

Effects of Bedding Change Frequency on Lipid Peroxidation, Antioxidant Status, and Histopathological Alterations in Rats

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ABSTRACT

The aim of this research was to evaluate the effect of bedding change frequency on oxidative stress parameters of rats. In the experiment; animals were randomly and equally divided into 4 groups, each group consisted of 6 male rats- Group 1; bedding change every day, Group 2; bedding change once in 2 days, Group 3; bedding change once in 4 days, and Group 4; bedding change once in a week. Dust-free wood shavings were used as bedding material and the study lasted for 2 months. At the end of experimental period, MDA levels were found to be increased in blood, liver, kidney, heart, brain, and lung of groups with longer intervals of bedding change whereas GSH levels of these tissues were decreased. It was also found that SOD and CAT activities were higher in erythrocyte and lung tissues in Group 1 than the other groups. Moreover, notable histopathological alterations were observed in the tissues of longer intervals of bedding change (especially, group 3 and 4). As a result, it has been determined that long periods of bedding change in animals causes oxidative stress, tissues damages, and these alterations adversely affect the life quality of laboratory animals.

Keywords: bedding, rat, oxidative stress

Sıçanlarda Altlık Değişim Sıklığının Lipid Peroksidasyon, Antioksidan Durum ve Histopatolojik Değişiklikler Üzerine Etkileri

ÖZ

Bu araştırmanın amacı sıçanlarda altlık değişim sıklığının oksidatif stres parametreleri üzerine etkisi değerlendirmektir. Çalışmada hayvanlar her grupta 6 erkek sıçan olacak şekilde rastgele ve eşit olarak 4 gruba ayrıldı. Gruplar: Grup 1; her gün altlığı değişen, Grup 2; 2 günde bir altlığı değişen, Grup 3; 4 günde bir altlığı değişen ve Grup 4; haftada bir altlığı değişen şeklinde oluşturuldu. Çalışma 2 ay olacak şekilde planlandı ve altlık malzemesi olarak tozsuz ağaç talaşı kullanıldı. Çalışmanın sonunda, daha uzun aralıklarla altlık değişimi olan gruplarda kan, karaciğer, böbrek, kalp, beyin ve akciğer dokularında MDA düzeylerinin arttığı, bu dokulardaki GSH düzeylerinin ise azaldığı görülmüştür. Ayrıca, eritrosit ve akciğer dokularında SOD ve CAT aktivitelerinin Grup 1'de, diğer gruplardan daha yüksek olduğu belirlendi. Ayrıca, daha uzun aralıklarla altlıkları değişen grupların (özellikle grup 3 ve 4) beyin, akciğer, kalp, karaciğer ve böbrek dokularında histopatolojik değişiklikler gözlemlendi. Sonuç olarak, hayvanlarda uzun aralıklarla altlık değişiminin oksidatif strese, doku hasarlarına yol açarak laboratuvar hayvanlarının yaşam kalitesini olumsuz etkilediği belirlenmiştir.

Anahtar Kelimeler: altlık, sıçan, oksidatif stress

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INTRODUCTION

One of the main considerations of animal welfare is animal microenvironment. Microenvironment comprises bedding change frequency, bedding material, and housing density (Domer et al. 2012). At the laboratory, rat cages are regularly cleaned between 2-3 times per week depending on animal facility conditions (Mason et al. 2006). In the cleaning process of animals, they are moved from one cage to another or their cages are transported with animals inside a laboratory or between laboratories which are routine animal facility procedures (Castelhano-Carlos and Baumans 2009). The cleaning process may affect acute behavioural and physiological responses of rats; they react to these procedures (Mason et al. 2006; Castelhano-Carlos and Baumans 2009). Bedding type, is a vital factor for laboratory animals, has an impact on the health and well-being of laboratory animals. Various bedding materials are preferred for laboratory animals such as wood shavings, paper, corn cobs, and chips (Potgieter and Wilke 1993; Yildirim et al. 2017). Bedding materials are closely related to aeration and moisture content which affects microbial quality (Potgieter and Wilke 1993). Wood shavings have recently been widely preferred as bedding material choice for laboratory animals. They are composed of fine particles of wood, also it is low-cost material. (Dean 1999; Yildirim et al. 2017).

