EFFECT OF CICHERIUM INTYBUS LEAVES ON N-NITROSODIETHYLAMINE INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT: Cichorium intybus (Asteraceae) is used as traditional medicine in India for various liver related disorders. The present study evaluates the hepatoprotective potential of leaf extract on N-nitrosodiethylamine induced hepatotoxicity, which is commonly present in foods, beverages, tobacco smoke, herbicides, pesticides, drinking water, and industrial pollution. The leaves were sorted as per their size (short, medium, large) and subjected to extraction with ethanol, water and ethanol: water (1:1 w/w) by cold maceration and hot soxlation. The extract having the highest extractive value 80.7% w/w was selected for animal studies. Group I, II, III served as control, toxic and standard. Group IV and V were post treatment receiving 400 mg/kg body weight and 800 mg/kg body weight respectively and group VI as pre treatment group receiving 800 mg/kg body weight of the extract before the induction of toxicity. The level of serum markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were significantly suppressed (P<0.0001) in both the groups receiving 400 mg/kg body weight and 800 mg/kg body weight of extract as compared to the toxic group. Activities of enzymic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR)) antioxidants were significantly increased (P<0.05) on supplementation with the extract. Histopathological studies also evidenced the liver protective effect of the plant extract. The study results show that treatment with 800 mg/kg body weight Cichorium intybus extract before or after NDEA provides protection against the hepatotoxicity caused by NDEA.

INTRODUCTION: Liver disease is currently one of the major health problems worldwide. According to the report of World Health Organization 75 % of the world population relies on the traditional medicines of plant origin1. Liver is the major site of metabolic activities. The detoxification of harmful chemicals occurs in the liver which in turn results in various hepatic diseases.

The general population may possibly be exposed to unknown quantities of N-nitrosodiethylamine (NDEA) present in foods, beverages, tobacco smoke, herbicides, pesticides, drinking water and industrial pollution. N-Nitrosodiethylamine is present in a variety of foods, including cheese at concentrations of 0.5 to 30 μg/kg, soybeans at 0.2 μg/kg, soybean oil at 4 μg/kg, various fish at 1 to 147 μg/kg, salt-dried fish at 1.2 to 21 mg/kg, cured meats at up to 40 μg/kg, and alcoholic beverages at 0.1 μg/kg.

N-Nitrosodiethylamine has been detected in tobacco smoke condensate at concentrations of 1.0 to 28 ng/cigarette. Upto 8.3 ng/cigarette were found in mainstream smoke and 8 to 73 ng/cigarette were...
found in sidestream smoke. An analysis of indoor air polluted with tobacco smoke indicated levels of up to 0.2 ng/L of N-nitrosodiethylamine. The compound has also been found at a concentration of 10 ng/m³ in the smoking compartment of a train.

Many Indian ancient medicinal texts emphasize on the hepatoprotective capacity of certain plants such as kalmegh (Andrographis paniculata), bhuia amla (Phyllanthus niruri), Indian bearberry (Berberis aristata), turmeric (Curcuma longa), kutki (Picrorhiza kurroa), mulethi (Glycyrrhiza glabra), punarnava (Boerhavia diffusa), tulsi (Ocimum sanctum), bhringa Raja (Eclipta alba), kanak champa (Pterospermum acerifolium), guduchi (Tinospora cordifolia), chirayata (Swertia chirata) besides chicory (Cichorium intybus). To validate the same various pharmacological and biological studies were previously performed on Cichorium intybus different plant parts like seeds, roots, leaves, stem etc.

For example (a) the alcoholic extract of seeds at the dose of 69.6 mg/kg bodyweight demonstrated hepatoprotective effect in chlorpromazine-induced hepatic damage in rats, (b) it is one of the herbal components of Liv-52, a traditional Indian tonic used widely for hepatoprotection. In a randomized, double blind clinical trial conducted on cirrhotic patients, Liv-52 medication reduced the serum levels of hepatic enzymes, namely alanine amino transferase and aspartate amino transaminase, another polyherbal formulation jigrine, contains the leaves of C. intybus as one of the 14 constituents. Jigrine was evaluated for its hepatoprotective activity against galactosamine induced hepatopathy in rats. The pretreatment of male Wistar albino rats with jigrine, significantly reduced the levels of alanine amino transferase and aspartate amino transaminase, and urea and increased the levels of blood and tissue glutathione. Histopathological examination of the liver revealed that the jigrine pretreatment prevented galactosamine toxicity and caused a marked decrease in inflamed cells.

