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Original article

Effect of *Moringa oleifera* Lam. on haematological and histopathological parameters in sodium flouride induced toxicity in wistar rats

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Abstract

The present investigation was undertaken to study the effect of *Moringa oleifera* Lam. on haematological and histopathological changes in the liver and kidney in wistar rats, induced with sodium fluoride toxicity and vitamin C. Thirty six female wistar rats were divided into six groups (T_1, T_2, T_3, T_4, T_5 and T_6), each comprising of six rats. T_1 served as negative control which received normal saline only. T_2 served as positive control, received sodium fluoride @ 20 mg/kg b.wt. only, T_3 received fluoride @ 20 mg/kg b.wt along with ascorbic acid @ 200 mg/kg b.wt and treatment was given to T_4, T_5 and T_6 with different doses, *viz*., 250, 500 and 750 mg/kg b.wt of aqueous extract of *M. oleifera*, respectively along with sodium fluoride. The blood was collected after 28 days for serum haematological parameters study and then all the rats were sacrificed for histopathological studies. On total RBC count, though values were noted in increasing direction from graph but statistically analysed were found non significant. The haemoglobin, WBC and PCV were shown to be restored significantly on induced toxicity rats. Histopathological alterations in T_3, T_4, T_5 and T_6 were observed milder degree as compared to T_2. The alterations in three (T_4, T_5 and T_6) groups when compared to the fluoride administered group showed high degree of protective effect treatment on kidney, by using aqueous extracts shows the toxic changes.

Key words: *Moringa oleifera* Lam., heamatological parameters, wistar rats, sodium fluoride toxicity, vitamin C, histopathological studies

1. Introduction

Fluorides are naturally occurring harmful contaminant in an environment (Raghuvansi *et al*., 2010). It is a cumulative poison and, thus leads to fluorosis, a serious public health problem. Fluoride causes damage not only to hard tissues of teeth and skeleton (Grynpas, 1990; WHO, 2002) but also to soft tissues, such as brain, liver, kidney, spleen and endocrine glands (WHO, 1984; Shanthakumari *et al*., 2004; Shashi and Thapar, 2001; Shashi *et al*., 2002; Zhan, 2006). Fluoride induced hepatotoxicity due to the formation of free radicals and decreased activity of the antioxidant system in hepatocytes of animals and humans have been reported (Chatterjee *et al*., 2016; Guo *et al*., 2003). Fluoride exposure also induces histopathological changes in liver involving focal necrosis, infiltration of leucocytes, swelling of kupffer cells, extensive vacuolization, hemorrhagic areas, ultrastructural alterations in hepatocytes and increased apoptosis in animals and humans (Chinoy *et al*., 2004; Basha and Rao, 2014).

The fluoride-treated group showed significant differences in several heamatological parameters, including decreases in WBC, RBC, and PLT counts and neutrophil ratio (Atmaca *et al*., 2014; Banu Priya *et al*., 1997). The healing and prophylactic effects of *M. oleifera* was observed on lead induced damage to haematological and bone marrow in adult wistar rat (Owolabi *et al*., 2012).

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The *M. oleifera* Lam. (syn. *M. ptreygosperma* Gaertn.) tree originated from Agra and Qudh in the northern eastern region of India, south of Himalayas (Mugal *et al*., 1999). Moringaceae is a single genus family with 13 known species. Among these, *oleifera* is most widely used and utilized species (Sengupta *et al*., 1956; Morton, 1991), commonly known as Drumstick or Horseradish (English), Shevga (Marathi), Muringa (Malayalam) and Sahjan (Hindi). It is a versatile tree useful, for human beings and animals and also has industrial values. It is one of the richest plant sources of Vitamins A, B, C, D, E and K (Anwar and Bhanger, 2003; Babu, 2000; Caceres *et al*., 1992; Dayrit *et al*., 1990; Delisle and Bakari, 1997). *Moringa* pod is an important commercial vegetable crop throughout India. Hence, the present study was undertaken to evaluate the haematological and histological parameters in wistar rats induced with or without the sodium fluoride toxicity and supplementation of Vitamin C.