The imbalance between the production of free radicals and antioxidants causes oxidative stress. This imbalance leads to biomolecular (lipids, proteins, and DNA) and cellular damage so it has an essential impact on the organism (Durackova 2010). The harmful effects of free radicals are balanced by the action of antioxidants (Valko 2006). One of the main biomarkers of oxidative stress parameter is malondialdehyde (MDA). Also, the measurements of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) take a pivotal place to determine oxidative status (Sisein 2014).

In this study, we aimed to evaluate the effect of bedding change frequency on live weight gain, oxidative stress parameters, and histopathological changes of tissues of rats.

MATERIAL and METHODS

In the study, dust-free wood shavings were used as bedding material. All reagents and chemicals were obtained from commercial sources. Healthy male 60 days of age Wistar albino rats (250–300 g) were obtained from Animal Breeding Laboratories of Afyon Kocatepe University, Turkey.

The rats were housed under optimal conditions (25 °C, 50–55% relative humidity, 12 h/12h light/dark cycle). Also, standard rodent diet and clean water

were given *ad libitum* to animals. In addition, animals were acclimatised to the condition of the animal breeding laboratory for 7 days before the experiment started.

The rats were randomly divided into 4 equal groups; each group included 6 male rats. The experimental period was 60 days and the experimental groups were as follows:

Group 1; the bedding was changed every day. Group 2; the bedding was changed every 2 days. Group 3; the bedding was changed every 4 days. Group 4; the bedding was changed one a week. The protocols of experimental design were also approved by the Animal Care and Use Committee of Afyon Kocatepe University (Reference Number: 49533702-73) and were consonant with the principles of NIH.

Erythrocytes preparation was performed according to the method of Winterbourn et al. (1975) and homogenates were prepared according to Kucukkurt et al. (2008).

Kidney, liver, heart, brain, and lung tissues from each animal were collected from rats. Then, their tissues stained with haematoxylin-eosin (H&E) which were analyzed under Olympus light microscope (Bx51) equipped with a camera (Olympus DP20 Tokyo, Japan).

MDA (Draper and Hardley 1990; Ohkawa et al. 1979) and GSH (Beutler et al. 1993) levels of whole blood and tissue homogenates were determined based on spectrophotometric methods. Also, SOD (Sun et al. 1988) and CAT (Luck 1955; Aebi 1974) activities of erythrocyte lysate and tissue homogenate were measured by spectrophotometrically. The haemoglobin and protein contents of tissues were assayed according to methods of Drabkin and Austin (1935), and Lowry et al. (1951), respectively.

Statistical analyses

Obtained data were analysed using one-way ANOVA by using SPSS (20.0). Data were stated as mean and standard deviation (\pm SD). Also, differences between the groups were specified by Duncan post-hoc test. A difference was considered to be significant as $p < 0.05$.

RESULTS

Effect on body weight gain

The effect of bedding change frequency on body weight gain of animals was investigated from the beginning of the study. It was found statistically insignificant ($p > 0.05$) and shown in Figure 1.

Effect on MDA and GSH

MDA is a naturally occurring end product of lipid peroxidation. The effect on bedding change

frequency on MDA levels of blood, kidney, liver, heart, brain, and lung tissues was investigated.

MDA levels of blood, kidney, liver, heart, brain, and lung tissues were found to be high level in the long interval of bedding change groups compared to group 1 ($p < 0.05$) and shown in Table 1. On the contrary, GSH levels of blood, kidney, liver, heart, brain, and lung tissues were found to be low in the long interval of bedding change groups compared to group 1 ($p < 0.05$) and shown in Table 2.

Effects on SOD and CAT activities

SOD and CAT (as antioxidant enzymes) activities were determined in erythrocyte, kidney, liver, heart, brain, and lung tissues of rats and they are shown in Table 3 and Table 4, respectively. SOD and CAT activities were found to be low in erythrocyte and lung in the long interval of bedding change groups compared to group 1 ($p < 0.05$). In addition, SOD and

CAT activities were found to be statistically insignificant in the other tissues.