The effect of N-nitroso compound was analyzed and it was found to cause breaking in the DNA strand of liver cells and also several enzymatic changes which induces hepatocellular carcinoma in experimental animals. Oxidative stress is considered as critical mechanism contributing to NDEA-induced hepatotoxicity and so the use of antioxidants reduces liver damage. NDEA is used either alone or in combination with other carcinogens like 2-acetylaminofluorine (2-AAF), phenobarbitol benzopyrene, ortic acid, N-amyl-N-methylnitrosoamine and carbontetrachloride.

To get an insight into the progression of the disease, hepatotoxicity can be accessed at three levels (i) alanine amino transferase (ALT) that is glutamyl oxaloacetic acid transaminase level which increases three fold in the serum. (ii) serum alkaline phosphates (ALP) level increases two fold and (iii) serum bilirubin(SBLN) level elevated two fold.

Researchers have investigated plant’s seed extract against acetaminophen and carbon tetrachloride-induced liver damage in mice. In analogous studies, the antihypototoxic activity of the alcoholic extract of the seeds and aqueous extracts of the roots and root callus of C.intybus was estimated. The oral administration of these extracts in albino rats lead to a marked decrease in the levels of hepatic enzymes. Also, histopathological examination of the liver showed no fat accumulation or necrosis after the treatment. The carbon tetrachloride and paracetamol-induced liver toxicities were also found to be counteracted by intraperitoneal administration of crude extracts and fractions of C. intybus. In the same study, toxicity was induced in rat hepatocytes by incubation with galactosamine and thioacetamide.

The hepatoprotective activity of C. intybus has been correlated to its ability to inhibit the free radical mediated damage. A fraction prepared from the ethanolic extract of the leaves was assessed for preventive action on the free radical mediated damage to the deoxyribose sugar of the DNA (obtained from calf thymus) when a dose dependent decrease in the DNA damage was observed.

In the present paper we tried to evaluate and validate the hepatoprotective effects of Cichorium intybus leaves on the basis of its size on NDEA induced toxicity in male wistar rats, so that in
future an effective formulation could be developed with reduced dose which will be specific for imparting hepatoprotection during such toxicities.

MATERIALS AND METHODS:

Collection of Plant Material:
The seeds of Cichorium intybus were procured from khari bawli market, Delhi, India and was sown in the botanical garden Hamdard university, New Delhi. The leaves were collected and segregated as per three sizes viz. short, medium, long. They were identified by the head, raw materials herbarium and museum, NISCAIR, New Delhi and a voucher specimen number NISCAIR/RHMD/Consult/-2010-11/1603/201 was deposited at the herbarium of national institute of science communication and information resources, New Delhi.

Animals:
Male wistar rats (130±10 g, 4–6-week old) were obtained from central animal house of hamdard university, New Delhi. They were housed in polypropylene cages in groups of six rats per cage and kept in a room maintained at 25±2°C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed (amrut laboratory, rat and mice feed, navmaharashtra chakan oil mills ltd., pune, India) and water *ad libitum*. Animal experimentation approval was granted from the Institutional animal ethics committee registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA).

Chemicals and reagents:
DEN and 2-AAF were purchased from sigma chemical company, USA, silymarin was purchased from Micro Labs, all other chemicals and reagents were of analytical grade, supplied by s.merck (India).

