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The ameliorative effect of α-lipoic acid on testicular dysfunction induced by gentamicin.

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ABSTRACT

Drug exposure is a common risk factor for male infertility and should be investigated carefully. This study aimed to evaluate the possible protective effect of α-lipoic acid against gentamicin-induced-testicular damage. Sixty Wister male rats aged 8 weeks and weighted 200±20 gm were randomly assigned to six groups of ten. Groups 1 and 2 were treated with 18.25 and 36.5mg/kg gentamicin, respectively. Groups 3, 4, and 5 were treated with 36.5mg/kg gentamicin plus 100mg/Kg/day α-lipoic acid (ALA), 200mg/Kg/day ALA, and 100mg/kg vitamin E, respectively. Group 6 served as control. All treatments were administered once daily for 14 days. Half the animals in each group were sacrificed on the 15th day, while the second half was sacrificed on the 60th day of the experiment. Blood samples were collected from the retro-orbital venous plexus just before sacrifice. Immediately after the sacrifice, reproductive organs were removed and weighed, and semen was collected for analysis. Groups treated with gentamicin and either ALA or vitamin E showed preserved reproductive organs weight and sperm parameters compared to gentamicin alone on both sacrifice days (p<0.05). Testosterone level was also preserved with either ALA or vitamin-E co-administrated with gentamicin compared to gentamicin alone (p<0.05). However, compared to gentamicin alone, gonadotropin levels showed variable levels with the cotreatments. The hormone levels of all groups were approximately normalized on the second sacrifice day. Pathological lesions induced by gentamicin were markedly reduced upon cotreatment with ALA. Adding α-lipoic acid or vitamin E to gentamicin therapy protects from gentamicin-induced reproductive system impairment in experimental animals.

1. INTRODUCTION

Male infertility is a significant health problem defined as an inability to have a pregnancy after at least one year of frequent and unprotected intercourse (Penzias et al., 2013). Chemical exposure is one of male infertility's most common etiological factors, including drug exposure (Bonde, 2013). Drugs such as anticancer are well-known as risk factors for male infertility (Ragheb and Sabanegh, 2010). However, other drugs, such as some antibiotics and analgesics, could impair male reproductivity based on limited evidence (Olayemi, 2010). An example of potential inducers of male reproductive function impairment is gentamicin antibiotic demonstrated by experimental animal studies (Khaki, 2015). Gentamicin belongs to aminoglycoside antibiotics which possess a broad spectrum against a wide range of aerobic bacteria, including *Pseudomonas aeruginosa* (Lin et al., 2020). It is widely used for human infectious diseases, including urinary tract infection, pneumonia, skin and soft tissue infection, infective endocarditis, bloodstream infection, CNS infection, osteomyelitis, peritonitis, sexually transmitted diseases, and neonatal sepsis (Chaves and Tadi, 2020). Gentamicin can be associated with many adverse events, including nephrotoxicity, ototoxicity, neuromuscular blocking, and respiratory paralysis (Halmagyi and Curthoys, 2018). It may be associated with testicular damage based on animal study data. The demonstrated adverse effects of gentamicin on the reproductive system were dose-dependent and reversible after the normal regeneration of testicular tissues and the production of new spermatocytes (Elsawah et al., 2020). The most proposed underlying mechanism is increasing testicular oxidative stress indicated by antioxidant enzymes level, oxidative stress bioproducts, and gene expression of the antioxidant enzymes (Elsawah et al., 2020). Antioxidants such as alpha lipoic acid (ALA) were studied as a protective agent from gentamicin-induced nephrotoxicity and showed good value (Darwish and El-Lateef, 2018). However, the protective effect of exogenous antioxidants from male reproductive organ impairment needs to be better studied. Therefore, we aimed to investigate the protective potential of ALA from testicular damage induced by gentamicin therapy in comparison to vitamin E indicated by testicular and other reproductive organ weights, sperm analysis, sexual hormone levels, and testicular histopathology.

