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Effects of 3 Rodent Beddings on Biochemical Measures in Rats and Mice

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Components of bedding might interact with experimental treatments and affect the outcome of various experiments. Here we studied the biochemical effects of 3 rodent bedding materials that are commonly used in Egypt. Male and female rats and mice were assigned randomly into 4 single-sex and single-species groups (10 animals per group). Three types of bedding—rice straw, wheat straw, and pine wood shavings—were evaluated. After 4 wk, animals were euthanized, and biochemical parameters were measured. In male and female rats given wood shavings, serum ALT activity and malondialdehyde concentration increased whereas catalase activity decreased compared with levels in the wheat straw group. In contrast, ALT activity and malondialdehyde concentrations decreased but CAT activity increased in rats housed on rice straw compared with wheat straw. Serum AST and ALT activities increased in male and female mice exposed to rice straw, whereas the malondialdehyde concentration increased and catalase decreased in the wood shavings group relative to the wheat straw group. In mice exposed to wheat straw, AST and ALT activities and malondialdehyde concentrations decreased and CAT activity increased compared with the other groups. Because our results showed that exposure to wood shavings affects some biochemical parameters of rats and mice, we do not recommend its use as laboratory animal bedding. We consider that, of the materials tested, rice straw bedding is the best bedding material for rats, whereas wheat straw is best for mice.

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Numerous factors can affect the microenvironment of rodents and thus study outcomes.[27] One of the most important factors affecting their microenvironment is bedding. Bedding material, which is a significant part of rodent housing, affects the health and wellbeing of laboratory animals.[33] Substances in beddings may bias results and increase variations in pharmacologic and toxicologic studies.[21] Moreover, bedding type selection may correlate to increased husbandry costs due to the need for increased cage changes and to potential negative effects on animal livelihood, including decreases in weight gain.[20] In addition, bedding components may interact with experimental treatments and affect the outcome of various types of experiments, such as those evaluating enzyme induction, the cytotoxicity and carcinogenicity of compounds, and anesthetics.[21] Bedding type and subsequently environmental microflora affect ammonia levels within cages.[12,26]

Rice is one of the most abundant crops in Egypt.[8] In Egypt, processing of rice in the river Nile delta yields large amounts of rice straw as residue. About 20% of this rice straw is used for other purposes, such as the production of ethanol, paper, fertilizers and fodders; the remainder is left on the fields and burned within 30 d. The resulting emissions significantly contribute to the air pollution called the "Black Cloud."[16]

In Egypt, the primary bedding materials used in laboratory animal units are wood shavings and wheat straw. One study[21] found that the corncob extracts, rice hulls, and straw used in a few laboratories were practically nontoxic. Recently, several Egyptian investigators have tended to use rice straw instead of wood shavings as bedding material for rats and mice. To enhance the comparability and reproducibility of results between experiments, the type of bedding should be standardized.[5] Therefore we evaluated the physiologic effects of 3 bedding materials commonly used in Egypt—rice straw, wheat straw, and wood shavings—on mice and rats.

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Materials and Methods

The experimental protocols and procedures used in this study were approved by the IACUC of the Faculty of Science, Cairo University (approval no. CUFS/ F / PHY / 41 / 14). All experimental procedures were performed in accordance with international guidelines regarding the care and use of laboratory animals.

The experimental animals used in this study were albino Wistar rats (*Rattus norvegicus*; weight, 150 to 160 g) and albino Swiss mice (weight, 23 to 25 g) that were obtained from the National Research Center (Dokki, Giza, Egypt). Rats and mice were maintained under a 12:12-h light:dark cycle at 22 to 25 °C in a well-ventilated animal facility (Department of Zoology, Faculty of Science, Cairo University). Autoclaved water and a commercial diet (Laboratory Rodent Diet 5001, LabDiet, St Louis, MO) were provided without restriction. The animals were grouped (10 per cage) and housed in static polycarbonate plastic cages (height, 20 cm; floor space, 860 cm^2). According to sentinel monitoring, rats and mice had conventional (undefined) gastrointestinal tract flora and were SPF for lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, mouse hepatitis virus, mouse parvovirus 1 and 2, mouse minute virus, reovirus 3, pneumonia virus of mice, polyoma virus, Sendai virus, Theiler encephalomyelitis virus, rat theilovirus, rat coronavirus (sialodacryoadenitis), ectromelia, hantavirus, *Bordetella bronchiseptica*, cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, and *Salmonella* spp., endoparasites (pinworms, tapeworms, and *Giardia muris*), and ectoparasites (mites).

