, multiple comparisons between all pairs of variables were performed with the Bonferroni test.

Differences between the groups were considered statistically significant when $P$ value was less than 0.05. Statistical analysis was performed using the statistical package SPSS version 12.0.1.

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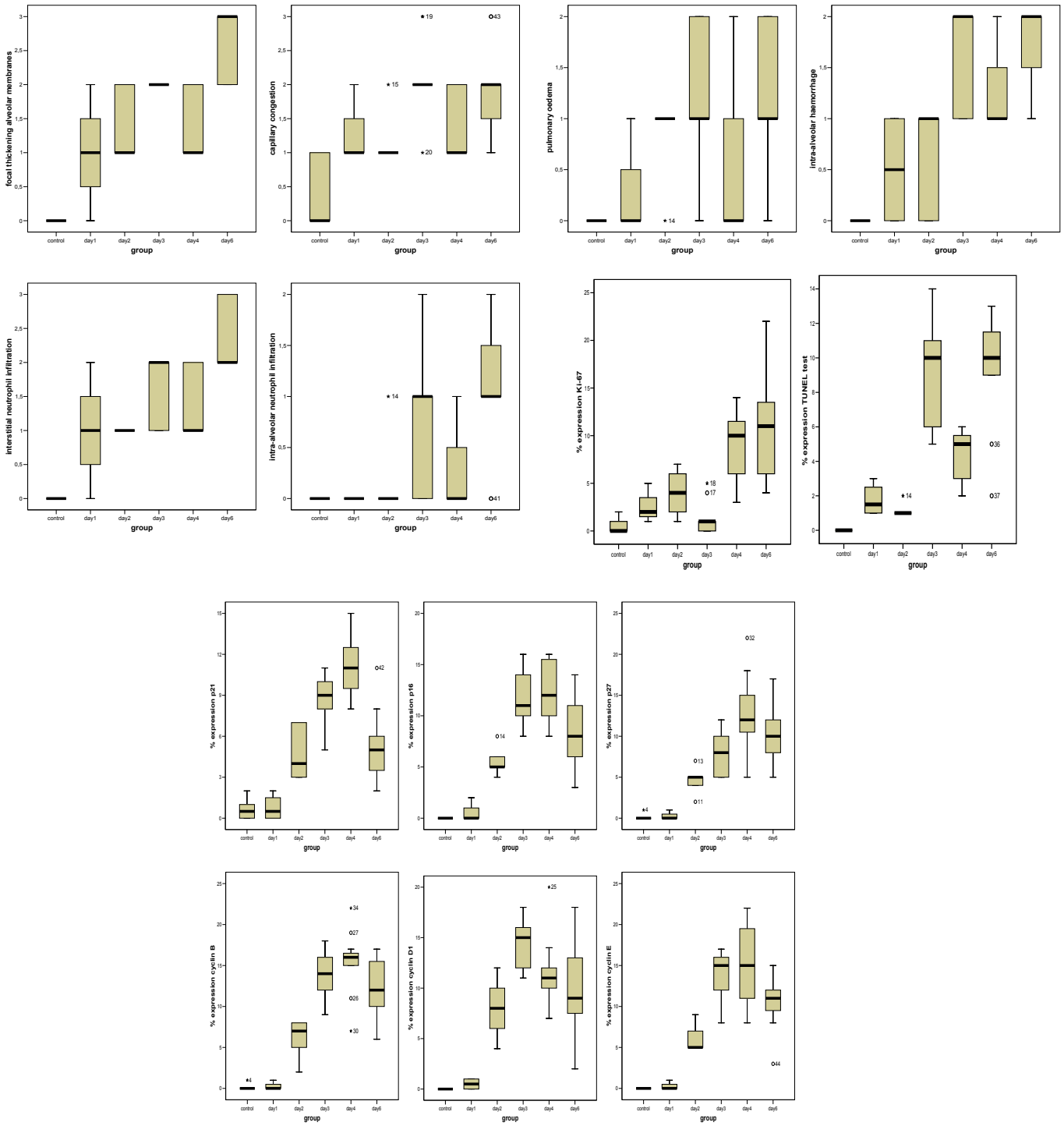