2. MATERIAL AND METHODS

2.1. Drug preparation:

Gentamicin was purchased under the brand name of Garamycin® ampoules 80 mg/2ml, Schering-Plough

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Corporation U.S.A. ALA was purchased under the generic name of Thiotacid® ampoules 300 mg/ 10 ml aqueous solvent manufactured by EVA Pharmaceutical Industries, Cairo, Egypt. Both gentamicin and ALA were given intraperitoneal with a 25-gauge needle with a final volume of 0.5ml per rat.Vitamin E was purchased under the generic Vitamin E 1000 mg®, Pharco pharmaceuticals. The capsules were evacuated, diluted with sunflower oil to a final volume of 1 ml per rat, and administered orally using 18-gauge soft gavage tubes.

2.2. Animals:
Sixty male Wister rats aged 8 weeks and weighted 200±20g were obtained from the Animal House of the Faculty of Veterinary Medicine, Benha University. The rats were housed at average room temperature (25 - 30°C) with humidity (40-60%) and 12h/12h light/ dark cycle before use in experimental protocols. The animals were fed laboratory formula and tap water ad libitum.

2.3. Study design:
After two weeks of acclimatization to the diet and house conditions, 60 rats were randomly assigned to six groups of ten. Rats in groups 1 and 2 received gentamicin OD (once daily) with 18.25 and 36.5 mg/kg, respectively. Rats in groups 3, 4, and 5 received 36.5 mg/kg gentamicin OD plus 100 mg/kg ALA, 200 mg/Kg ALA /day, and 100 mg/kg vitamin E OD respectively. Group 6 served as a control and received intraperitoneal 0.5 ml NS OD. All treatments were administered for 14 days. The given doses were the average weight-based and body surface area-based doses (Nair and Jacob, 2016). Half the animals in each group were sacrificed by decapitation on day 15th from the start of the treatment (first sacrifice day), while the second half was sacrificed on the 60th day (second sacrifice day). Immediately after sacrifice, the testis, epididymis, prostate, and seminal vesicle were removed and weighed (absolute and relative to body weight. The experiment was carried out in the Departments of Pharmacology and Theriogenology, Faculty of Veterinary Medicine, Benha University. The faculty Ethics committee approved the study with an ethical approval number of 080422.

2.4. Blood sample collection:
Blood samples were collected from the retro-orbital venous plexus located at the medial canthus of the eye utilizing heparinized capillary tubes. Plasma samples were kept frozen (-20°C) for the analysis of sex hormone levels, including testosterone, FSH, and LH, using rat ELISA kits under the commercial name of Fine-Test kits (Wuhan Fine Biotech Co., 2022).

2.5. Histopathology of the testis:
The testis was quickly fixed in a 10% formalin solution. The fixed tissues were dehydrated in graded ascending strengths of ethanol, cleared in xylol, embedded in paraffin, and then 5 µm thick sections were prepared and stained with hematoxylin and eosin (H and E) (Bancroft and Gamble, 2008).

2.6. Sperm parameter assessment:
The tails of the two epididymides were cut into small pieces in a petri dish containing 2 ml of saline (0.9% NaCl) to give sperm. To evaluate sperm count, the epididymal contents extracted in 2 ml normal saline was diluted to 10 ml normal saline and kept for 24 hours to let sperms distribute in the solution (Hackett and Macpherson, 1965). To evaluate progressive motility, a cover slip was placed onto the semen drop, and the percentage of progressively motile sperms was estimated microscopically at 400x magnification. To evaluate sperm morphology and viability, Carnoy's solution and Eosin and Nigrosine stain were used, respectively.
2.7. Statistical analysis:
A one-way analysis of variance (ANOVA) test was used to perform the multi-group comparisons, followed by post hoc Tukey's test for pairwise comparison at 0.05 level of significance.

3. RESULTS

Organ weight:
On the first sacrifice day, there was a significant reduction in the testis weight of animals treated with double dose gentamicin alone compared to double dose ALA-cotreated-, vitamin-E cotreated-, and placebo-treated groups (p<0.05). On the second sacrifice day, the group treated with double-dose gentamicin showed the lowest weight (p<0.05). On both the first and second sacrifice days, the group treated with double-dose gentamicin showed a significant reduction in the epididymis weights compared to the other groups (p<0.05). The group treated with double-dose gentamicin alone showed a significant reduction in the seminal vesicle weights compared to the other groups (p<0.05) on the first sacrifice day. However, on the second sacrifice day, all weights were restored. (Table 1).