2. Materials and Methods

The present study was carried out in the Department of Pharmacology and Toxicology, Nagpur Veterinary College, Nagpur Maharashtra Animal and Fishery Sciences University, Nagpur-440 001, Maharashtra, India. The study has been designed to assess the ameliorative effect of the aqueous extract of the dried seed powder of the plant, *M. oleifera* on sodium fluoride induced toxicity in wistar rats. The parameters like hematological and histophalogical were studied. The study was carried out for the period of 28 days.

The Institutional Animal Ethical Committee (IAEC) approved the experimental protocol (Reg No. 244/CPCEA). The experimental

protocol met the national guidelines as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The present research work was carried out on thirty six rats of albino wistar strain, which were procured from the recognized Laboratory of Animal Breeding Centre, NIN, Hyderabad, Telangana State, India. Rats weighing around 150-200 g were used for the present study. The plant material, *i.e., M. oleifera* seeds were procured from local market and aqueous extract of dried *M. oleifera* seeds was prepared and was used as herbal medicine. The *M. oleifera* seeds were procured from Nagpur region and were authenticated by Dr. Alka Chaturvedi, Professor, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Mahatma Phule Educational Campus, Amravati Road, Nagpur-440003 (Maharashtra). The voucher specimen was deposited in the department. The seeds were dried at room temperature and powdered. The powder was stored in glass bottle in a cool and dry place away from direct sunlight and used for preparation of aqueous extract. The aqueous extract was prepared by the method described by Rosenthaler (1930). The freshly prepared *M. oleifera* seed powder (50 g) was immersed in 200 ml distilled water in a flask stoppered tightly with cotton plug and was kept at room temperature for 72 h at 150 rpm in an orbital shaker. The contents of the flask were filtered through muslin cloth. The residue left in the flask was rinsed with little quantity of distilled water and filtered through the muslin cloth. The filtrate, thus obtained was filtered through Whatman filter paper No. 1. Final filtrate, so obtained was transferred to already weighed petridish and was evaporated *in vaccuo* using rotary film evaporated. After complete storage of the solvent, the petridish was once again weighed to know the amount of extract. The per cent extractability was determined and was stored at – 20ºC and diluted to a desired concentration with distilled water just before use. The extract was stored in a desiccator in cool and dry place until further used in this study.

A total of thirty six female wistar rats, used in this study were divided into six groups containing six rats in each group. The rats were acclimatized for 15 days to the environment, before the start of the experiment. Each rat was given separate identification mark by using picric acid. Six groups formed were labeled as T_1, T_2, T_3, T_4, T_5 and T_6. Group T_1 served as negative control and was treated with normal saline. Group T_2 served as positive control, which received sodium fluoride only @ 20 mg/kg b.wt. Group T_3 received sodium fluoride only @ 20 mg/kg b.wt and Vit C@ 200 mg/kg b.wt. as a referral standard and Groups T_4, T_5 and T_6 were treated with *M. oleifera* @ 250, 500, 750 mg/Kg b.wt, respectively along with sodium fluoride only @ 20 mg/kg b.wt. For induction of sodium fluoride toxicity, sodium fluoride was used @ 20 mg/kg body weight dissolved in distilled water.

The rats from all experimental groups were properly anaesthetized and euthanized after 28th day of experiment. Prior to sacrifice, the rats were weighed and fasted for 12 h. Blood was collected by retro bulbar method in glass vials containing 1% ethylenediamine tetra acetic acid (EDTA) for hematological estimation. After 28th day of experiment, the collected blood samples in 1% EDTA were subjected to hematological studies such as hemoglobin concentration (Hb), using Sahli's Method (acid hematin), total erythrocyte count (TEC), total leukocyte count (TLC) and packed cell volume (PCV) by Microtube method as mentioned by Benjamin (1965). After collection of blood for haematology, the animals were sacrificed for histopathological examinations. The liver and kidney of each rat were examined grossly. Thereafter, liver and kidney were removed for histological study. The tissues were washed with normal saline and immersion fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5 µm sections and stained with hematoxylin and eosin for histopathological examination according to standard procedure as described by Ross *et al.* (1989).