**Figure 1**: Boxplots of the values of each variable, for all groups of animals.

| | Focal thickening of alveolar membranes | Capillary congestion | Pulmonary oedema | Intra-alveolar haemorrhage | Interstitial neutrophil infiltration | Intra-alveolar neutrophil infiltration | Ki-67 % | TUNEL test % | p21 % | p16 % | p27 % | Cyclin B % | Cyclin D1 % | Cyclin E % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | 0 | 0 (0-1) | 0 | 0 | 0 | 0 | 0 (0-2) | 0 | 0.5 (0-2) | 0 | 0 (0-1) | 0 (0-1) | 0 | 0 |
| Day 1 | 1 (0-2) | 1 (1-2) | 0 (0-1) | 0.5 (0-1) | 1 (0-2) | 0 | 2 (1-5) | 1.5 (1-3) | 0.5 (0-2) | 0 (0-2) | 0 (0-1) | 0 (0-1) | 0 (0-1) | 0 (0-1) |
| Day 2 | 1 (1-2) | 1 (1-2) | 1 (0-1) | 1 (0-1) | 1 | 0 (0-1) | 4 (1-7) | 1 (1-2) | 4 (3-7) | 5 (4-8) | 5 (2-7) | 7 (2-8) | 8 (4-12) | 5 (5-9) |
| Day 3 | 2 | 2 (1-3) | 1 (0-2) | 2 (1-2) | 2 (1-2) | 1 (0-2) | 1 (0-5) | 10 (5-14) | 9 (5-11) | 11 (8-16) | 8 (5-12) | 14 (9-18) | 15 (11-18) | 15 (8-17) |
| Day 4 | 1 (1-2) | 1 (1-2) | 0 (0-2) | 1 (1-2) | 1 (1-2) | 0 (0-1) | 10 (3-12) | 5 (2-6) | 11 (8-15) | 12 (8-16) | 12 (5-22) | 16 (7-22) | 11 (7-20) | 15 (8-22) |
| Day 6 | 3 (2-3) | 2 (1-3) | 1 (0-2) | 2 (1-2) | 2 (2-3) | 1 (0-2) | 11 (4-22) | 10 (2-13) | 5 (2-11) | 8 (8-14) | 10 (5-17) | 12 (6-17) | 9 (2-18) | 11 (3-15) |

**Table 1:** Median and range of values for all variables.

| | focal thickening of alveolar membranes | capillary congestion | pulmonary oedema | intra-alveolar haemorrhage | interstitial neutrophil infiltration | intra-alveolar neutrophil infiltration | % expression Ki-67 | % expression TUNEL test | % expression p21 | % expression p16 | % expression p27 | % expression cyclin B | % expression cyclin D1 | % expression cyclin E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chi-Square | 33,523 | 21,803 | 18,236 | 28,149 | 31,234 | 19,196 | 32,011 | 36,001 | 36,042 | 32,825 | 33,272 | 31,454 | 30,130 | 32,874 |
| *P* value | **0.000** | **0.001** | **0.003** | **0.000** | **0.000** | **0.002** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** |