Table1 Reproductive organ weights comparison among the groups treated with gentamicin with/without alpha-lipoic acid or vitamin-E, and control.

Sacrifice	Groups	Testis (mg)	Epididymis (mg)	Seminal Vesicle (mg)	Ventral Prostate (mg)
1st	1	1134 ± 20	432 ± 12^{CDE}	448 ± 14^{CE}	472 ± 21
	2	902 ± 30^{CE}	384 ± 11^{CE}	394 ± 15^{CE}	520 ± 13
	3	1125 ± 252	561 ± 8.6^{D}	501 ± 12^{CD}	490 ± 20
	4	1438 ± 41^{D}	592 ± 6.3^{D}	562 ± 13^{D}	498 ± 20
	5	1440 ± 38^{D}	569 ± 12^{D}	550 ± 16^{D}	468 ± 14
	6	1520 ± 30^{D}	556 ± 16^{D}	572 ± 14^{D}	486 ± 18
2nd	1	1588 ± 30^{CD}	876 ± 17^{CDE}	640 ± 22	602 ± 17
	2	1430 ± 3^{CE}	770 ± 23^{CE}	670 ± 40	584 ± 30
	3	1684 ± 41^{D}	1122 ± 17^{D}	670 ± 27	606 ± 28
	4	1740 ± 37^{D}	1184 ± 13^{D}	738 ± 27	628 ± 24
	5	1730 ± 32^{D}	1138 ± 25^{D}	704 ± 52	572 ± 16
	6	1740 ± 30^{D}	1110 ± 32^{D}	738 ± 27	602 ± 19

Groups 1 and 2 received therapeutic and double dose gentamicin. Groups 3, 4 and 5 received double dose gentamicin plus therapeutic dose alpha-lipoic acid, double dose alpha-lipoic acid, and vitamin-E, respectively. Group 6 served as a control. Comparisons among groups were conducted using one-way ANOVA, followed by post hoc Tukey's test. ^{C}: Significant difference in comparison with control. ^{D}: Significant difference in comparison with double dose gentamicin. ^{E}: Significant difference in comparison with double dose gentamicin plus vitamin-E.

Sperm parameters:

On the first and second sacrifice days, there was a significant reduction in the sperm count and progressive motility among animals treated with double dose gentamicin alone compared to animals cotreated with either double dose or therapeutic dose ALA, vitamin E, or placebo group (p<0.05). The reduction in sperm viability was only found among animals treated with double-dose gentamicin alone compared to the other groups (p<0.05). This effect was found on the first and second sacrifice days. A significant increase in head abnormalities was found among animals treated with double-dose gentamicin alone compared to the therapeutic-dose gentamicin and control groups (p<0.05). On the second sacrifice day, the head abnormalities of all treated groups were improved. A significant increase in tail abnormalities was found in the animals treated with therapeutic or double-dose gentamicin alone compared to the other groups (p<0.05). (Table 2). Head and tail abnormalities included tapered, bent, pyriform, small, large, or amorphous head; and short, multiple, hairpin, broken, irregular width, or coiled tails. (Figure1).

Table 2 Sperm analysis comparison among the groups treated with gentamicin with/without alpha-lipoic acid or vitamin-E, and control.