Histopathological examination

Histopathological alterations in brain, lung, heart, liver, and kidney of experimental groups were shown in Figure 2. Neuronal degeneration and focal gliosis in brain (Figure 2A4), oedema and interalveolar septal thickening in lung (Figure 2B3 and B4), slight hyaline degeneration in heart (Figure 2C4), sinusoidal hyperemia (Figure 2D3), degeneration and necrosis in vena centralis of liver (Figure 2D4) have been observed in group 3 and group 4. Also, there was a focal mononuclear cell infiltration in kidney and necrotic changes in tubulus (Figure 2E4). In group 1 and group 2, no fundamental histopathological changes were observed in brain, lung, heart, liver, and kidney of rats (Figure 2A-E1, and 2A-E2, respectively).

Table 1. Effects of bedding change frequency on glutathione levels in blood, kidney, liver, heart, brain, and lung tissues of male rats.

| | Blood (nmol/ml) | Kidney (nmol/g tissue) | Liver (nmol/g tissue) | Heart (nmol/g tissue) | Brain (nmol/g tissue) | Lung (nmol/g tissue) |
|---------|-------------------------|---------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Group 1 | 3.32±0.52 ^d | 4.52±0.59 ^b | 3.25±0.49 ^b | 3.49±0.83 ^b | 6.55±1.15 ^b | 4.16±0.52 ^b |
| Group 2 | 4.58±0.26 ^c | 4.54±0.43 ^b | 4.24±0.62 ^a | 4.33±0.76 ^b | 6.86±0.86 ^b | 4.33±0.76 ^b |
| Group 3 | 6.01±0.91 ^b | 4.53±0.71 ^b | 4.22±0.48 ^a | 9.08±1.12 ^a | 7.08±0.55 ^b | 9.21±1.76 ^a |
| Group 4 | 11.15±1.25 ^a | 5.42±0.57 ^a | 4.54±0.56 ^a | 9.26±0.91 ^a | 8.74±1.00 ^a | 9.26±0.91 ^a |

Values are mean ± Standard deviations; n = 6.

^{a,b,c,d} In the same column values with different letters show statistically significant differences ($p < 0.05$).

Table 2. Effects of bedding change frequency on malondialdehyde levels in blood, kidney, liver, heart, brain, and lung tissues of male rats.

| | Blood (nmol/ml) | Kidney (nmol/g tissue) | Liver (nmol/g tissue) | Heart (nmol/g tissue) | Brain (nmol/g tissue) | Lung (nmol/g tissue) |
|---------|-------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Group 1 | 47.41±4.54 ^a | 23.91±4.07 ^a | 21.61±2.03 ^a | 20.07±1.72 ^a | 19.54±2.05 ^a | 21.14±1.85 ^a |
| Group 2 | 45.09±5.02 ^a | 22.77±5.09 ^{ab} | 19.24±1.37 ^b | 20.00±1.13 ^a | 17.43±1.45 ^b | 19.19±1.45 ^{ab} |
| Group 3 | 34.62±4.55 ^b | 18.65±2.35 ^{bc} | 18.87±1.33 ^b | 19.79±1.59 ^a | 17.19±0.80 ^b | 18.96±3.61 ^{ab} |
| Group 4 | 23.11±3.67 ^c | 18.06±1.18 ^c | 16.63±1.43 ^c | 17.27±1.15 ^b | 15.83±1.19 ^b | 16.85±1.42 ^b |

Values are mean ± Standard deviations; n = 6.

^{a,b,c} In the same column values with different letters show statistically significant differences ($p < 0.05$).