**Table 2:** Comparisons of the median values of all variables with the non-parametric Kruskal-Wallis test.

| Dependent Variable | (I) group | (J) group | Mean Difference (I-J) | Std. Error | *P* value |
|---|---|---|---|---|---|
| focal thickening alveolar membranes | control | day 1 | -1.000 | .297 | .025 |
| | | day 2 | -1.400 | .279 | .000 |
| | | day 3 | -2.000 | .243 | .000 |
| | | day 4 | -1.455 | .234 | .000 |
| | | day 6 | -2.636 | .234 | .000 |
| | day 1 | control | 1.000 | .297 | .025 |
| | | day 2 | -.400 | .309 | 1.000 |
| | | day 3 | -1.000 | .277 | .012 |
| | | day 4 | -.455 | .269 | 1.000 |
| | | day 6 | -1.636 | .269 | .000 |
| | day 2 | control | 1.400 | .279 | .000 |
| | | day 1 | .400 | .309 | 1.000 |
| | | day 3 | -.600 | .257 | .368 |
| | | day 4 | -.055 | .248 | 1.000 |
| | | day 6 | -1.236 | .248 | .000 |
| | day 3 | control | 2.000 | .243 | .000 |
| | | day 1 | 1.000 | .277 | .012 |
| | | day 2 | .600 | .257 | .368 |
| | | day 4 | .545 | .207 | .178 |
| | | day 6 | -.636 | .207 | .057 |
| | day 4 | control | 1.455 | .234 | .000 |
| | | day 1 | .455 | .269 | 1.000 |
| | | day 2 | .055 | .248 | 1.000 |
| | | day 3 | -.545 | .207 | .178 |
| | | day 6 | -1.182 | .196 | .000 |
| | day 6 | control | 2.636 | .234 | .000 |
| | | day 1 | 1.636 | .269 | .000 |
| | | day 2 | 1.236 | .248 | .000 |
| | | day 3 | .636 | .207 | .057 |
| | | day 4 | 1.182 | .196 | .000 |
| | | | -.917 | .374 | .279 |
| capillary congestion | control | day 1 | | | |
| | | day 2 | -.867 | .351 | .267 |
| | | day 3 | -1.778 | .305 | .000 |
| | | day 4 | -1.121 | .294 | .007 |
| | | day 6 | -1.576 | .294 | .000 |
| | day 1 | control | .917 | .374 | .279 |
| | | day 2 | .050 | .388 | 1.000 |
| | | day 3 | -.861 | .348 | .265 |
| | | day 4 | -.205 | .338 | 1.000 |
| | | day 6 | -.659 | .338 | .874 |
| | day 2 | control | .867 | .351 | .267 |
| | | day 1 | -.050 | .388 | 1.000 |
| | | day 3 | -.911 | .323 | .111 |
| | | day 4 | -.255 | .312 | 1.000 |
| | | day 6 | -.709 | .312 | .429 |
| | day 3 | control | 1.778 | .305 | .000 |
| | | day 1 | .861 | .348 | .265 |
| | | day 2 | .911 | .323 | .111 |
| | | day 4 | .657 | .260 | .236 |
| | | day 6 | .202 | .260 | 1.000 |
| | day 4 | control | 1.121 | .294 | .007 |
| | | day 1 | .205 | .338 | 1.000 |
| | | day 2 | .255 | .312 | 1.000 |
| | | day 3 | -.657 | .260 | .236 |
| | | day 6 | -.455 | .247 | 1.000 |
| | day 6 | control | 1.576 | .294 | .000 |
| | | day 1 | .659 | .338 | .874 |
| | | day 2 | .709 | .312 | .429 |
| | | day 3 | -.202 | .260 | 1.000 |
| | | day 4 | .455 | .247 | 1.000 |
| pulmonary oedema | control | day 1 | -.250 | .400 | 1.000 |
| | | day 2 | -.800 | .375 | .587 |
| | | day 3 | -1.111 | .326 | .023 |
| | | day 4 | -.455 | .314 | 1.000 |
| | | day 6 | -1.273 | .314 | .003 |
| | day 1 | control | .250 | .400 | 1.000 |
| | | day 2 | -.550 | .416 | 1.000 |
| | | day 3 | -.861 | .372 | .389 |
| | | day 4 | -.205 | .362 | 1.000 |
| | | day 6 | -1.023 | .362 | .109 |
| | day 2 | control | .800 | .375 | .587 |
| | | day 1 | .550 | .416 | 1.000 |
| | | day 3 | -.311 | .346 | 1.000 |
| | | day 4 | .345 | .334 | 1.000 |
| | | day 6 | -.473 | .334 | 1.000 |
| | day 3 | control | 1.111 | .326 | .023 |
| | | day 1 | .861 | .372 | .389 |
| | | day 2 | .311 | .346 | 1.000 |
| | | day 4 | .657 | .278 | .350 |