Sacrifice	Groups	Sperm count (10^6/epid.)	Progressive Motility	Viability (%)	Head Abnormality (%)	Tail Abnormality (%)
1st	1	12.5 ± 9.51^{CE}	41 ± 3.62^{CE}	71 ± 2.12	7.8 ± 2.05	70.2 ± 2.21^{CE}
	2	10.3 ± 0.34^{CE}	29 ± 3.71^{CE}	61 ± 3.61^{C}	15.8 ± 3.4^{C}	68.4 ± 3.6^{CE}
	3	27.6 ± 1.6^{D}	61 ± 3.4^{D}	71 ± 1.5^{D}	15.6 ± 2^{C}	29.2 ± 2^{D}
	4	30.5 ± 0.95^{D}	74 ± 3.0^{D}	69 ± 3.2	13.2 ± 1.8^{C}	27.2 ± 1.3^{D}
	5	30.9 ± 0.95^{D}	74 ± 4.4^{D}	71 ± 1.8	12 ± 0.9^{C}	25.2 ± 2.5^{D}
	6	29.5 ± 0.75^{D}	73 ± 4.23^{D}	75 ± 1.72^{D}	3.0 ± 0.63^{DE}	20.6 ± 2.48^{D}
2nd	1	16.3 ± 0.60^{CE}	50 ± 4.70^{CE}	59 ± 0.01^{CD}	5.4 ± 0.01	54.6 ± 0.02^{CE}
	2	14.4 ± 0.42^{CE}	44 ± 2.72^{CE}	45 ± 0.02^{CE}	4.6 ± 0.01	61 ± 0.01^{CE}
	3	33.9 ± 0.54^{DE}	68 ± 4.1^{D}	63 ± 2.0^{D}	7.0 ± 1.2^{E}	39.6 ± 2.1^{CD}
	4	36.9 ± 0.68^{D}	74 ± 4.0^{D}	66 ± 3.0^{D}	5.4 ± 0.4	10.4 ± 3.7^{DE}
	5	37.2 ± 0.95^{D}	77 ± 2.0^{D}	62 ± 1.2^{CD}	3.2 ± 0.7	30.8 ± 1.4^{CD}
	6	36.8 ± 1.09^{D}	76 ± 2.73^{D}	71 ± 0.04^{DE}	5.8 ± 0.01	19.2 ±0.02^{DE}

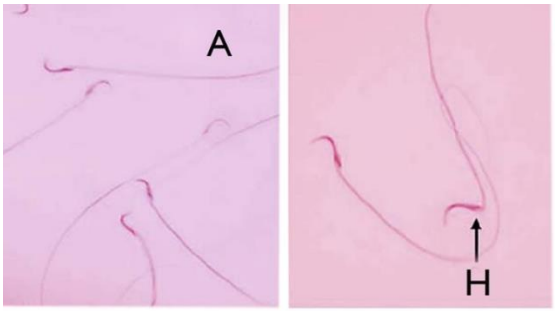

Figure 1 Epidydimal sperms fixed in Carnoy's solution, showing (A) normal sperm morphology, (H) sperm with bent head. (x1000).

Hormones level:

A significant reduction in testosterone level was recorded among animals treated with double dose gentamicin alone compared to animals cotreated with double dose ALA, vitamin E, or placebo on the first sacrifice day (p<0.05). On the second sacrifice day, all testosterone levels obtained from the studied animals were restored compared to the control group. Treatment with double-dose gentamicin alone significantly increased FSH and LH plasma levels compared to the control. However, co-treating with ALA or vitamin E showed variable degrees of restoration for the FSH and LH levels (p<0.05). However, on the second sacrifice day, FSH and LH levels obtained from all the studied animals were normalized compared to the control group (Table 3).

Table 3 Sexual hormones level comparison among the groups treated with gentamicin with/without alpha-lipoic acid or vitamin-E, and control.

Sacrifice	Groups	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)
1st	1	0.42 ± 0.02^{CE}	3.19 ± 0.2^{C}	1.78 ± 0.01^{C}
	2	0.37 ± 0.02^{CE}	3.28 ± 0.3^{C}	1.81 ± 0.3^{C}
	3	0.3 ± 0.02^{CE}	2.0 ± 0.03^{CD}	1.05 ± 0.01^{CDE}
	4	0.68 ± 0.04^{CDE}	3.2 ± 0.07^{C}	1.5 ± 0.12^{C}
	5	0.9 ± 0.02^{D}	2.6 ± 0.06^{C}	1.7 ± 0.07^{C}
	6	0.95 ± 0.06^{D}	1.2 ± 0.1^{DE}	0.65 ± 0.1^{DE}
2nd	1	0.97 ± 0.05	3.68 ± 0.1	2.56 ± 0.5
	2	0.80 ± 0.05	3.48 ± 0.5	2.28 ± 0.3
	3	0.78 ± 0.06	2.98 ± 0.21	1.8 ± 0.02
	4	1.07 ± 0.09	4.58 ± 0.5	3.4 ± 0.4^{C}
	5	0.92 ± 0.22	4.24 ± 1.01	2.44 ± 0.6
	6	0.84 ± 0.01	3.44 ± 0.1	1.46 ± 0.1

Histopathological examination:

