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Nephroprotective role of eugenol against cisplatin-induced acute kidney injury in mice

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Abstract: Cisplatin (CP) is a choice of drug for cancer chemotherapy. Its nephrotoxic effects are well reported and limit its use. In this study, we aim to assess the nephroprotective potential of eugenol on cisplatin-induced acute kidney injury (AKI). Thirty male, BALB/c mice were randomly divided into five groups. Normal saline was given to controls in normal group (group 1), CP group (group 2) received CP injection (20 mg/kg), and other two groups 3 and 4 were treated with eugenol at doses of 50 and 100 mg/kg respectively for four days before receiving cisplatin. While group 5 (positive control) received ascorbic acid (200 mg/kg) for four days before CP injection. Blood samples were collected after 72 hours of CP injection and kidneys were dissected out. Blood urea and creatinine values were increased significantly ($p < 0.001$) in CP group. However, significant improvement ($p < 0.001$) was seen in eugenol treated groups. Similar pattern was observed in kidney injury score ($p < 0.001$) which markedly improved after eugenol treatment. Improvement in protein cast in tubular lumen and tubular brush borders was also observed. Eugenol has an ameliorative role in AKI induced by cisplatin in mice. This compound is recommended for further evaluation of its mechanism(s) of action.

Keywords: Cisplatin, acute kidney injury, eugenol.

INTRODUCTION

One of the most common clinical problem encountered while dealing with critically ill patients, geriatric patients, patients on anti-cancer therapy is nephrotoxicity. It occurs when bioactive molecules in various therapeutic agents causes significant damage to the structure of nephron, which is the functional unit of kidney. Due to this damage, kidneys are unable to perform their excretory function adequately, resulting in the inability to excrete urine and electrolytes. This results in accumulation of waste products and elevation of electrolytes like magnesium and potassium in blood (Arunkumar, 2012). Varieties of therapeutic agents are known to cause kidney damage leading to chronic interstitial nephritis, acute renal failure, nephrotic syndrome. These therapeutic agents include chemotherapeutic agents, aminoglycoside antibiotics, NSAIDS. All of these are the recent addition in therapeutic arsenal. Exposure to chemicals comprising of heavy metals like cadmium, mercury, platinum, arsenic also causes significant damage to the structure of nephron. Early recognition of these compounds, cessation of toxic drug and use of minimal dose only in essential therapy can be few options which can minimize nephrotoxic effects and warrants exploration (Paueksakon and Fogo (2017).

Cisplatin (CP) is an intravenously administered, platinum-based anti-cancer drug (Sooriyaarachchi *et al.*, 2016). It is used in the chemotherapeutic treatment of different type of cancers like ovarian cancer, bladder cancer, germ cell tumors, lymphomas and small cell lung cancer and some others (Gomez-Ruiz *et al.*, 2012). It is included in the essential list of medicines required for a health system recommended by World Health Organization (World Health Organization, 2015). It produces its cytotoxic effect by binding to DNA and damaging it, thus preventing DNA replication (Rybak *et al.*, 2007; Slattery *et al.*, 2014). One of the most important adverse effects of CP is its nephrotoxicity (Oun *et al.*, 2018). Among CP receiving patients, about 33% are reported to demonstrate clinically the nephrotoxic effect of the drug (Mishra *et al.*, 2004). It reduces the glomerular filtration rate, dysregulation in the serum levels of urea, creatinine and electrolytes (Pabla and Dong, 2008). CP is a well-known chemotherapeutic agent, causing AKI by creating an imbalance of anti-oxidant and pro-oxidant like reactive nitrogen species (RNS) and reactive oxygen species (ROS) resulting in oxidative stress. ROS and RNS are known to have greater reactivity with vital molecules like carbohydrate, lipid, DNA, RNA and proteins (Mishra *et al.*, 2015; Hagar *et al.*, 2015). Any imbalance or alteration in ROS and RNS can result in the damage of cellular structures composed of these vital molecules. Histopathological evidence suggests that despite of low rate of growth of proximal tubules of nephrons, significant damage of epithelial cells of proximal tubules of nephrons is caused by CP (Karasawa and Steyger, 2015). Therefore, there is a need to explore the preventive measures by which the nephrotoxicity of CP can be reduced.