2.1 Statistical analysis

All the values in the test are presented as means ± SEM. Statistical differences between the means of the various groups were evaluated using Completely Randomized Design. A '*p*' value of more than 5% was considered to be statistically significant ($p < 0.05$). The data generated were analysed statistically by standard statistical procedure (Snedecor and Cochron, 1980).

3. Results and Dicussion

3.1 Haematological parameters

Haematological parameters like total erythrocyte count (TEC), haemoglobin (Hb) concentration, PCV and TLC count were estimated in control and treatment groups and are presented in Table I. In haematological parameters, the results have been found significant indicating ameliorative effect of *M. oleifera* on sodium fluoride induced toxicity. On TEC though values were noted in increasing direction from graph but statistically analysed were found non-significant. The haemoglobin, TLC and PCV were shown to be restored significantly on induced toxicity rats.

Haematological parameters, the Hb, PCV and TLC were significantly improved when compared to control, indicative of amelioration against sodium fluoride toxicity. In present study, fluoride decreased the haemoglobin concentration, TEC, TLC and PCV values (T_2) as compared to control (T_1) in which only normal saline was administered. Similar findings were reported by Sharma and Vaghela (2010) who found significant alterations in TEC, Hb, TLC and PCV content after exposure of rats to 5.8 ppm of sodium fluoride. We have reported similar findings in present study except RBC which was found no significantly altered because in present study 20 mg/kg b. wt. did not alter the total erythrocyte count. The present study, revealed that the altered blood values due to fluoride toxicity were restored to normal significantly in Hb, TLC and PCV except TEC (Figures 1, 2, 3 and 4). The present observation was not exactly identical with the review collected but it is in partial agreement to it.

3.2 Histopathological changes

Section of liver from T_2 group showed hydropic degeneration of hepatocytes, particularly at periportal region and their cytoplasm showed fine vacuolations (Figure 5). Focal necrosis was frequent in this section (Figure 6). The groups T_3, T_4 and T_5 revealed various degenerative changes and the vacuolization of hepatocyte was prominent in these groups. However, the group T_6 revealed periportal necrosis and aggregation of round cells. The hepatic architexture was normal and indicate the protective action of *M. oleifera* in group T_6 at 750 mg/kg b.wt. (Figure 7).

Table 1: Effect of aqueous extract of *M. oleifera* on haematological parameters on sodium fluoride induced toxicity in wistar rats

Groups	Treatment	Hb (g/dl)	TEC (×10⁶ cells/ dl)	PCV (%)	TLC (×10³ cells/ dl)
T_1	Normal saline @ 0.5 ml	12.43±0.146[a]	6.1±0.209	37.3±0.429[a]	12.28±0.106[a]
T_2	Sodium fluoride only @20mg/kg b.wt	11.56±0.121[b]	5.66±0.187	34.7±0.365[b]	9.95±0.124[d]
T_3	Sodium fluoride + L-Ascorbic acid @200 mg/kg b.wt.	12.33±0.066[a]	5.88±0.137	37±.0.2[a]	11.61±0.129[b]
T_4	Sodium fluoride + Aq. Ext *M. oleifera* @250 mg/kg b.wt.	11.46±0.083[b]	5.76±0.166	34.4±0.259[b]	11.01±0.199[c]
T_5	Sodium fluoride + Aq. Ext *M. oleifera* @500 mg/kg b.wt.	12.26±0.15[a]	5.93±0.176	36.8±0.45[a]	11.36±0.082[bc]
T_6	Sodium fluoride + Aq. Ext *M. oleifera* @750 mg/kg b.wt.	12.36±0.121[a]	6.06±0.165	37.1±0.365[a]	11.51±0.107[b]

Hb-haemoglobin, TEC-Total Erythrocyte Count, PCV-Packed Cell Volume, TLC-Total Leucocyte Count, Mean values carrying different superscripts a, b, c… in columns differ significantly ($p \leq 0.05$)