Preparation of Leave extract:
The leaves were collected from the herbal garden in the month of January and were segregated as per three sizes viz short (5 mm), medium (5-7 mm) and large (7 mm & above). They were washed with distilled water and dried away from direct light, and then powdered. The powder was kept in three different closed container at 4°C. The powdered material (500 g) was extracted three times in three different solvents viz. water, ethanol, water-ethanol(1:1,v/v) by two methods viz hot soxlation and by cold maceration, the cold extracts were vacuum dried while the hot extracts were concentrated under reduced pressure. The dried extract was then suspended in three solvents mentioned above and the volume adjusted to 500 ml. (1 g plant powder per ml). All the eighteen concentrated extract was divided in 25 ml aliquots, labeled properly and kept at -20 °C for further investigation.

Preliminary Phytochemical Screening of the extract:
Preliminary phytochemical screening of all the eighteen extracts of Cichorium intybus leaves were performed to detect the presence of saponins, sterol, triterpines, alkaloids, coumarins, and Flavonoids. Phenolic acids tests were carried by various chemical tests as mentioned in I.P (1966) by thin layer chromatography. Determination of total Flavonoid content was performed in the three extracts whose extractive values was found to be highest, amongst the eighteen extracts from the three sized leaves viz short, medium and large as prepared above.

Animal Study Protocol:
Rats were randomly divided into six groups with six animals in each group. The extract having the highest extractive value was codified as ‘NCP 72’ for use in animal studies. The experimental design and treatment protocol were as follows:

Group I (Normal Control):
Orally administered only CMC 1% solution

Group II (Toxic Control):
Received i.p.100 mg/kg body weight NDEA

Group III (Standard Control):
Received a single dose of NDEA (100 mg/kg body weight, i.p.) followed by orally administered silymarin (25 mg/kg body weight for 10 days)
Received a single dose of NDEA (100 mg/kg body weight, i.p.) followed by orally administered plant extract ‘NCP72’ (400 mg/kg body weight for 10 days)
Mathur et al., JIPS, 2015; Vol. 6(9): 3861-3871.

**Group V (800 mg/kg Post Treatment)**
Received a single dose of NDEA (100 mg/kg body weight, i.p.) followed by orally administered plant extract ‘NCP72’ (800 mg/kg body weight for 10 days)

**Group VI (800 mg/kg Pre Treatment)**
Orally administered plant extract ‘NCP72’ (800 mg/kg body weight for 10 days) followed by a single dose of NDEA (100 mg/kg body weight, i.p)

Rats were then sacrificed by cervical decapitation at the end of treatment and blood samples were collected. Serum was then separated by centrifugation at 1,200×g for 10 min and stored at –80ºC for 12 hrs before analysis. Liver was removed and washed in ice cold normal saline and divided into 2 pieces; one piece is stored in 10% formalin solution at room temperature for histopathological analysis and second is preserved in 0.1N phosphate buffer, pH 7.4 for biochemical studies. Liver homogenate was made in ice cold 0.15 M KCl solution using motor driven teflon pestle. The supernatant was further centrifuged at 10,000×g for 20 min at 4°C to get the post-mitochondrial supernatant (PMS), which was used for various biochemical assays.

**Biochemical estimations:**

**Assay for serum AST and ALT activity:**
The serum enzymes were assayed using diagnostic kits provided by Span, and the AST and ALT activity was determined.

**Assay for alkaline phosphatase activity:**
Alkaline phosphatase (ALP) activity was determined using the Span diagnostic kit.

**Liver TBARS and Protein estimation:**
TBARS was used as a measure of lipid peroxidation and measured by the modified method. Total protein in the tissue homogenate was also estimated. The levels of TBARS were expressed as nmol MDA/mg protein.

**Determination of reduced glutathione:**
Blood and liver glutathione were estimated by 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) and expressed as mg% and μmol/g of protein respectively.

**Assay for catalase:**
Catalase (CAT) activity was analyzed. The assay mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M hydrogen peroxide (H₂O₂) and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. CAT activity was expressed as nanomoles H₂O₂ consumed per minute per milligram protein.