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The compounds which show protective activities against the damage of the structure of nephrons are referred as nephroprotective agents. Various herbal compounds have been reported to possess nephroprotective effects. Co-administration of these nephroprotective compounds along with minimal dose of nephrotoxic drug can minimize damage of nephron. Eugenol (1-allyl-4-hydroxy-3-methoxybenzene, $C_{10}.H_{12}.O_2$) is widely used in foods as a flavoring agent (Giuliani, 2014). It is DNA protective. Anti-oxidant and anti-inflammatory properties were also reported in thioacetamide-induces liver injury model (Yogalakshmi *et al.*, 2010). Its kidney protective effects are also reported in chromium, gentamicin and glycerol-induced model of acute kidney injury (Barhoma, 2018; Said, 2011; Saifullah *et al.*, 2019). Its effect on cisplatin-induced model of kidney injury is not reported. The current study aimed to assess the kidney protective effects of eugenol on AKI induced by Cisplatin *in vivo*.

MATERIAL AND METHODS

Animals

Thirty BLAB/c mice, male in gender were recruited for study. Weight of the mice ranged between 20 to 30 gm. Animals were bought from the University of Karachi, Animal House of International Center for Chemical and Biological Sciences (ICCBS). Approval from Animal Ethics Committee (AEC) of Ziauddin University was taken before the start of animal experiments (Approval Protocol No.2019-003). The mice were kept in sterile cages, made up of plastic. The cages were kept in room temperature between 21 to 25℃ at 12 hours light diminished cycles. Mice were provided with free access to water and food. Acclimatization of mice with the environment was assured before the start of experiment.

Treatment regime

Five groups of animals were randomly formed having six (6) animals per group i.e. normal control (Group 1), CP group (Group 2), CP + eugenol 50 mg/kg (Group 3), CP + eugenol 100 mg/kg group (Group 4) and CP + ascorbic acid 200 mg/kg group (Group 5). CP (20 mg/kg) was administered in animals of group 2, 3, 4 and 5 after pre-treatment with compounds for four days (Ekinci Akdemir *et al*., 2019; Siddiqui *et al*., 2019). After 72 hours of CP injection to all animals, except the animals of group 1 which were given normal saline, animals were sacrificed under anesthesia to collect blood and tissue samples for evaluation.

Serum urea and creatinine estimation

Cardiac puncture technique was used to collect blood samples in test tubes in which no additives were used. After centrifugation, serum was separated for the estimation of serum creatinine and urea by spectrophotometric method using Microlab 300 (ELITechGroup).

Histopathological evaluation of kidney tissues

Animal dissection was done to collect kidneys, which were longitudinally cut. The specimen was fixed in Bouin's fixative for 4 hours after which deionized water was used for washing. The specimen was then placed in 70% isopropanol at room temperature for overnight. On next day, tissues were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax.3-4µm thick tissue sections were cut and hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) staining were done using standard protocol. Nikon Ts2R-FL inverted microscope was used for microscopic examination of the tissue sections. All images were captured and processed by Nikon Elements D software (Siddiqui *et al*., 2019*)*

STATISTICAL ANALYSIS

Data was evaluated using SPSS version 20 using one–way ANOVA. Data was presented as mean ±SD, and p value <0.05 was considered statistically significant.

RESULTS

Effect of eugenol on blood urea and creatinine levels

A significant increase (*p<0.001) in the levels of serum urea and creatinine in CP group as compared to the normal group, were observed demonstrating the nephrotoxic effect of CP. Significant improvement (#p<0.001) in the levels of serum urea and creatinine were observed in the groups of animals which were pre-treated with eugenol after the injection of CP, demonstrating its kidney protective effect at dose dependent manner. Similarly, significant improvement (#p<0.001) in the levels of serum urea and creatinine were observed in the mice treated with ascorbic acid, which were positive controls (fig. 1).

Effect of eugenol on kidney injury score

Statistically significant increase in injury score (*p<0.001) in CP treated group was observed as compared to normal group which shows the tubular damage caused by CP. Statistically significant improvements (#p<0.001) in the damage scores were observed in groups of animals which were treated with eugenol before CP injection. This results show progressive improvement in the damage of nephron with the increased dosage of eugenol, indicating its mitigating effect on kidneys. Statistically better improvements (#p<0.001) in the injury scores, were observed in eugenol groups as compared to the ascorbic acid group which shows better performance of eugenol in ameliorating kidney damage (fig. 2). Thus suggesting eugenol as a better nephroprotective agent then ascorbic acid.