Histopathological examination of the negative control group testes showed the typical histological structure of seminiferous tubules, interstitial tissues, and tunica albuginea. Testes from rats treated with double-dose gentamicin revealed congestion of the testicular blood vessels and interstitial capillaries with marked atrophy of seminiferous tubules. However, the co-treatment with double-dose ALA revealed mild pathological changes. Tunica albuginea of the testes of most investigated animals appeared normal, except one case showed slight thickening of tunica albuginea with congestion of sub-capsular blood vessels. Moreover, most of the seminiferous tubules were compact with each other in association with the typical histological structure of the germinal epithelium of seminiferous tubules and interstitial tissues, and normal spermatogenesis was demonstrated in most animals. On the other hand, the group co-treated with vitamin E showed that tunica albuginea and interstitial tissues appeared normal in most examined animals. Most of the seminiferous tubules were compact with each other and restored their normal histological structure, and the spermatogenesis processes are normal in most examined cases. (Figures 2–5).

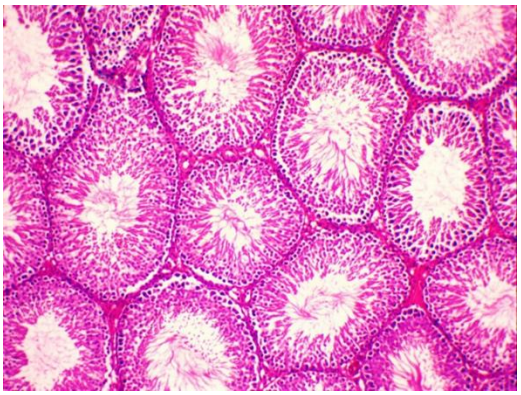

Figure 2 Testes of control rat showing normal histological structures of seminiferous tubules and interstitial tissues. H&E stain x100.

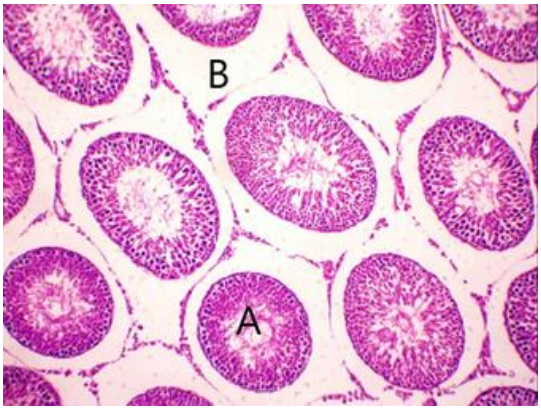

Figure 3 Testes obtained from rat treated with double dose gentamicin, showing atrophy of seminiferous tubules(A) with inter-tubular edema(B). H&E stain x100.

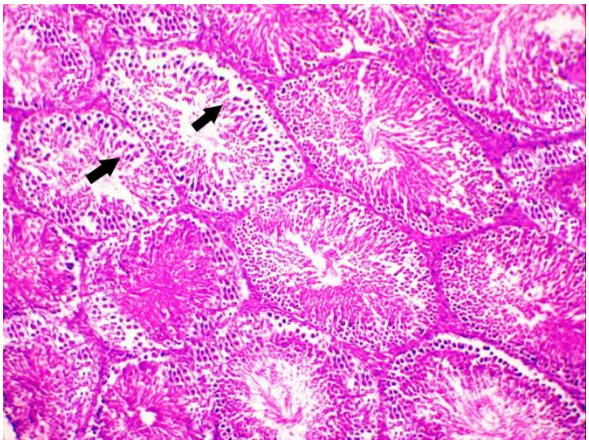
Figure 4 Testes obtained from rat treated with gentamicin plus high dose ALA, showing normal histological structure of the germinal epithelium of most seminiferous tubules and interstitial tissues except mild degeneration of germ cells in few tubules (arrows). H&E stain x100.