| | | | | | |
|---|---|---|---|---|---|
| | | day 6 | -.162 | .278 | 1.000 |
| | day 4 | control | .455 | .314 | 1.000 |
| | | day 1 | .205 | .362 | 1.000 |
| | | day 2 | -.345 | .334 | 1.000 |
| | | day 3 | -.657 | .278 | .350 |
| | | day 6 | -.818 | .264 | .053 |
| | day 6 | control | 1.273 | .314 | .003 |
| | | day 1 | 1.023 | .362 | .109 |
| | | day 2 | .473 | .334 | 1.000 |
| | | day 3 | .162 | .278 | 1.000 |
| | | day 4 | .818 | .264 | .053 |
| intra-alveolar haemorrhage | control | day 1 | -.500 | .303 | 1.000 |
| | | day 2 | -.600 | .284 | .612 |
| | | day 3 | -1.556 | .247 | .000 |
| | | day 4 | -1.273 | .238 | .000 |
| | | day 6 | -1.727 | .238 | .000 |
| | day 1 | control | .500 | .303 | 1.000 |
| | | day 2 | -.100 | .314 | 1.000 |
| | | day 3 | -1.056 | .282 | .008 |
| | | day 4 | -.773 | .274 | .110 |
| | | day 6 | -1.227 | .274 | .001 |
| | day 2 | control | .600 | .284 | .612 |
| | | day 1 | .100 | .314 | 1.000 |
| | | day 3 | -.956 | .261 | .011 |
| | | day 4 | -.673 | .253 | .167 |
| | | day 6 | -1.127 | .253 | .001 |
| | day 3 | control | 1.556 | .247 | .000 |
| | | day 1 | 1.056 | .282 | .008 |
| | | day 2 | .956 | .261 | .011 |
| | | day 4 | .283 | .211 | 1.000 |
| | | day 6 | -.172 | .211 | 1.000 |
| | day 4 | control | 1.273 | .238 | .000 |
| | | day 1 | .773 | .274 | .110 |
| | | day 2 | .673 | .253 | .167 |
| | | day 3 | -.283 | .211 | 1.000 |
| | | day 6 | -.455 | .200 | .426 |
| | day 6 | control | 1.727 | .238 | .000 |
| | | day 1 | 1.227 | .274 | .001 |
| | | day 2 | 1.127 | .253 | .001 |
| | | day 3 | .172 | .211 | 1.000 |
| | | day 4 | .455 | .200 | .426 |
| interstitial neutrophil infiltration | control | day 1 | -1.000(*) | .311 | .039 |
| | | day 2 | -1.000 | .292 | .022 |
| | | day 3 | -1.556 | .254 | .000 |
| | | day 4 | -1.364 | .245 | .000 |
| | | day 6 | -2.364 | .245 | .000 |
| | day 1 | control | 1.000 | .311 | .039 |
| | | day 2 | .000 | .324 | 1.000 |
| | | day 3 | -.556 | .290 | .938 |
| | | day 4 | -.364 | .282 | 1.000 |
| | | day 6 | -1.364 | .282 | .000 |
| | day 2 | control | 1.000 | .292 | .022 |
| | | day 1 | .000 | .324 | 1.000 |
| | | day 3 | -.556 | .269 | .683 |
| | | day 4 | -.364 | .260 | 1.000 |
| | | day 6 | -1.364 | .260 | .000 |
| | day 3 | control | 1.556 | .254 | .000 |
| | | day 1 | .556 | .290 | .938 |
| | | day 2 | .556 | .269 | .683 |
| | | day 4 | .192 | .217 | 1.000 |
| | | day 6 | -.808 | .217 | .009 |
| | day 4 | control | 1.364 | .245 | .000 |
| | | day 1 | .364 | .282 | 1.000 |
| | | day 2 | .364 | .260 | 1.000 |
| | | day 3 | -.192 | .217 | 1.000 |
| | | day 6 | -1.000 | .206 | .000 |
| | day 6 | control | 2.364 | .245 | .000 |
| | | day 1 | 1.364 | .282 | .000 |
| | | day 2 | 1.364 | .260 | .000 |
| | | day 3 | .808 | .217 | .009 |
| | | day 4 | 1.000 | .206 | .000 |
| | | | .000 | .345 | 1.000 |
| intra-alveolar neutrophil infiltration | control | day 1 | | | |
| | | day 2 | -.200 | .324 | 1.000 |
| | | day 3 | -.778 | .282 | .131 |
| | | day 4 | -.273 | .271 | 1.000 |
| | | day 6 | -1.091 | .271 | .004 |
| | day 1 | control | .000 | .345 | 1.000 |
| | | day 2 | -.200 | .359 | 1.000 |
| | | day 3 | -.778 | .321 | .303 |
| | | day 4 | -.273 | .312 | 1.000 |
| | | day 6 | -1.091 | .312 | .018 |
| | day 2 | control | .200 | .324 | 1.000 |
| | | day 1 | .200 | .359 | 1.000 |
| | | day 3 | -.578 | .298 | .899 |
| | | day 4 | -.073 | .289 | 1.000 |
| | | day 6 | -.891 | .289 | .055 |
| | day 3 | control | .778 | .282 | .131 |
| | | day 1 | .778 | .321 | .303 |
| | | day 2 | .578 | .298 | .899 |
| | | day 4 | .505 | .240 | .630 |
| | | day 6 | -.313 | .240 | 1.000 |
| | day 4 | control | .273 | .271 | 1.000 |
| | | day 1 | .273 | .312 | 1.000 |
| | | day 2 | .073 | .289 | 1.000 |
| | | day 3 | -.505 | .240 | .630 |
| | | day 6 | -.818 | .228 | .014 |
| | day 6 | control | 1.091 | .271 | .004 |
| | | day 1 | 1.091 | .312 | .018 |
| | | day 2 | .891 | .289 | .055 |
| | | day 3 | .313 | .240 | 1.000 |
| | | day 4 | .818 | .228 | .014 |
| % expression Ki-67 | control | day 1 | -2.000 | 2.292 | 1.000 |
| | | day 2 | -3.500 | 2.150 | 1.000 |
| | | day 3 | -.944 | 1.871 | 1.000 |
| | | day 4 | -8.409 | 1.802 | .001 |
| | | day 6 | -10.136 | 1.802 | .000 |
| | day 1 | control | 2.000 | 2.292 | 1.000 |
| | | day 2 | -1.500 | 2.382 | 1.000 |
| | | day 3 | 1.056 | 2.133 | 1.000 |
| | | day 4 | -6.409 | 2.073 | .054 |
| | | day 6 | -8.136 | 2.073 | .005 |
| | day 2 | control | 3.500 | 2.150 | 1.000 |
| | | day 1 | 1.500 | 2.382 | 1.000 |
| | | day 3 | 2.556 | 1.980 | 1.000 |
| | | day 4 | -4.909 | 1.915 | .213 |
| | | day 6 | -6.636 | 1.915 | .019 |