Effect of eugenol on kidney tissue architecture

Fig. 3A shows normal structure of renal cortex with normal architecture of renal corpuscles and tubules.

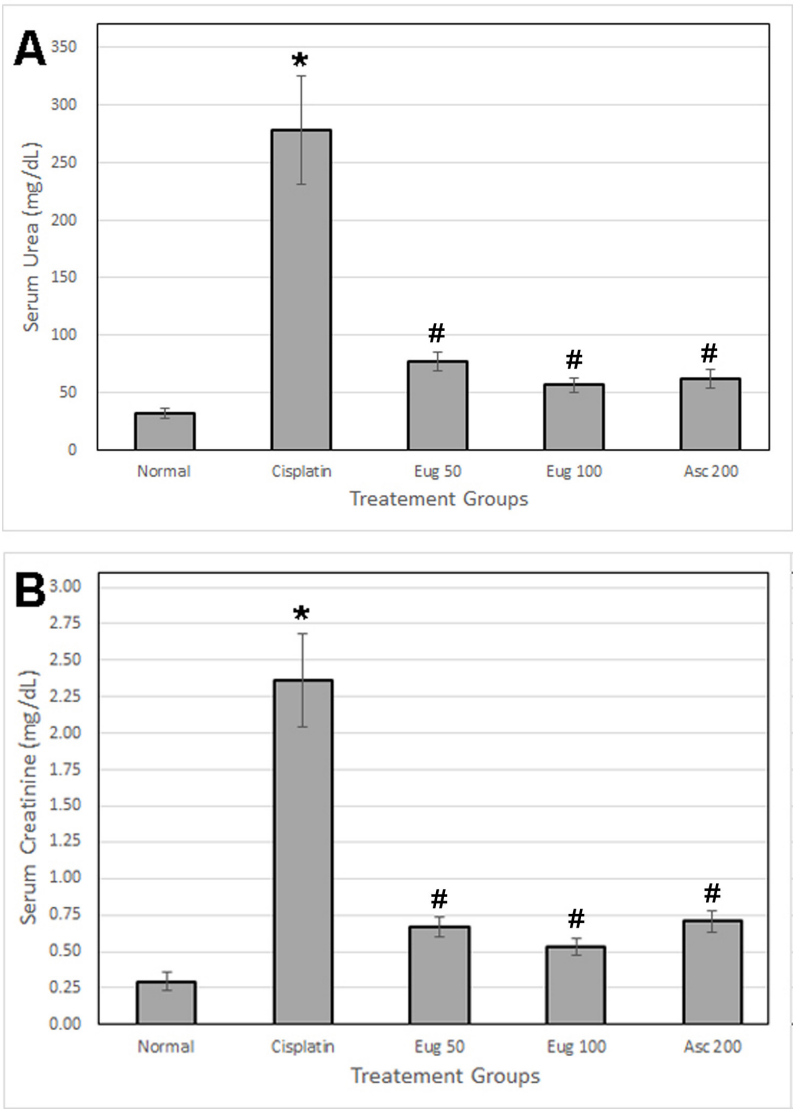

Fig. 1: Demonstrating the levels of serum urea (A) and creatinine (B) in all groups.