**Assay for superoxide dismutase:**
Superoxide dismutase (SOD) activity was measured with some modifications. The reaction mixture contained 0.8 ml of 50 mmol/l glycine buffer (pH 10.4), and 0.2 ml PMS. The reaction was initiated by the addition of 0.02 ml of a 20mg/ml solution of (−) epinephrine. Absorbance was recorded at 480 nm in a spectrophotometer. SOD activity was expressed as nanomoles of (−) epinephrine protected from oxidation by the sample as compared with the corresponding readings in the blank cuvette. The molar extinction coefficient of 4.02×10³ M⁻¹ cm⁻¹ was used for calculations.

**Histological studies:**
Liver sections from various groups were stained with haematoxylin and eosin and were preserved in buffered formalin (10%). Histological liver sections were prepared as described by.

**Data Analysis:**
Results are expressed as mean ± SEM. Statistical analysis of the data was obtained via analysis of variance, followed by Turkey’s test. P<0.05 was considered statistically significant.

**RESULTS:**

**Extractive values:**

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Ethanol-water</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short leaves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot</td>
<td>17.54</td>
<td>44.45</td>
<td>64.32</td>
</tr>
<tr>
<td>Cold</td>
<td>15.34</td>
<td>27.91</td>
<td>48.35</td>
</tr>
<tr>
<td><strong>Medium leaves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot</td>
<td>66.71</td>
<td>80.7</td>
<td>71.83</td>
</tr>
<tr>
<td>Cold</td>
<td>35.02</td>
<td>49.81</td>
<td>65.16</td>
</tr>
</tbody>
</table>

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**TABLE 1: EXTRACTIVE VALUES (%w/w) OF CICHERIUM INTYBUS LEAVES**

International Journal of Pharmaceutical Sciences and Research
The extract prepared from medium sized leaves by hot soxlation yielded highest extractive value 80.7\% w/w and so was chosen for administration to rats for animal studies. It was codified as ‘NCP 72’. Total flavonoid content in 1mg/ml sample of the extract ‘NCP 72’ was found to be 39.2 mg of Quercetin equivalent/gm of extract.\textsuperscript{31} (Mathur et al., 2014)

**Morphological Changes:**
Body weights of control group rats were slightly higher than those in NDEA-treated group. Body weight of the groups, control, NDEA-treated and protected was measured weekly to assess the effect of NDEA & efficacy of plant extract NCP72 against NDEA on general metabolism of experimental animals.

Usually NDEA administration causes weight loss in rats but in rats it was observed that although the body weight increased in all the groups, but the increase in weight in NDEA treated group was slow as compared to the control & protected, who gained their body weight throughout 3 weeks time (Fig. 1). At the start of experiment it was seen that the body weight was 140g and 150g for control and treated respectively. Within the course of experiment the body weight of control increased to 190 g and that of treated was 170 g.

**Biochemical parameters:**
Rats treated with NDEA showed significant hepatic damage as observed from increase in serum enzymes (SGOT, SGPT, and ALP) and lipid peroxidation Fig.3–5 and glutathione reductase, SOD and catalase Fig. 6-8. However, pretreatment of rats with NCP72 extract (800mg/kg dose) followed by NDEA, afforded protection by lowering the serum enzymes. In addition, pretreatment of extract normalized the level of antioxidant enzymes Fig. 6-8. Protection was observed maximally with the highest dose of the extract.
Note: - Fig. 2, 3 Values are expressed as mean ± SEM (n=12). Diseased group showed a significant increase in AST, ALT, ALP levels as compared to the control group.
[P<0.0001 disease vs. control group].

Fig. 4, 5, 6, 7 Analysis of hepatic oxidative stress parameters like lipid peroxidation, SOD, CAT [P<0.05 disease vs. control group].

Histopathological observations:
Sections from all the six groups were examined. The histological examination of H&E –stained control liver sections showed normal architecture of hepatocytes (Fig.9a, b). The NDEA followed by 400 mg Cichorium intybus extract group showed moderate sinusoidal dilatation (Fig.9e, f).

The groups that received silimarin (Fig.9c, d) after NDEA or 800 mg Cichorium intybus extract (Fig.9g, h) and the pre-treatment group showed nearly normal liver tissue (Fig.9i). The post-treatment group also shows the centronal area with no sinusoidal dilatation in the liver section (Fig. 9j).

