NEUROPROTECTIVE POTENTIAL OF *CRESCENTIA CUJETE* AGAINST MPTP INDUCED NEUROTOXICITY IN PARKINSON'S RAT BRAIN

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ABSTRACT

The aim of the present study was to evaluate neuroprotective effect of ethanolic extracts of *Crescentia cujete* (Bignoniaceae) in MPTP (1-methyl,4-phenyl-1,2,3,6-tetrahydropyridine) mouse model. *Crescentia cujete leaves* extracts was administered at different doses of 250 & 500 mg/kg b.wt (P.O) in different groups once a day for fourteen days and the first dose was given 30 min prior to first MPTP injection. Mice were sacrificed by decapitation and whole brain was analysed for Norepinephrine, Epinephrine, dopamine, serotonin, GABA, glutamate, Acetylcholinesterase and MAO-A and B levels. Ethanolic extract of *C.cujete* significantly ($P<0.001$) improved the brain neurochemical status like epinephrine, norepinephrine, dopamine and serotonin and also improved the neuroexcitatory contents by increase in GABA and acetylcholinesterase levels and decrease in glutamate as compared to MPTP control group in a dose dependent manner, The increased level of MAO A and B were decreased significantly upon the administration of *C.cujete.* The results of the present study indicated that the ethanolic extract of *Crescentia cujete* leaves possess significant neuroprotection activity.

Key words: Parkinson disease, *Crescentia cujete*, Dopamine, Norepinephrine, Serotonin, GABA, Monoamines

INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disorder affecting 1-3 percent of people over the age of 65, frequently impairing the motor skills, voice, and other functions of the sufferer. The disorder is named for a pharmacist from England. James Parkinson, who explained the condition in detail in his essay: "A Shaking Palsy Essay." Parkinson's disease develops when nerve cells, or neurons, are damaged and/or die in an area of the brain that regulates motion. Such neurons usually secrete significant brain chemicals known as dopamine's. When the neurons die or get damaged, they release a smaller amount of dopamine, which causes Parkinson's movement discomfort (Toulouse *et al.,* 2008). Other symptoms include tremor, rigidity, bradykinesia, postural instability, gait, festination, dystonia, hypophonia, micrographia and dysphagia. Secondary symptoms are mood, cognitive, sleep, sensational disturbances, weight loss, incontinence and constipation (Liu *et al.,* 2012). The medications such as levodopa–carbidopa, dopamine agonists, monoamine oxidase B inhibitors, and amantadine are currently the most widely used PD treatment. The

current therapeutic strategy is mainly to increase the activity of dopaminergic neurons or to reduce the cholinergic effects on the striatum. Available prescription medications currently only provide temporary relief for the PD (Ramirez and Molho, 2014).

Over the last decade, numerous studies have been published exploring the anti-PD therapeutic potential of herbs, fruits, vegetables and spices, ornamental and parasite plants, and fungi. Empirical evidence includes phytochemicals that can play a role in preventing and reducing some of the PD's intractable signs and symptoms (Ven Murthy *et al*. 2010).

Traditionally, *Crescentia cujete* have been reported to be used among many tribes and different culture around the world to treat different aliments (Pravin *et al*., 2015). Studies have suggested antiangiogenic, neutralizing snake venom, CNS depressant, cytotoxic, wound healing, antiplatelet aggregation, antidiabetic, antibacterial, antimycobacterial, anti-inflammatory, anthelmintic properties (Musbau *et al*., 2018). In the present investigation, an attempt has been made to study the effect of ethanolic extract of *crescentia cujete* leaves against the neurochemical changes induced by 1-methyl-4-phenyl-1,2,3,6–tetra hydropyridine in mice.

MATERIALS AND METHODS:

Preparation of ethanolic extract of *Crescentia cujete* (EECC) leaves

The leaves of *Crescentia cujete* were collected from different localities of Coimbatore District and authenticated by Botanical Survey of India (BSI) of “Tamil Nadu Agricultural University” Coimbatore with a voucher specimen (No: BSI/SRC/5/23/2017/Tech 2021).The leaves were washed with running tap water and separated before being chopped into pieces. They were oven dried at 45°C for 2 days and ground to powder form. The powdered material of *Crescentia cujete* was extracted with ethanol solvent using Soxhlet extractors exhaustively for 20-24 hours. The extract was concentrated at reduced pressure and temperature regulated (40-50 ° C) to dryness.

Selection of animals for MPTP induced Parkinson evaluation

40 Male Swiss albino mice 3 months of age, and 25-30 g body weight were offered by Nandha College of Pharmacy, Erode. All the rats were kept at room temperature and allowed to Acclimatization in the animal house under standard conditions of less than 12 hours light/12 hours dark period. They were fed on a commercial pellet rat chow and water ad libitum, acclimatized to animal house conditions. Experimental animals were treated under university and institutional law, governed by the Committee for Animal Experimental

Control and Supervision (CPCSEA), Ministry of Social Justice and Empowerment, Government of India with the Reg No: 688/PO/Re/S/02/CPCSEA

Grouping of animals

Experimental design

A total of 40 rats were used. The animals were divided into five groups of eight animals each as follows.