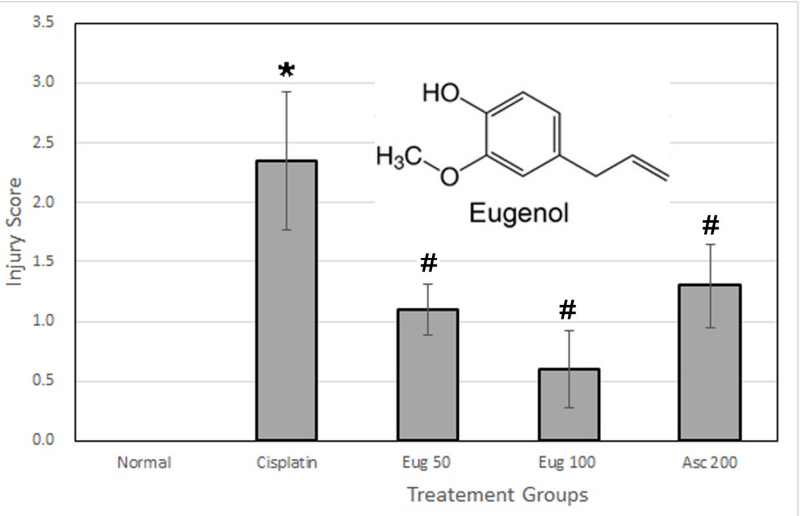

Fig. 2: Demonstrating the kidney injury score in all groups.

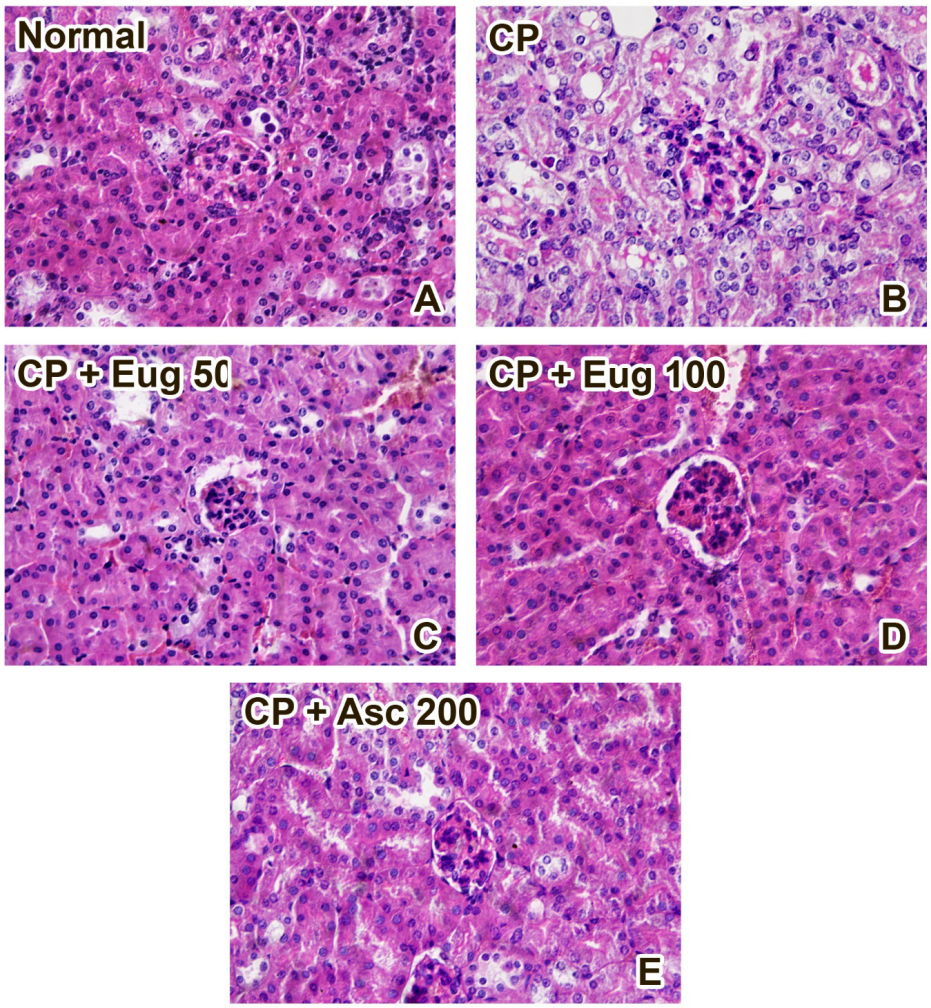

Fig. 3: H&E stained photomicrographs showing kidney cortex of normal (A); CP (B); CP+Eug 50 mg/kg (C); CP+Eug 50 mg/kg (D) and CP+Asc 200 mg/kg (E) (Magnification 400x).