These results show that treatment with 800 mg Cichorium intybus extract before or after NDEA provides protection against the hepatotoxicity caused by administration of NDEA similar to that achieved by use of silimarin.
Histopathological sections:

(a) Normal Control (100X)

(b) Normal Control (400X)

(c) Standard Control (10X)

(d) Standard Control (40X)

(e) NDEA + 400mg extract (10X)

(f) NDEA + 400mg extract (40X)

(g) NDEA + 800mg extract (10X)

(h) NDEA + 800mg extract (40X)
FIG. 8: HISTOPATHOLOGICAL OBSERVATIONS OF THE EFFECT OF CICHORIUM INTYBUS LEAVE EXTRACT ON NDEA INTOXICATED MALE WISTAR RATS.

Captions:
(a) Liver 10x. Control group showing normal liver architecture, PT portal triad, CV central vein. (HE x 100X).
(b) Liver 40x. Same section showing details of a normal PT; PV portal vein, BD bile duct, HA hepatic artery (HE x 400X).
(c) Liver 10x. Low power photomicrograph of liver from animal in NDEA followed by Silymarin group showing normal arrangement of cells in the liver lobule. PT = Portal Triad and CV = Central vein. (HE x 100X).
(d) Liver 40x. High power photomicrograph of liver from animal in NDEA followed by Silymarin group showing a portal triad. PV=Portal Vein, HA=Hepatic Artery, BD=Bile Duct (HE x 400X).
(e) Liver 10x. Low power photomicrograph of liver from animal in NDEA followed by 400 mg C.I Extract group showing normal arrangement of cells in the liver lobule. There is moderate sinusoidal dilatation seen. PT = Portal Triad and CV = Central vein. (HE x 100X).
(f) Liver 40x. High power photomicrograph of liver from animal in NDEA followed by 400 mg C.I Extract group showing the centrilobular area with sinusoidal dilatation seen. CV=Central Vein. (HE x 400X).
(g) Liver 10x. Low power photomicrograph of liver from animal in NDEA followed by 800 mg C.I Extract group showing normal arrangement of cells in the liver lobule. There is no sinusoidal dilatation seen. PT = Portal Triad and CV = Central vein. (HE x 100X).
(h) Liver 40x. High power photomicrograph of liver from animal in NDEA followed by 800 mg C.I Extract group showing the centrilobular area with no sinusoidal dilatation seen. CV=Central Vein. (HE x 400X).
(i) Liver 10x. Low power photomicrograph of liver from animal in 800 mg C.I Extract followed by NDEA (Pre-Treatment) group showing normal arrangement of cells in the liver lobule. There is no sinusoidal dilatation seen. PT = Portal Triad and CV = Central vein. (HE x 100X).
(j) Liver 40x. High power photomicrograph of liver from animal in NDEA followed by 800 mg C.I Extract group (Post-Treatment) showing the centrilobular area with no sinusoidal dilatation seen. CV=Central Vein. (HE x 400X).

DISCUSSION: The ethnobotanical studies of Cichorium intybus have proven its uses in skin diseases, calculus, enlargement of liver, as stomachic, digestive, tonic, mild diuretic. Leaves are specifically used to cure jaundice & liver disorders. Leaves and roots are also effective in curing swelling of joints. A study was conducted to determine the suitable age of harvest of green tops of chicory for extraction of edible leaf protein by, the age of 150 to 155 d was found suitable for obtaining optimum yield of leaf protein from green tops of chicory. The study also revealed that the level of antioxidants was maximal between 40 and 50 d after sowing, indicating the optimal period of harvesting.