Figure 1. Different types of bedding materials.

In this study, 60 rats (30 male and 30 female) and 60 mice (30 male and 30 female) were assigned randomly into 6 groups (10 rats or mice per group). Three types of bedding—rice straw, wheat straw, and pine wood shavings—were sterilized at 130 °C for 20 min, applied to a depth of 2 cm in cages, and changed weekly (Figure 1). After 4 wk, animals were euthanized under deep anesthesia with sodium pentobarbital, blood was collected by cardiocentesis into centrifuge tubes, and tubes were centrifuged at 1008 × *g* for 20 min. Liver and kidney were removed, blotted by using filter paper to remove traces of blood, and homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl (pH 7.4). The homogenate was centrifuged at 1008 × *g* for 15 min at 4 °C. Serum and homogenate supernatants were stored at –80 °C until biochemical analysis. Appropriate kits (Bio-Diagnostics, Dokki, Giza, Egypt) were used for the determination of AST and ALT,[23] albumin,[29] urea,[9] and uric acid,[29] as previously described. In addition, we used appropriate kits (Bio-Diagnostics) to determine the quantities of malondialdehyde[19] and catalase,[1] which are markers of oxidative stress, in the supernatants of the liver and kidney homogenates.

Statistical analysis. Values are expressed as mean ± SE. To evaluate differences between groups, 2-way ANOVA with Duncan posthoc testing (version 15.0, SPSS for Windows) was used to compare group means, with significance defined as a *P* value less than 0.05.

Results

In rats, serum AST, albumin, urea, and uric acid levels did not differ among types of bedding (Table 1). However, ALT activity was higher ($P < 0.05$) in the wood-shavings group and lower in rats bedded on rice straw compared with wheat straw.

In mice, serum albumin, urea, and uric acid levels did not differ among types of bedding (Table 2). However, serum AST and ALT activities were significantly higher ($P < 0.05$) mice housed on rice straw and significantly lower ($P < 0.05$) in wheat straw groups compared with the wood-shavings group.

The renal and hepatic concentrations of malondialdehyde were increased ($P < 0.05$) in rats on wood shavings and decreased ($P < 0.05$) in those on rice straw (Table 3) compared with wheat straw. In contrast, catalase activity in liver and kidney was increased ($P < 0.05$) in rats exposed to rice straw but decreased ($P < 0.05$) in those given wood shavings compared with wheat straw.

Malondialdehyde concentrations in the kidney and liver of mice were significantly higher ($P < 0.05$) when animals were housed on wood shavings but were lower ($P < 0.05$) when they were on wheat straw compared with rice straw groups. In contrast, the renal and hepatic catalase activities were higher ($P < 0.05$) in mice housed on wheat straw but lower ($P < 0.05$) in the wood-shavings groups (Table 4) compared with rice straw groups.

Discussion

The present study was performed to evaluate the biochemical profiles of male and female mice and rats housed on rice straw, wheat straw, and wood shavings as bedding materials. Environmental conditions such as housing and husbandry have a major effect on laboratory animals throughout their lives and thereby influence the outcomes of animal experiments.[4,30] Bedding is one environmental factor that can affect the outcomes of studies using laboratory rodents.[27] Rodent beddings serve to absorb moisture from excrements and provide nesting material. The type of bedding material affects not only the microenvironment of the animal cage[13] but also the environment of the animal room.[25] Furthermore, bedding components may interact with experimental treatments and influence the data from various types of experiments, such as those evaluating enzyme induction, the cytotoxicity and carcinogenicity of compounds, and anesthetics.[21] In addition, previous rodent studies have demonstrated enzyme induction due to direct bedding exposure.[7]