However, fig. 3B demonstrates the damaged tubular architecture particularly in proximal tubules caused by Cisplatin. Increase in vacuolization in the cytoplasm of proximal tubular cells can also be observed which indicates cellular injury. Fig. 3C and D show progressive improvements in the extent of damage of proximal tubules with different dosage of eugenol. Decrease in cytoplasmic vacuolization of proximal tubular cells can also be appreciated which demonstrate the protective effects of eugenol on nephron. Fig. 3E shows kidney sections of ascorbic acid treated group, showing less damage to the architecture of proximal tubular cells with marked decreased in cytoplasmic vacuolization. This ascorbic acid treated group was used as positive control.

Effect of eugenol on kidney tubular brush borders

Fig. 4A shows normal brush borders of loop of Henle (LH) and proximal convoluted tubules (PCT). However, fig. 4B shows extensive damage in the brush border of LH and PCT, with increased protein cast in the tubular lumen and increased cytoplasmic vacuolization caused by CP. Figs. 4C and D show lesser extent of damage in the brush border of LH and PCT and decreased cytoplasmic vacuolization in the eugenol treated groups with progressive improvement with different dosage of eugenol representing its mitigating effects on the damage of tubular epithelial cells. Figure 4E shows sections of ascorbic acid treated group.

DISCUSSION

Nephrotoxicity of therapeutic agents is a very common problem encountered when dealing with geriatric, critically ill patients and patients on anti-cancer treatment. It results in accumulation of waste products in the body thus affecting the choice of drug selection and severely affecting the morbidity and mortality of the patient worldwide. Inclusion of heavy metal based compounds in

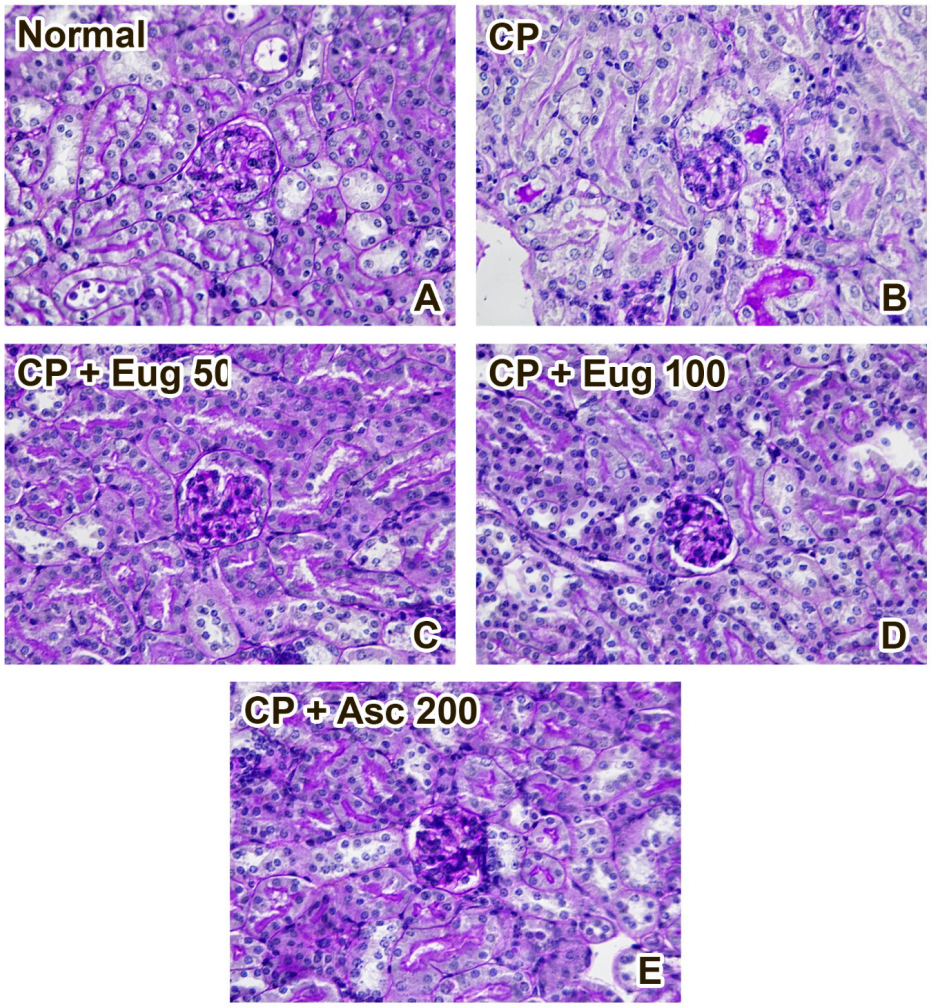

Fig. 4: PAS stained photomicrographs showing kidney cortex of normal (A); CP (B); CP+Eug 50 mg/kg (C); CP+Eug 50 mg/kg (D) and CP+Asc 200 mg/kg (E) (Magnification 400x).