These studies have initiated the idea that the collection of leaves at different time intervals and...
thus grading them according to their sizes (short, medium, large) may influence its activity as a hepatoprotective agent. Three different solvent system viz. water, ethanol-water (1:1 %v/v), ethanol, and two extraction process viz. hot and cold were choosen to extract all possible bioactive ingredients in the leaves in this range of solvent system. Thus eighteen extracts were prepared. Amongst these eighteen extracts, the extract having the highest extractive value was choosen for preliminary phytochemical studies and was used further for animal and histopathological studies. Our results have shown that the highest extractive value was found in the extract prepared from medium sized leaves by hot soxlation process using water-ethanol (1:1, v/v) as a solvent. Further, this extract was used for animal and histopathological studies. Two doses were selected for study based on previous research work conducted on chicory 39.

In our study we selected NDEA (N-nitrosodiethylamine) toxicity as NDEA is a major environmental carcinogen responsible for increasing the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury 40. NDEA is found in a wide variety of foods such as cheese, soybeans, salted and dried fish, cured meat and alcoholic beverages 41. Metabolism of certain therapeutic drugs is also reported to produce N-nitrosodiethylamine 42. Since liver is the main site of NDEA metabolism, the production of ROS in the liver may be responsible for its carcinogenic effects 43. For the assessment of drug induced hepatotoxicity, new powerful in vitro models are being developed, besides the in vivo models to study carcinogenesis in liver 44, 45, 46, 47.

Research Literature shows that NDEA can be administered via different routes like oral, subcutaneous, and intraperitoneal etc. We have selected intraperitoneal route for NDEA administration to rats on the basis of our previous research work 48. However, plant extract was administered orally, as based on these findings we aim to develop an oral formulation for future use.

ALT, AST, ALP are liberated into blood whenever liver cells are damaged and their increased levels in the blood are index of liver damage 49. Thus, by measuring the activities of serum marker enzymes one can make assessment of liver function 50, 51. While NDEA has carcinogenicity, it is also potent alkylating agent and shows toxicity. Our results have shown that the elevated levels of ALP, AST and ALT after NDEA administration in rats, confirming the liver damage caused by this compound. Other parameters assayed were lipid peroxidation (TBARS), reduced glutathione level, Catalase (CAT), superoxide dismutase (SOD) activity. Literature review has revealed that oxidative stress is one of the key factors during carcinogenesis 52 and Lipid peroxidation is one of the most studied biologically relevant free radical chain reactions which is initiated by the attack of a free radical on a fatty acid or fatty acyl side chain of any chemical species that has sufficient reactivity to extract a hydrogen atom from a methylene carbon side chain.

Lipid peroxidation may lead to the formation of several toxic byproducts such as malondialdehyde and 4-hydroxynonenal, which can attack cellular targets including DNA, inducing mutagenecity and carcinogenicity 52, 53. Administration of NDEA has been reported to generate lipid peroxidation products in general 54 and the same was also reported in the NDEA treated animals. Chemically induced liver carcinoma is associated with changes in oxygen radical metabolism in liver. The changes in hepatic oxygen radical metabolism were demonstrated by measurement of antioxidant enzymes such as SOD and catalase 55.

Tumor cells show a decrease in the activities of SOD and CAT though the mechanism is still unclear. As CAT and SOD are the two major scavenging enzymes that remove radicals in vivo, a decrease in activity of these antioxidants can lead to an excess availability of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (·OH), resulting in initiation and propagation of lipid peroxidation. SOD catalyzes dismutation of O_2^- to H_2O_2, which is then deactivated to H_2O by CAT 56, 57.

In the present study, we report that the levels of these antioxidative enzymes were also decreased in experimental groups with the progression of disease as compared to control groups, suggesting...
the protective effect of the *Cichorium intybus* leaf extract in liver.

**CONCLUSION:** Our study aimed at identifying that particular leaf extract of *Cichorium intybus* which is effective against NDEA induced toxicity in male wistar rats. Literature review has revealed that *Cichorium intybus* (family Asteraceae) contains coumarins and sesquiterpene lactones, further they can be isolated and characterized for future formulation of this extract for easy and effective dose management.

**ACKNOWLEDGEMENT:** The authors are grateful to Dr. Ashok K. Chauhan, Hon’ble Founder President, Amity University Uttar Pradesh, Noida for providing facilities for conducting the research.

**REFERENCES:**


How to cite this article:

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