Serum transaminases (ALT and AST) play key roles in animal amino acid metabolism.[24] Serum aminotransferases activities are considered sensitive indicators of liver injury, in that elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of hepatic cell membranes.[19] Furthermore, serum ALT activity is regarded as a reliable and sensitive marker of liver disease.[17]

Table 1. Effects of bedding type on serum biomarkers in rats (n = 10 per group)

	Bedding	AST (U/mL)	ALT (U/mL)	Albumin (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Male rats	Wood shavings	21.35 ± 5.47	50.97 ± 11.62^{b}	3.53 ± 0.26	58.18 ± 1.69	1.76 ± 0.02
	Wheat straw	25.24 ± 1.89	22.65 ± 4.22^{a}	3.19 ± 0.09	52.32 ± 1.27	1.82 ± 0.02
	Rice straw	22.00 ± 1.89	14.88 ± 3.17^{a}	2.83 ± 0.21	56.10 ± 3.25	1.82 ± 0.07
Female rats	Wood shavings	23.78 ± 5.48	32.12 ± 3.26^{b}	3.10 ± 0.12	46.63 ± 3.44	1.75 ± 0.04
	Wheat straw	16.82 ± 3.14	28.65 ± 4.51^{a}	3.10 ± 0.13	45.05 ± 2.98	1.82 ± 0.02
	Rice straw	25.88 ± 2.71	22.65 ± 3.83^{a}	3.37 ± 0.08	48.43 ± 2.27	1.77 ± 0.04

Within each sex and biochemical marker, different superscripted letters indicate significantly ($P < 0.05$) different values.

Table 2. Effects of bedding type on serum biomarkers in mice (n = 10 per group)

	Bedding	AST (U/mL)	ALT (U/mL)	Albumin (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Male mice	Wood shavings	23.82 ± 1.63^{b}	38.17 ± 10.02^{b}	2.37 ± 0.10	54.61 ± 4.66	0.98943 ± 0.44
	Wheat straw	18.59 ± 1.46^{a}	14.25 ± 3.00^{a}	2.40 ± 0.28	53.46 ± 3.78	0.23341 ± 0.10
	Rice straw	29.87 ± 1.65^{c}	64.82 ± 4.47^{c}	2.78 ± 0.13	58.91 ± 2.91	0.12260 ± 0.05
Female mice	Wood shavings	24.95 ± 1.61^{b}	24.15 ± 5.53^{b}	2.41 ± 0.38	49.93 ± 1.77	1.91 ± 0.00
	Wheat straw	19.52 ± 1.45^{a}	10.41 ± 2.37^{a}	2.58 ± 0.12	52.70 ± 4.34	2.12 ± 0.13
	Rice straw	30.23 ± 3.88^{c}	30.41 ± 4.81^{c}	2.95 ± 0.22	54.71 ± 5.44	1.98 ± 0.08

Within each sex and biochemical marker, different superscripted letters indicate significantly ($P < 0.05$) different values.

Table 3. Effects of bedding type on oxidative stress markers in rats (n = 10 per group)

	Bedding	Liver malondialdehyde (nmol/g tissue)	Kidney malondialdehyde (nmol/g tissue)	Liver catalase (U/g protein)	Kidney catalase (U/g protein)
Male rats	Wood shavings	59.17 ± 2.14^{c}	39.45 ± 1.27	1.38 ± 0.13^{a}	3.40 ± 0.11^{a}
	Wheat straw	49.36 ± 1.96^{b}	37.38 ± 1.33	2.31 ± 0.18^{b}	5.98 ± 1.23^{b}
	Rice straw	38.26 ± 1.81^{a}	36.16 ± 1.43	9.24 ± 0.12^{c}	13.82 ± 2.80^{c}
Female rats	Wood shavings	48.51 ± 3.44^{c}	55.45 ± 3.05^{b}	1.60 ± 0.06^{a}	3.29 ± 0.22^{a}
	Wheat straw	42.84 ± 1.65^{b}	47.86 ± 2.61^{a}	2.29 ± 0.11^{b}	9.29 ± 0.37^{b}
	Rice straw	37.64 ± 2.35^{a}	45.22 ± 1.84^{a}	9.35 ± 0.24^{c}	13.59 ± 2.86^{c}

Within each sex and biochemical marker, different superscripted letters indicate significantly ($P < 0.05$) different values.