the therapeutic arsenal in the past few years have further aggravated this problem as many heavy metal based compounds like platinum, cadmium, arsenic are known to cause nephrotoxicity (Paueksakon and Fogo (2017).

CP is a platinum-based chemotherapeutic agent used to treat many cancers. Its nephrotoxic effects are well documented and limits it use (Miller and Tadagavadi, 2010). Limited number of researches on the exact mechanism of injury caused by CP makes it an interesting area for research. Limiting the extent of damage caused by CP will benefit the patient by improving their morbidity. Quest for the means of improvements in the adverse outcomes of chemotherapeutic agents is the need of the time (Oh *et al*., 2014). Current study was aimed to assess the renal protective effects of eugenol on AKI caused by CP.

Our study demonstrates the extensive nephrotoxic effects caused by CP with significant increase in serum creatinine and urea with higher kidney injury scores. Histological evidence also confirmed these findings with marked disruption of normal architecture of epithelial cells of LH and PCT with deposition of protein cast in the tubular lumen. Our findings are in agreement with studies reporting decrease in glomerular filtration rate, increase in serum creatinine and urea with extensive damage in proximal tubules (Özülker *et al*., 2011; Ravindra *et al*., 2010). A literature suggests that CP causes these effects by causing imbalance in the levels pro-oxidants and anti-oxidants (Noori and Mahboob, 2010). Multiple pro-inflammatory cytokines are activated with increase infiltration of inflammatory cells to the kidneys resulting in tubular necrosis (Ozkok and Edelstein, 2014). Xu *et al*. has suggested increase in TNF-α, IFN-γ and increase in the expression of RIP1 and RIP3 could be a cause of tubular necrosis (Xu *et al*., 2015).

Nephroprotective substances which minimize and reverse the damage of nephrotoxic compounds needs to be

explored. The nephroprotective compounds can be co-administered with drug to reduce its nephrotoxic effects. Among such nephroprotective compounds, eugenol has been reported to show nephroprotective effects in rat kidney injury models. However its potential role as nephroprotective agent in CP induced kidney injury is rarely reported which was explored in this study. Our study demonstrated the significant protective effects of eugenol in the group of animals treated with eugenol before the injection of CP. Significant progressive improvements were observed in serum urea, serum creatinine and kidney injury score levels with the increasing dose of eugenol. Histological evidence showing improvements in the structure of LH and PCT protein cast in the tubular lumen also complement these improvements in renal biomarker levels. These findings are in accordance with the findings in literature reporting the mitigating effects of eugenol on the tubular cast and epithelial structure of renal tubules (Garud and Kulkarni 2017; Said, 2011). Another study suggests anti-inflammatory and anti-oxidant characteristics of eugenol in minimizing the injury (Ekinci Akdemir *et al*., 2019).

The present study suggests that the eugenol exerts significant improvement in the acute kidney injury caused by CP, with marked improvements in renal tubular histology and renal biomarker levels. This compound may have a potential role in minimizing the harmful effects of CP may be by altering inflammatory cytokines. Further studies should be done to find the exact mechanism(s) by which eugenol improves these adverse effects caused by CP.

CONCLUSION

The study revealed that eugenol mitigate the renal tubular damage caused by CP with significant improvements in the urea levels, creatinine levels, injury scores in eugenol treated group. Histological evaluation also demonstrates improvements in the architecture of proximal convoluted tubular cells with marked improvements in cytoplasmic vacuolization in eugenol treated group. This nephroprotective effect of eugenol progressively increases with the dose of eugenol. Additional studies are required to find out the specific mechanism(s) involved and to evaluate the potential of eugenol as a compound for reduction in cisplatin-induced AKI.

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