| | | | | | |
|---|---|---|---|---|---|
| | day 3 | control | .944 | 1.871 | 1.000 |
| | | day 1 | -1.056 | 2.133 | 1.000 |
| | | day 2 | -2.556 | 1.980 | 1.000 |
| | | day 4 | -7.465 | 1.596 | .000 |
| | | day 6 | -9.192 | 1.596 | .000 |
| | day 4 | control | 8.409 | 1.802 | .001 |
| | | day 1 | 6.409 | 2.073 | .054 |
| | | day 2 | 4.909 | 1.915 | .213 |
| | | day 3 | 7.465 | 1.596 | .000 |
| | | day 6 | -1.727 | 1.514 | 1.000 |
| | day 6 | control | 10.136 | 1.802 | .000 |
| | | day 1 | 8.136 | 2.073 | .005 |
| | | day 2 | 6.636 | 1.915 | .019 |
| | | day 3 | 9.192 | 1.596 | .000 |
| | | day 4 | 1.727 | 1.514 | 1.000 |
| % expression TUNEL test | control | day 1 | -1.750 | 1.480 | 1.000 |
| | | day 2 | -1.200 | 1.389 | 1.000 |
| | | day 3 | -9.222 | 1.209 | .000 |
| | | day 4 | -4.364 | 1.164 | .008 |
| | | day 6 | -9.455 | 1.164 | .000 |
| | day 1 | control | 1.750 | 1.480 | 1.000 |
| | | day 2 | .550 | 1.538 | 1.000 |
| | | day 3 | -7.472 | 1.378 | .000 |
| | | day 4 | -2.614 | 1.339 | .870 |
| | | day 6 | -7.705 | 1.339 | .000 |
| | day 2 | control | 1.200 | 1.389 | 1.000 |
| | | day 1 | -.550 | 1.538 | 1.000 |
| | | day 3 | -8.022 | 1.279 | .000 |
| | | day 4 | -3.164 | 1.237 | .217 |
| | | day 6 | -8.255 | 1.237 | .000 |
| | day 3 | control | 9.222 | 1.209 | .000 |
| | | day 1 | 7.472 | 1.378 | .000 |
| | | day 2 | 8.022 | 1.279 | .000 |
| | | day 4 | 4.859 | 1.031 | .000 |
| | | day 6 | -.232 | 1.031 | 1.000 |
| | day 4 | control | 4.364 | 1.164 | .008 |
| | | day 1 | 2.614 | 1.339 | .870 |
| | | day 2 | 3.164 | 1.237 | .217 |
| | | day 3 | -4.859 | 1.031 | .000 |
| | | day 6 | -5.091 | .978 | .000 |
| | day 6 | control | 9.455 | 1.164 | .000 |
| | | day 1 | 7.705 | 1.339 | .000 |
| | | day 2 | 8.255 | 1.237 | .000 |
| | | day 3 | .232 | 1.031 | 1.000 |
| | | day 4 | 5.091 | .978 | .000 |
| % expression p21 | control | day 1 | -.083 | 1.319 | 1.000 |
| | | day 2 | -4.133 | 1.237 | .027 |
| | | day 3 | -8.222 | 1.077 | .000 |
| | | day 4 | -10.515 | 1.037 | .000 |
| | | day 6 | -4.515 | 1.037 | .001 |
| | day 1 | control | .083 | 1.319 | 1.000 |
| | | day 2 | -4.050 | 1.371 | .078 |
| | | day 3 | -8.139 | 1.228 | .000 |
| | | day 4 | -10.432 | 1.193 | .000 |
| | | day 6 | -4.432 | 1.193 | .009 |
| | day 2 | control | 4.133 | 1.237 | .027 |
| | | day 1 | 4.050 | 1.371 | .078 |
| | | day 3 | -4.089 | 1.140 | .014 |
| | | day 4 | -6.382 | 1.102 | .000 |
| | | day 6 | -.382 | 1.102 | 1.000 |