Table 3. Effects of bedding change frequency on superoxide dismutase activities in erythrocyte, kidney, liver, heart, brain, and lung tissues of male rats.

| | Erythrocyte (U/gHb) | Kidney (U/μg protein) | Liver (U/μg protein) | Heart (U/μg protein) | Brain (U/μg protein) | Lung (U/μg protein) |
|---------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Group 1 | 29.18±1.10 ^a | 1.97±0.61 | 2.25±0.60 | 3.70±0.87 | 5.20±1.49 | 6.06±0.92 ^a |
| Group 2 | 18.94±2.65 ^b | 1.75±0.59 | 2.08±0.80 | 4.01±1.20 | 4.44±1.53 | 3.73±0.96 ^b |
| Group 3 | 17.49±1.18 ^b | 2.07±0.78 | 1.78±0.30 | 4.24±1.16 | 3.57±0.83 | 3.66±1.08 ^b |
| Group 4 | 12.41±1.61 ^c | 1.84±0.56 | 2.39±0.50 | 2.97±0.90 | 3.45±0.67 | 3.44±0.88 ^b |

Values are mean ± Standard deviations; n = 6.

^{a,b,c} In the same column values with different letters show statistically significant differences ($p < 0.05$).

Table 4. Effects of bedding change frequency on catalase activities in erythrocyte, kidney, liver, heart, brain, and lung tissues of male rats.

| | Erythrocyte (k/gHb) | Kidney (k/ μ g protein) | Liver (k/ μ g protein) | Heart (k/ μ g protein) | Brain (k/ μ g protein) | Lung (k/ μ g protein) |
|---------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| Group 1 | 15.43 \pm 2.26 ^a | 1.04 \pm 0.27 | 0.81 \pm 0.13 | 0.55 \pm 0.07 | 0.35 \pm 0.10 | 2.47 \pm 0.79 ^a |
| Group 2 | 14.46 \pm 3.86 ^a | 1.26 \pm 0.36 | 0.69 \pm 0.15 | 0.56 \pm 0.07 | 0.26 \pm 0.07 | 1.89 \pm 0.17 ^b |
| Group 3 | 8.82 \pm 1.89 ^b | 1.31 \pm 0.24 | 0.61 \pm 0.07 | 0.55 \pm 0.09 | 0.29 \pm 0.09 | 1.77 \pm 0.34 ^b |
| Group 4 | 4.12 \pm 1.16 ^c | 1.34 \pm 0.39 | 0.74 \pm 0.12 | 0.49 \pm 0.07 | 0.23 \pm 0.05 | 1.67 \pm 0.20 ^b |

Values are mean \pm Standard deviations; n = 6.

^{a,b,c} In the same column values with different letters show statistically significant differences ($p < 0.05$). k; nmol/min

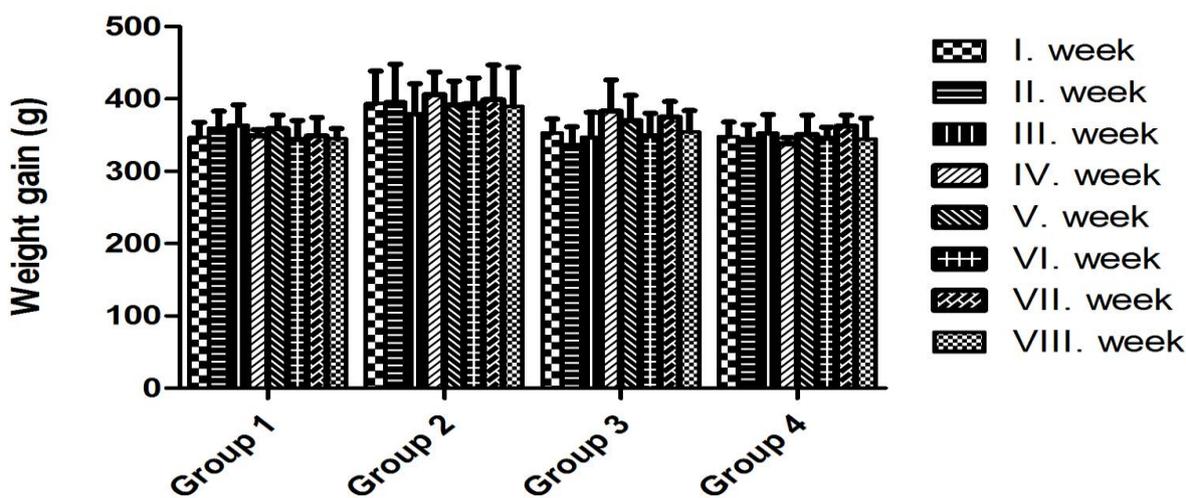


Figure 1. Weight gain of male rats during the experimental period (Mean \pm Standard deviations; n = 6).