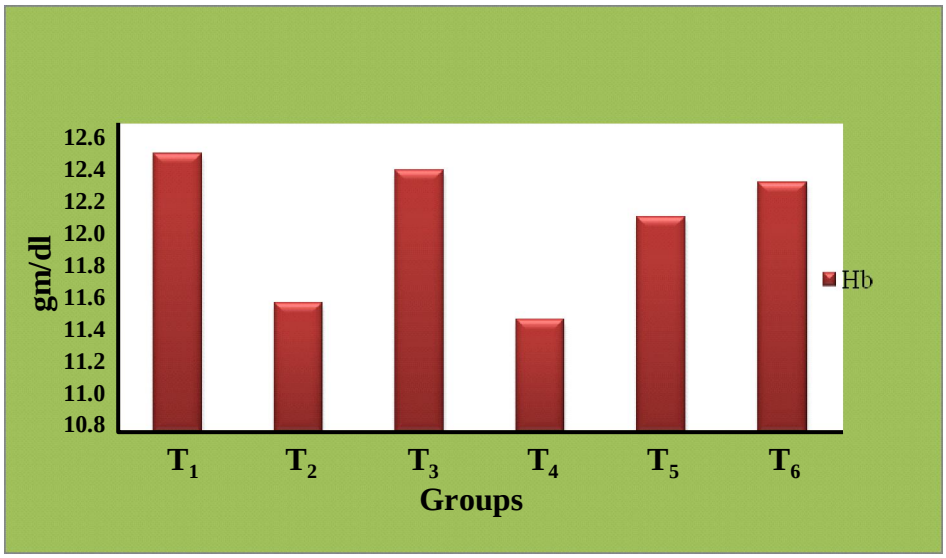

Figure 1: Effect of *M. oleifera* on haemoglobin in sodium fluoride induced toxicity in wistar rats.

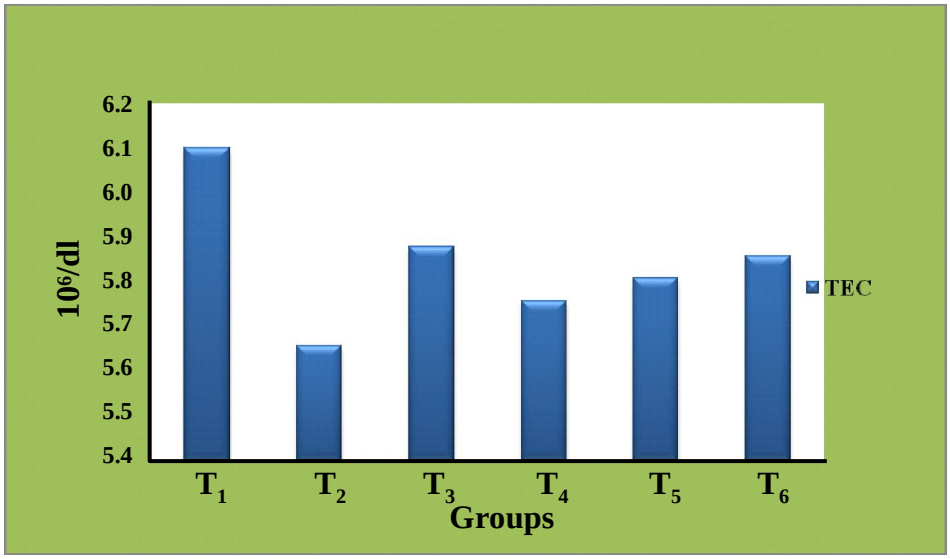

Figure 2: Effect of *M. oleifera* on TEC in sodium fluoride induced toxicity in wistar rats.