| | | | | | |
|---|---|---|---|---|---|
| | day 3 | control | 8.222 | 1.077 | .000 |
| | | day 1 | 8.139 | 1.228 | .000 |
| | | day 2 | 4.089 | 1.140 | .014 |
| | | day 4 | -2.293 | .919 | .252 |
| | | day 6 | 3.707 | .919 | .004 |
| | day 4 | control | 10.515 | 1.037 | .000 |
| | | day 1 | 10.432 | 1.193 | .000 |
| | | day 2 | 6.382 | 1.102 | .000 |
| | | day 3 | 2.293 | .919 | .252 |
| | | day 6 | 6.000 | .871 | .000 |
| | day 6 | control | 4.515 | 1.037 | .001 |
| | | day 1 | 4.432 | 1.193 | .009 |
| | | day 2 | .382 | 1.102 | 1.000 |
| | | day 3 | -3.707 | .919 | .004 |
| | | day 4 | -6.000 | .871 | .000 |
| % expression p16 | control | day 1 | -.500 | 1.691 | 1.000 |
| | | day 2 | -5.600 | 1.586 | .016 |
| | | day 3 | -11.778 | 1.381 | .000 |
| | | day 4 | -12.273 | 1.329 | .000 |
| | | day 6 | -8.364 | 1.329 | .000 |
| | day 1 | control | .500 | 1.691 | 1.000 |
| | | day 2 | -5.100 | 1.757 | .090 |
| | | day 3 | -11.278 | 1.574 | .000 |
| | | day 4 | -11.773 | 1.529 | .000 |
| | | day 6 | -7.864 | 1.529 | .000 |
| | day 2 | control | 5.600 | 1.586 | .016 |
| | | day 1 | 5.100 | 1.757 | .090 |
| | | day 3 | -6.178 | 1.461 | .002 |
| | | day 4 | -6.673 | 1.413 | .000 |
| | | day 6 | -2.764 | 1.413 | .862 |
| | day 3 | control | 11.778 | 1.381 | .000 |
| | | day 1 | 11.278 | 1.574 | .000 |
| | | day 2 | 6.178 | 1.461 | .002 |
| | | day 4 | -.495 | 1.177 | 1.000 |
| | | day 6 | 3.414 | 1.177 | .091 |
| | day 4 | control | 12.273 | 1.329 | .000 |
| | | day 1 | 11.773 | 1.529 | .000 |
| | | day 2 | 6.673 | 1.413 | .000 |
| | | day 3 | .495 | 1.177 | 1.000 |
| | | day 6 | 3.909 | 1.117 | .017 |
| | day 6 | control | 8.364 | 1.329 | .000 |
| | | day 1 | 7.864 | 1.529 | .000 |
| | | day 2 | 2.764 | 1.413 | .862 |
| | | day 3 | -3.414 | 1.177 | .091 |
| | | day 4 | -3.909 | 1.117 | .017 |
| % expression p27 | control | day 1 | -.083 | 1.998 | 1.000 |
| | | day 2 | -4.433 | 1.874 | .344 |
| | | day 3 | -7.500 | 1.631 | .001 |
| | | day 4 | -12.652 | 1.571 | .000 |
| | | day 6 | -10.288 | 1.571 | .000 |
| | day 1 | control | .083 | 1.998 | 1.000 |
| | | day 2 | -4.350 | 2.076 | .638 |
| | | day 3 | -7.417 | 1.860 | .004 |
| | | day 4 | -12.568 | 1.807 | .000 |
| | | day 6 | -10.205 | 1.807 | .000 |
| | day 2 | control | 4.433 | 1.874 | .344 |
| | | day 1 | 4.350 | 2.076 | .638 |
| | | day 3 | -3.067 | 1.726 | 1.000 |
| | | day 4 | -8.218 | 1.669 | .000 |
| | | day 6 | -5.855 | 1.669 | .017 |