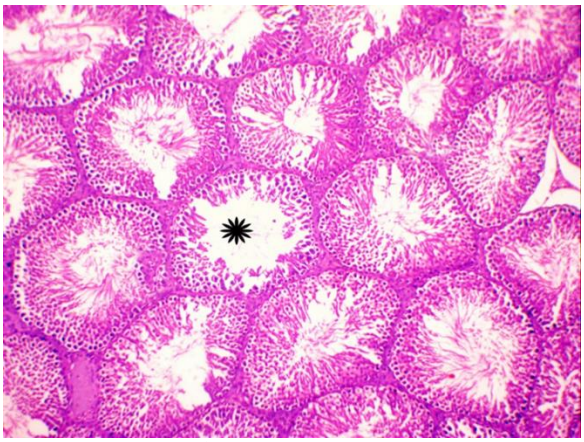
Figure 5 Testes obtained from rat treated with gentamicin plus vitamin E, showing normal histological structure of the germinal epithelium of seminiferous tubules and interstitial tissues. Notice also, degeneration of germinal layers within complete spermatogenesis (asterisks). H&E stain x100.

4. DISCUSSION

We conducted a novel study on the protective potential of ALA against gentamicin's adverse effects on male reproductive organs. Because the gentamicin-induced male reproductive impairment was associated with increased testicular oxidative stress (Elsawah et al., 2020), ALA could protect from these adverse effects via its antioxidant effect (Pershadsingh, 2007). Additionally, ALA is a coenzyme of the mitochondrial pyruvate dehydrogenase complex, is a component of the mitochondrial glycine cleavage system (Bilska and Wlodek, 2005), modulates AMP-dependent kinase (AMPK) in peripheral tissues, activates PPAR-α and PPAR-γ have redox effects (Pershadsingh, 2007). vitamin-E is a potent antioxidant that exerts its protective effect via antioxidant effect (Traber and Atkinson, 2007). It has been demonstrated that ALA could protect from gentamicin-induced nephrotoxicity (Tahira et al., 2012), cochlear damage (Conlon et al., 1999), and lipid peroxidation (Sandhya and Varalakshmi, 1997). To our knowledge, no studies have been conducted on the potential protective effect of either ALA or vitamin E from a gentamicin-induced male reproductive impairment, except one recent study which evaluated the protective effect of ALA only (Yahya et al., 2019). We found that gentamicin reduced testis and epididymis weights and impaired sperm parameters. However, co-administered ALA or vitamin E could preserve the organ weights and sperm parameters, and when comparing ALA to vitamin E, it gave approximal protection. Consistently, the previously mentioned study reported that oral 600 mg/kg ALA co-treatment with 80 mg/kg gentamicin persevered sperm count, viability, motility, and abnormalities (Yahya et al., 2019). Other studies reported the adverse effects of gentamicin on male gonads, but with other protective agents such as ginger (Zahedi et al., 2012), melatonin (Kim et al., 2014), and dandelion (ÖMÜR et al., 2016), or without protection (Aly and Hassan, 2018). In the present study, the testosterone level was markedly decreased with gentamicin treatment in association with LH and FSH elevation that could be nearly normalized with the co-administration of vitamin E or ALA. The reduction in testosterone level was due to Leydig cells' affection, as demonstrated by our histopathological findings. Steroidogenic enzymes were also inhibited by gentamicin, that decreased testosterone synthesis (Ghosh and Dasgupta, 1999). LH and FSH elevation could be secondary to testosterone reduction (McNeilly et al., 2003). The study by Yahya et al. (2019) consistently reported that testosterone was decreased with gentamicin treatment and nearly normalized with ALA-co-treatment. Moreover, testosterone level reduction was reversible on the 35th day of the experiment after treating rats with 40 mg/kg gentamicin for 4 days without protective agents (Carageorgiou et al., 2005). Testicular histopathological examination in the present study revealed structural alterations that could be prevented by ALA or Vitamin E co-treatment. Consistently, atrophy, degeneration, and loss of spermatogenesis in some of the seminiferous tubules were found with gentamicin treatment (Aly, 2019). Additionally, depletion of germ cells, especially in spermatogonia, and abnormal Leydig cell appearance were also reported (Khaki et al., 2009). However, ALA co-treatment could protect from histopathological alterations (Yahya et al., 2019).

5. CONCLUSION

Gentamicin impairs male reproductive organs, especially the testis and epididymis, and sperm function, in addition to plasma testosterone level reduction in Wister rats. These effects are mostly reversible and more predominant with the double dose. However, they can be mitigated by ALA-co-administration with gentamicin.

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