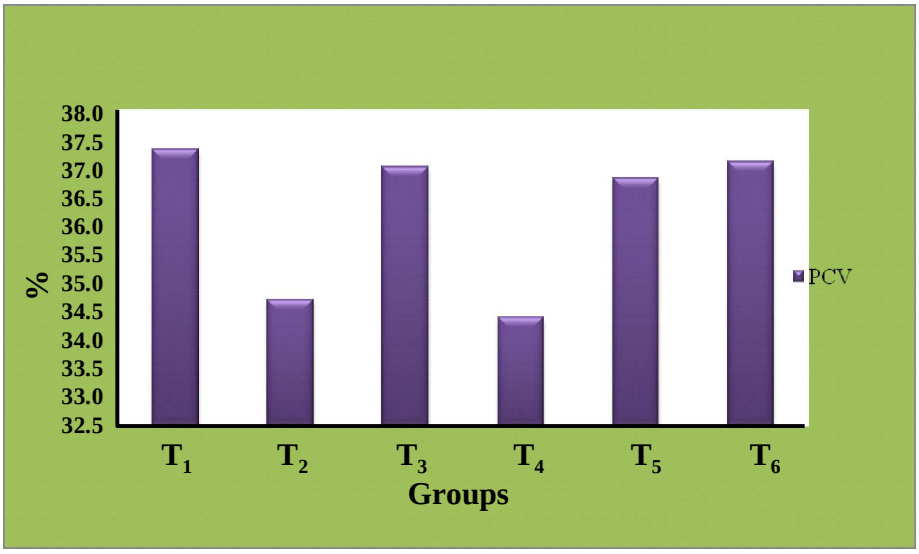

Figure 3: Effect of *M. oleifera* on PCV in sodium fluoride induced toxicity in wistar rats.

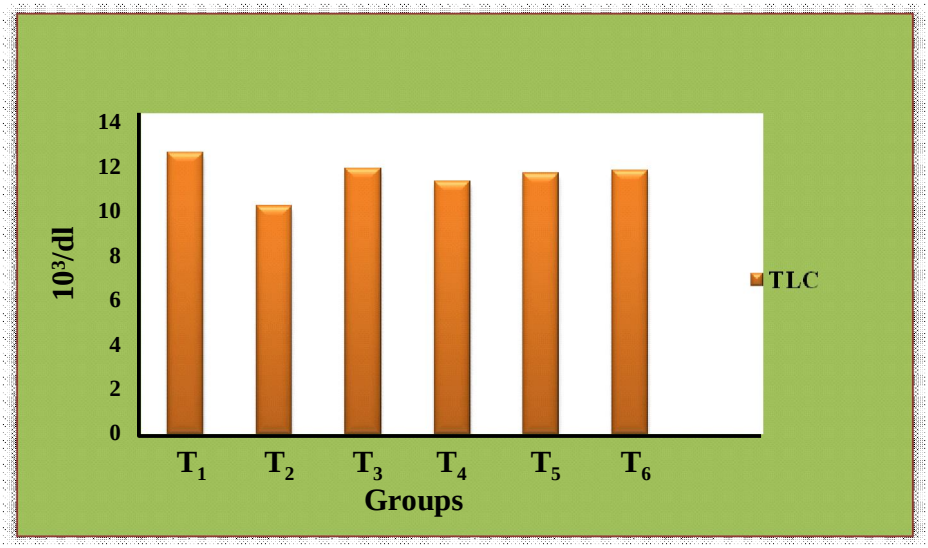

Figure 4: Effect of *M. oleifera* on TLC in sodium fluoride induced toxicity in wistar rats,

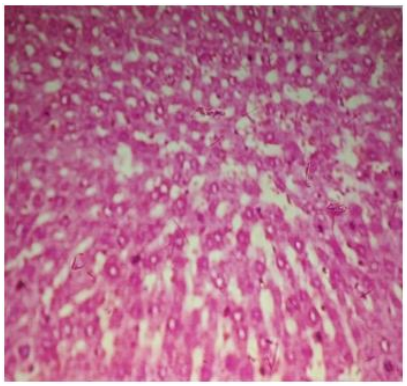

Figure 5: Degenerative changes and vacuolization of hepatocytes in liver of rats from T_2 group (H and E, 200X).

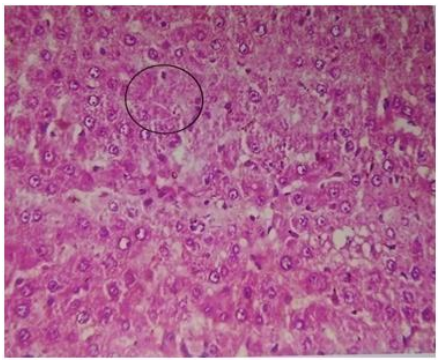

Figure 6: Focal necrosis in liver of from T_2 group (H and E, 200X).