| | | | | | |
|---|---|---|---|---|---|
| | day 3 | control | 7.500 | 1.631 | .001 |
| | | day 1 | 7.417 | 1.860 | .004 |
| | | day 2 | 3.067 | 1.726 | 1.000 |
| | | day 4 | -5.152 | 1.391 | .010 |
| | | day 6 | -2.788 | 1.391 | .778 |
| | day 4 | control | 12.652 | 1.571 | .000 |
| | | day 1 | 12.568 | 1.807 | .000 |
| | | day 2 | 8.218 | 1.669 | .000 |
| | | day 3 | 5.152 | 1.391 | .010 |
| | | day 6 | 2.364 | 1.320 | 1.000 |
| | day 6 | control | 10.288 | 1.571 | .000 |
| | | day 1 | 10.205 | 1.807 | .000 |
| | | day 2 | 5.855 | 1.669 | .017 |
| | | day 3 | 2.788 | 1.391 | .778 |
| | | day 4 | -2.364 | 1.320 | 1.000 |
| Dependent Variable | (I) group | | | | |
| % expression cyclin B | control | | | | |
| | | day 2 | -5.833 | 1.858 | .048 |
| | day 1 | | | | |
| | | day 2 | -5.750 | 2.058 | .119 |
| | | day 3 | -13.528 | 1.843 | .000 |
| | | day 4 | -15.114 | 1.791 | .000 |
| | | day 6 | -12.205 | 1.791 | .000 |
| | day 2 | control | 5.833 | 1.858 | .048 |
| | | day 1 | 5.750 | 2.058 | .119 |
| | | day 3 | -7.778 | 1.711 | .001 |
| | | day 4 | -9.364 | 1.655 | .000 |
| | | day 6 | -6.455 | 1.655 | .005 |
| | day 3 | control | 13.611 | 1.617 | .000 |
| | | day 1 | 13.528 | 1.843 | .000 |
| | | day 2 | 7.778 | 1.711 | .001 |
| | | day 4 | -1.586 | 1.379 | 1.000 |
| | | day 6 | 1.323 | 1.379 | 1.000 |
| | day 4 | control | 15.197 | 1.557 | .000 |
| | | day 1 | 15.114 | 1.791 | .000 |
| | | day 2 | 9.364 | 1.655 | .000 |
| | | day 3 | 1.586 | 1.379 | 1.000 |
| | day 6 | day 6 | 2.909 | 1.308 | .478 |
| | | control | 12.288 | 1.557 | .000 |
| | | day 1 | 12.205 | 1.791 | .000 |
| | | day 2 | 6.455 | 1.655 | .005 |
| | | day 3 | -1.323 | 1.379 | 1.000 |
| | | day 4 | -2.909 | 1.308 | .478 |
| % expression cyclin D1 | control | day 1 | -.500 | 2.124 | 1.000 |
| | | day 2 | -8.000 | 1.992 | .004 |
| | | day 3 | -14.222 | 1.734 | .000 |
| | | day 4 | -11.455 | 1.670 | .000 |
| | | day 6 | -9.818 | 1.670 | .000 |
| | day 1 | control | .500 | 2.124 | 1.000 |
| | | day 2 | -7.500 | 2.207 | .023 |
| | | day 3 | -13.722 | 1.977 | .000 |
| | | day 4 | -10.955 | 1.921 | .000 |
| | | day 6 | -9.318 | 1.921 | .000 |
| | day 2 | control | 8.000 | 1.992 | .004 |
| | | day 1 | 7.500 | 2.207 | .023 |
| | | day 3 | -6.222 | 1.835 | .024 |
| | | day 4 | -3.455 | 1.774 | .879 |
| | | day 6 | -1.818 | 1.774 | 1.000 |
| | day 3 | control | 14.222 | 1.734 | .000 |
| | | day 1 | 13.722 | 1.977 | .000 |
| | | day 2 | 6.222 | 1.835 | .024 |
| | | day 4 | 2.768 | 1.479 | 1.000 |
| | | day 6 | 4.404 | 1.479 | .074 |
| | day 4 | control | 11.455 | 1.670 | .000 |
| | | day 1 | 10.955 | 1.921 | .000 |
| | | day 2 | 3.455 | 1.774 | .879 |
| | | day 3 | -2.768 | 1.479 | 1.000 |
| | | day 6 | 1.636 | 1.403 | 1.000 |
| | day 6 | control | 9.818 | 1.670 | .000 |
| | | day 1 | 9.318 | 1.921 | .000 |
| | | day 2 | 1.818 | 1.774 | 1.000 |
| | | day 3 | -4.404 | 1.479 | .074 |
| | | day 4 | -1.636 | 1.403 | 1.000 |
| % expression cyclin E | control | day 1 | -.250 | 2.111 | 1.000 |
| | | day 2 | -6.200 | 1.980 | .049 |
| | | day 3 | -13.889 | 1.723 | .000 |
| | | day 4 | -15.364 | 1.660 | .000 |
| | | day 6 | -10.455 | 1.660 | .000 |
| | day 1 | control | .250 | 2.111 | 1.000 |
| | | day 2 | -5.950 | 2.194 | .147 |
| | | day 3 | -13.639 | 1.965 | .000 |
| | | day 4 | -15.114 | 1.909 | .000 |
| | | day 6 | -10.205 | 1.909 | .000 |
| | day 2 | control | 6.200 | 1.980 | .049 |
| | | day 1 | 5.950 | 2.194 | .147 |
| | | day 3 | -7.689 | 1.824 | .002 |
| | | day 4 | -9.164 | 1.764 | .000 |
| | | day 6 | -4.255 | 1.764 | .308 |
| | day 3 | control | 13.889 | 1.723 | .000 |
| | | day 1 | 13.639 | 1.965 | .000 |
| | | day 2 | 7.689 | 1.824 | .002 |
| | | day 4 | -1.475 | 1.470 | 1.000 |
| | | day 6 | 3.434 | 1.470 | .368 |
| | day 4 | control | 15.364 | 1.660 | .000 |
| | | day 1 | 15.114 | 1.909 | .000 |
| | | day 2 | 9.164 | 1.764 | .000 |
| | | day 3 | 1.475 | 1.470 | 1.000 |
| | | day 6 | 4.909 | 1.394 | .016 |
| | day 6 | control | 10.455 | 1.660 | .000 |
| | | day 1 | 10.205 | 1.909 | .000 |
| | | day 2 | 4.255 | 1.764 | .308 |
| | | day 3 | -3.434 | 1.470 | .368 |
| | | day 4 | -4.909 | 1.394 | .016 |