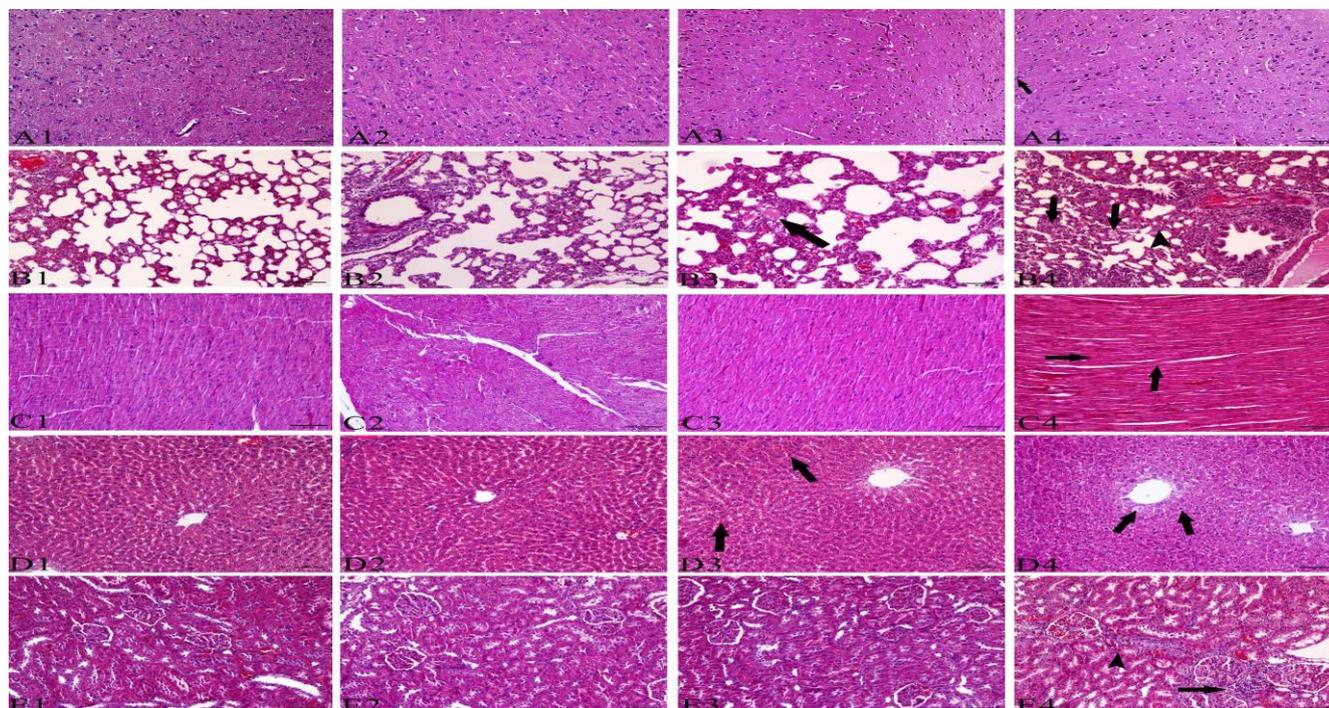


Figure 2. Effect of bedding change frequency in the brain (A), lung (B), heart (C), liver (D), and kidney tissues of male rats. Representative figures were stained with H&E. The original magnification was 20x and the scale bars represent 100 μ m. Arrows and arrowheads indicate degeneration and focal gliosis in neurons of brain (Figure 2A4), oedema in lung (arrow) and thickness in interalveolar septal tissue (arrowheads) (Figure 2B3 and B4), mild hyaline degeneration in heart (Figure 2C4), sinusoidal hyperemia in liver (Figure 2D3), necrosis and degeneration in vena centralis (Figure 2D4), focal mononuclear cell infiltration (arrow) and necrotic alterations in tubules of kidney (arrowhead) (Figure 2E4). (1) Group 1; bedding change every day, (2) Group 2; bedding change once in 2 days, (3) Group 3; bedding change once in 4 days and (4) Group 4; bedding change once in a week.