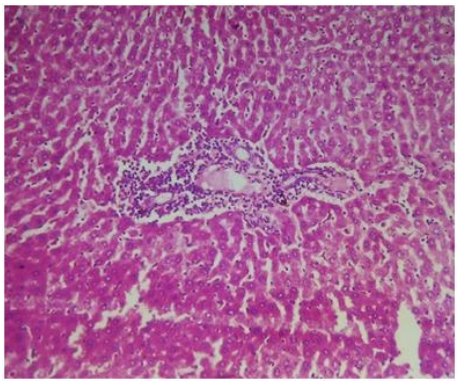

Figure 7: Periportal necrosis and aggregation of round cells in liver of rats from T_6 group (H and E, 100X).

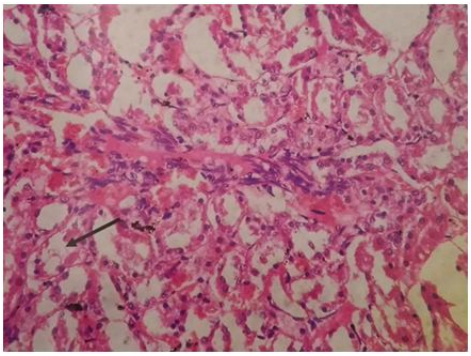

Figure 8: Vacuolar changes in tubular epithelium in kidney of rats from T_2 group (H and E, 200X).

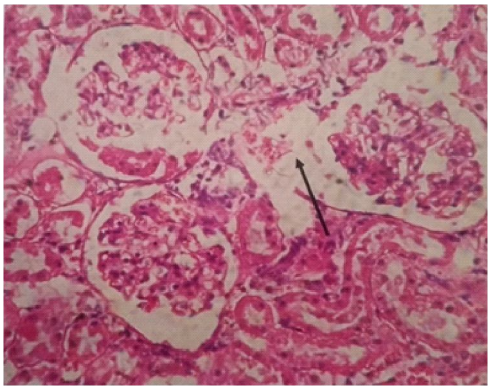

Figure 9: Distension of bowman's space with hemorrhages in kidney of rats from T_4 groups (H and E, 200X).

Section of kidney group T_2 revealed nephrotic changes including degenerative changes in tubular epithelium with presence of degenerated cellular cast in the lumen of tubule. Interstitial haemorrhage was prominent in this group (Figure 8). The glomeruli showed vacuolar changes and destruction of glomeruli. Bowman's space was found distended with haemorrhage (Figure 9). Groups T_3, T_4, T_5 and T_6 revealed mild nephritic changes and haemorrhages. *M. oleifera* and Vit C showed a less degeneration of tubules and almost normal glomeruli.

Fluoride induced morphological changes in liver and kidney. No marked histopathological alterations were noticed in any of the vital organ in normal rats. Predominant hepatic histopathological alterations were severe degenerative changes resulting in reduction of sinusoidal space, focal necrosis and vacuolization of hepatocytes. In kidney, nephrotic changes including degenerative changes in tubular epithelium, interstitial haemorrhage, vacuolar changes and destruction of glomeruli were observed. Bowman's space was also found to be distended with haemorrhage was noticed in fluoride administered group.

4. Conclusion

In conclusion, all the treatment groups produced significant results in haematological parameters by increasing TLC, Hb and PCV level while no significant changes were seen in RBC count. Fluoride induced morphological changes in liver and kidney. Predominant hepatic histopathological alterations were severe degenerative changes resulting in reduction of sinusoidal space, focal necrosis and vacuolization of hepatocytes. In kidney, nephrotic changes including degenerative changes in tubular epithelium, interstitial haemorrhage, vacuolar changes and destruction of glomeruli. Bowman's space was also found to be distended with haemorrhage were noticed in fluoride administered group. Histopathological alterations in T_3, T_4, T_5 and T_6 were similar to T_2 but of milder degree. By using aqueous extract, complete reversal of the toxic changes were obtained. The overall inference of the study is that the aqueous extract at all the doses, *viz*., 250, 500 and 750 mg/kg b.wt used showed ameliorative effect on the damage, caused by fluoride induced toxicity. No marked histopathological alterations were noticed in any of the vital organ in normal rats. The hepatic architexture was normal and indicate the protective action of *M. oleifera* Lam.

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Conflict of interest

We declare that we have no conflict of interest.

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