Table 4. Effects of bedding type on oxidative stress markers in mice (n = 10 per group)

	Bedding	Liver malondialdehyde (nmol/g tissue)	Kidney malondialdehyde (nmol/g tissue)	Liver catalase (U/g protein)	Kidney catalase (U/g protein)
Male mice	Wood shavings	55.52 ± 2.56^{c}	50.92 ± 1.20^{c}	1.32 ± 0.12^{a}	3.73 ± 0.07^{a}
	Wheat straw	42.87 ± 2.38^{a}	40.90 ± 0.86^{a}	5.68 ± 0.36^{c}	13.47 ± 2.89^{c}
	Rice straw	49.19 ± 3.42^{b}	44.49 ± 2.09^{b}	2.06 ± 0.06^{b}	5.29 ± 0.26^{b}
Female mice	Wood shavings	56.53 ± 1.72^{c}	57.99 ± 2.37^{c}	2.12 ± 0.07^{a}	3.46 ± 0.23^{a}
	Wheat straw	41.35 ± 1.74^{a}	42.32 ± 2.40^{a}	9.16 ± 0.18^{c}	13.59 ± 2.86^{c}
	Rice straw	48.53 ± 1.90^{b}	50.68 ± 1.75^{b}	4.17 ± 0.54^{b}	6.94 ± 0.14^{b}

Within each sex and biochemical marker, different superscripted letters indicate significantly ($P < 0.05$) different values.

Our results revealed significantly higher levels of ALT activity in rats housed on pine wood shavings. Several studies[11,15,31,33] are in agreement with our results. These findings may due to the highly cytotoxic effect of wood shavings.[20] In contrast, rats housed on rice showed significantly lower levels of as compared with the wood-shavings groups. In addition, rats have been shown to prefer bedding with a large particle size, such as rice straw.[6]

In mice, despite the potential toxicity of wood shavings, ALT and AST were higher in the rice-straw groups. This effect may be due to the influence of light. Wild mice are typically nocturnal and generally avoid brightly lit areas; therefore most food is consumed during the dark phase.[10] Because rice straw is large, it provides shading that protects mice from most of the light in the housing room, thereby increasing their protein intake and leading to increases in AST and ALT activities relative to those in mice on other beddings.[3,20] This effect may also explain the higher albumin and urea (end products of protein metabolism) in the rice straw groups. However, further study needed to confirm our hypothesis that mice on rice straw consumed more feed than those given wood shavings.

Oxidative stress is defined as an imbalance between prooxidants and antioxidants or results from an impaired antioxidation system.[32] Lipid peroxides, which are derived from polyunsaturated fatty acids, are unstable and can decompose to form a complex series of compounds,[11] including reactive carbonyl compounds, of which the most abundant is malondialdehyde.[2] Catalase is an important enzyme in the defense against reactive oxygen species. Catalase catalyzes the reduction of hydrogen peroxide, thereby protecting cells against oxidative damage.[22]

In our rats and mice, oxidative stress, as measured malondialdehyde catalase levels, was more pronounced in animals given wood shavings as bedding. Ammonia production is very high in wood shavings compared with other bedding materials.[28,32] Ammonia is a highly toxic waste product that is implicated in the pathogenesis of hepatic and kidney injury. The mechanisms of ammonia toxicity involve oxidative stress and mitochondrial dysfunction.[14]

In conclusion, our results showed that wood shavings cause changes in the biochemical parameters of rats and mice. In mice, rice straw bedding increased levels of liver enzymes. Of the materials tested, we conclude that rice straw is preferable for rats and wheat straw for mice.

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