DISCUSSION

Cage cleaning is important in terms of hygiene. Also, long term of cage cleaning and/or bedding change causes illness in animals. Burn et al. (2006) reported that totally 320 male Sprague Dawley and Wistar rats were kept in cages containing aspen wood or absorbent paper for 5 months and these cages were cleaned twice a week, once a week and once every two weeks. In the study, non-aggressive behaviours were determined at the highest level in once a week cage cleaned group. The incidence of sneezing and the pathological alterations of the lungs in aspen bedding animals have been reported to be higher than those used in paper bedding. At the end of the study, it has been stated that the frequency of cage cleaning affects the rats in terms of social housing but the types of bedding have a significant effect on rat health. On the other hand, Yildirim et al. (2017) performed a study with 48 male and female Sprague-Dawley rats and evaluated the effect bedding types (wood shavings, perlite, and corn cobs) on body weight. They observed that bedding material had no significant effect on body weight among the groups. In accordance with our study, it was observed that weight gain in rats was not affected by bedding change frequency. This may be due to the type of bedding or the duration of the study.

Totally, 48 female Sprague Dawley rats were divided into 4 equal groups and animals were kept in pine chips, eucalyptus pulp, vermiculite, and wire meshed bedding for 14 days. After this period, GSH levels were determined in heart, lung, and liver tissue of rats. Liver and lung GSH levels were observed at the highest level in the pine chips group and the level of heart GSH was the highest in the vermiculite group. Moreover, 48 male and 48 female Sprague-Dawley rats were kept with different types of beddings such as wood shavings, perlite, and corn cob for 30 days. At the end of the study CAT, SOD, GSH, GPx, and MDA parameters of blood samples were evaluated. It was reported that CAT, GPx, SOD, and GSH levels decreased significantly in the perlite groups, whereas MDA levels increased in the perlite groups (Yildirim et al. 2017). In the present study, it was determined that oxidative stress parameters increased in the long interval of bedding change groups. Also, it was observed that intracellular antioxidant enzyme levels in lung and blood tissues were depleted with long interval of bedding change. This situation shows that oxidation is the result of long interval of bedding change and it has a negative effect on the living conditions of animals.

Horn et al. (2012) performed a study which evaluated the effect of bedding type (grinded with poplar, cellulose and 50:50 mixture for 60 days) on lung histopathology of rats (192 male and 192 female). Peribronchial lymphoid hyperplasia was observed in

all groups. The perivascular eosinophilic filtration and metaplasia in the cellulose group, multifocal foreign body bronchopneumonia were observed in aspen and cellulose groups. Focal interstitial pneumonia was also observed in the cellulose group. Effects of sanitation frequency, cage density, and bedding type have effects on animal wellbeing and health of animals. Ferrecchia et al. (2014) evaluated four different bedding materials (1/4 of irradiated corn cob, aspen wood chips, reclaimed wood pulp, and recycled newspaper) in 100 mice. No lesions were detected in a corn cob, paper chip, or aspen chip beddings. However, mice housed on reclaimed wood pulp demonstrated significant nasal pathology which included epithelial necrosis, multifocal submucosal oedema, inflammatory cell infiltrates, haemorrhage, and congestion. In the present study, it was observed that long interval of bedding change caused histopathological changes in the brain, lung, heart, liver, and kidney tissues of the rats.

In conclusion, it was determined that long interval of bedding change in animals triggered oxidative stress, caused cellular damage in tissues and adversely affected the quality of life of the animals.

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