**Table 3:** Multiple comparisons between all pairs of variables with the Bonferroni test.

## Results

Median and range of values for all variables are presented in Table 1 whereas Figure 1 contains the boxplots of each variable for all groups of animals. As shown in Tables 2 and 3, all variables were significantly altered in all the study groups as compared with the control group suggesting the deleterious effect of the paraquat inhalation. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes.

Multiple comparisons showed significantly higher mean differences

in TUNEL staining and in the expression of ki-67, cyclins and CDKIs particularly after 2-3 days from the paraquat inhalation.

## Discussion

ALI is a complex multifactorial process with wide heterogeneity of disease manifestations and outcomes. To optimize the understanding of the genetic basis for ALI, a major focus has been on candidate genes that are prominently involved in the primary pathophysiology of the disease, which includes widespread endothelial and epithelial disruption leading to increased permeability, protein-rich pulmonary oedema, neutrophilic inflammation, and surfactant dysfunction [4,5]. A common feature, regardless of the aetiological basis for disease, is an imbalance between pro- and anti-inflammatory cytokines, oxidants and antioxidants, procoagulants and anticoagulants, neutrophil recruitment/activation and clearance, proteases and protease inhibitors [1]. The pathogenesis of ALI is similar to that of sepsis, as both involve uncontrolled host defense responses that lead to inflammation, endothelial damage, enhanced coagulation, diminished fibrinolysis, and fibroproliferation [6]. Acute lung injury produced by paraquat causes progressive pulmonary insufficiency. Because of selective accumulation in the lungs, it causes severe lung injury manifested by edema, hemorrhage, interstitial inflammation, and progressive fibrosis [1,3]. Paraquat-induced lung injury is described on day 5 after PQ exposure in mice as pulmonary oedema, inflammatory cell recruitment, hemorrhage and light fibrosis [3]. Excessive productions of oxygen free radicals as well as lipid peroxidation are contributing factors for PQinduced acute lung injury [7]. The cell cycle is regulated by various proteins among which cyclins play a keyrole. Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (CDK) enzymes. On the other hand, cyclin-dependent kinase inhibitors (CDKIs) are proteins that bind to and inhibit the activity of CDKs. CDKIs are capable of suppressing growth and there is strong evidence suggesting that at least some CDKIs may be tumor suppressor proteins [8]. In the present study, the expression of cyclins B, D1 and E was examined as well as the expression of CDKIs p21, p16 and p27. The Ki-67 protein is a cellular marker for proliferation [9]. It is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). The TdT mediated dUTP nick end-labeling (TUNEL) method has been employed widely to demonstrate apoptotic cells in routinely prepared paraffin sections.

In the present study, an experimental model of paraquat-induced ALI was applied. Focal thickening of alveolar membranes and intra-alveolar haemorrhage were the more significant pathological changes. Two to three days after the lung damage, cellular proliferation was significantly increased as reflected by the expression of Ki-67. The expression of cyclins (B, D1, E) and CDKIs (p21, p16, p27) was also found at high levels. Furthermore, TUNEL test suggested increased apoptosis. These findings implicate that lung tissue damage is characterized by enhanced cellular proliferation and apoptosis both being regulated by complex interactions of cyclins and CDKIs. The outcome of the above process is remodeling of the lung architecture. Apoptosis is a form of regulated cell death in which activation of specific intracellular serine rich proteases (carpases) leads to cleavage and cell death. The literature confirms the presence of apoptosis in the alveolar and bronchial epithelial cells as an early feature in ALI [10]. Alveolar cell apoptosis likely contributes to ALI pathogenesis in response to various environmental stimuli, by induction of endothelial and epithelial barrier dysfunction [11]. While apoptosis may induce barrier dysfunction early in ALI, increasing evidence suggests that apoptosis plays a beneficial role during ALI resolution. In fact, apoptosis may limit the duration of pulmonary inflammation by shortening neutrophil lifespan [12]. The beneficial influence of apoptosis in ALI can be further explained by the proregenerative role of clearance of apoptotic cells. This beneficial effect is mediated via the production of growth factors, including vascular endothelial growth factor (VEGF), from macrophages engulfing apoptotic cells [13].

Further studies need to elucidate the complex interactions of cyclins and CDKIs in ALI pathogenesis.

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