Group I: Normal rats given 1ml of saline water for 14 days

Group II: Parkinson was induced in overnight fasted animals by twice dose of MPTP (15 mg/kg b.wt) by intraperitoneally with 4 h interval each for 14 days

Group III: Parkinson induced rats (as in group II) treated with EECC leaves (LD-250 mg/kg, p.o) for 14days

Group IV: Parkinson induced rats (as in group II) treated with EECC leaves (LD-500 mg/kg, p.o) for 14 days

Group V: Parkinson induced rats (as in group II) treated with Standard (Levodopa 12 mg/kg + Benzerazide 3mg/kg i.p) for 14 days

Assessment of brain Neurochemical status in the experimental animals

Estimation of Epinephrine, Nor epinephrine, Dopamine and Serotonin

Rats were killed at the end of the experiment, and dissected the entire brain. Weighed 0.25 g of tissue and homogenized to approximately 1 min in 5 mL HCl–butanol with motor-driven Teflon coated homogenizer. Afterwards the sample was centrifuged at 2000 rpm for 10 min. An aliquot supernatant (1 mL) step was removed and applied to the heptane (2.5 mL) and 0.1 mHCl (0.31 mL) centrifuge tube. The tube was centrifuged under the same conditions as above after 10 min of intense shaking to separate the two stages, and the organic overlaying phase was discarded. Then the aqueous phase was taken for either 5-HT or NA and DA assays. For 1 ml of aqueous phase, 0.25 ml 0.4 M HCl and 0.5 mL Sodium acetate buffer (pH 6.9) were added, followed by an oxidation solution of 0.5 ml iodine (0.1 M in ethanol). With the addition of 0.5 ml Na_2SO_3 solution the reaction was stopped after 2 min. After 1.5 min, 0.5 ml Acetic acid was added. The solution was then heated up for 6 min to 100 ° C. The sample was read from the spectrofluorimeter when it reached room temperature, excitation, and emission spectrums. The measurements were taken in dopamine at 330-375 nm and in nor adrenaline at 395-485 nm. Blanks were prepared for the assay by adding oxidation stage reagents in reversed order (sodium sulphite before iodine) 1 mg/ml was used as standard (Margret Schlumfjf *et al.*, 1984). 1.75 mL of O-phthalaldehyde reagent was added to 1.4 ml of aqueous extract. The fluorophore was designed for 10 min with heating up to 100 ° C. After the samples achieved equilibrium with the ambient temperature,

readings in the spectrofluorimeter were taken at 360-470 nm. The condensed HCI was taken as blank without an OPT. As normal, serotonin (1 mg / mL) was used at different concentrations (Dilip Kumar Pal 2009).

Estimation of brain Gamma-amino butyricacid (GABA) content

0.1 ml of homogenous tissue was put in a 0.2 ml 0.14 M ninhydrin solution in a 0.5 M carbonate-bicarbonate buffer (pH 9.95) and held for 30 minutes in a water bath at 60 ° C. It was then cooled and treated with 5 ml of tartarated reagent in copper. It was reported in a spectrofluorometer after 10 min fluorescence at 377/455 nm (Raju *et al*., 2004)

Estimation of brain neuroexcitatory glutamate (Glut) and Acetylcholinesterase levels

Enzyme expression for both the samples (brain homogenates) and the blanks was calculated in duplicate. 100 µl of buffer substrates (0.1 M sodium phosphate buffer, pH 8.0, plus acetyl thiocholine iodide) is pipetted into a glass tube and 5 µl of homogenate was added. By adding 15 µl of 2.4 N perchloric acid to the tubes before incubation, blanks were obtained. This was achieved to precipitate the protein thus inhibiting thiocholine formation. The tubes were incubated at a water bath at 37^0C for 30 min with continuous stirring. When perchloric acid was added, the enzyme activity tubes were centrifuged at 2,250 g at 0^oC for 15 min. A 50 µl aliquot was pipetted into another tube, and Ellman's reagent was added to 500 µl. After 15 min, after 1 min and 5 min, the samples were read into a spectrophotometer using micro cuvettes at 412 nm. Protein was measured using standard bovine serum albumin using the Lowry *et al*. process.AChE activity is stated as nmol thiocholine produced per min per mg of protein AChE activity was measured using the formulation below:

R = O.D. X volume of assay/E X mg of protein

Where R=rate of enzyme activity in “n” moles of acetylcholine iodide hydrolyzed/min/mg protein and O.D=change in absorbance/min, E=extinction coefficient= 13600/M/cm (Ellman *et al*., 1961).

Weighed brain section was homogenized by weight of perchloric acid with 2 parts, and centrifuged at 3000 rpm for 10 min. 3.0 ml supernatant fluid with 1.0 ml phosphate solution was balanced to the pH 9. It was allowed to stand in an ice bath for 10 minutes, and then was filtered through a thin, fluted filter paper. It was further heated to RT and diluted with phosphate solution in 1:10 ratio; pH 9.0 (buffer) and 1.0 ml of this was taken for assay.

The absorbance was 340 nm. Likewise, they reported a blank reading at 340 nm. The glutamate levels were expressed as µmol per g of brain tissue. (Titus *et al*., 2007).

Estimation of Monoamine Oxidase A and B levels

Procedure for estimation of MAO- A

250 µl of the homogenate was added to 250 µl of serotonin and 250 µl of buffer. The reaction tube was placed at 37°C for 20 minutes and the reaction was arrested by the addition of 200 µl of 1M HCl. The reaction product was extracted with 5 ml of Butyl acetate. The organic phase was separated and measured at 280 nm for estimation of MAO- A and 242 nm for estimation of MAO- B using a spectrophotometer. Blank samples were prepared by adding 1M HCl (200 µl) prior to reaction and the reaction was carried out. The MAO-A and B is expressed in n moles/ mg protein (Charles & McEwen, 1977)

RESULTS AND DISCUSSION

Estimation of Nor-epinephrine, Epinephrine, dopamine and serotonin

Fig 1.1: Effect of ethanolic leaves extract of *Crescentia cujete* on brain Norepinephrine and Epinephrine level in MPTP treated mice

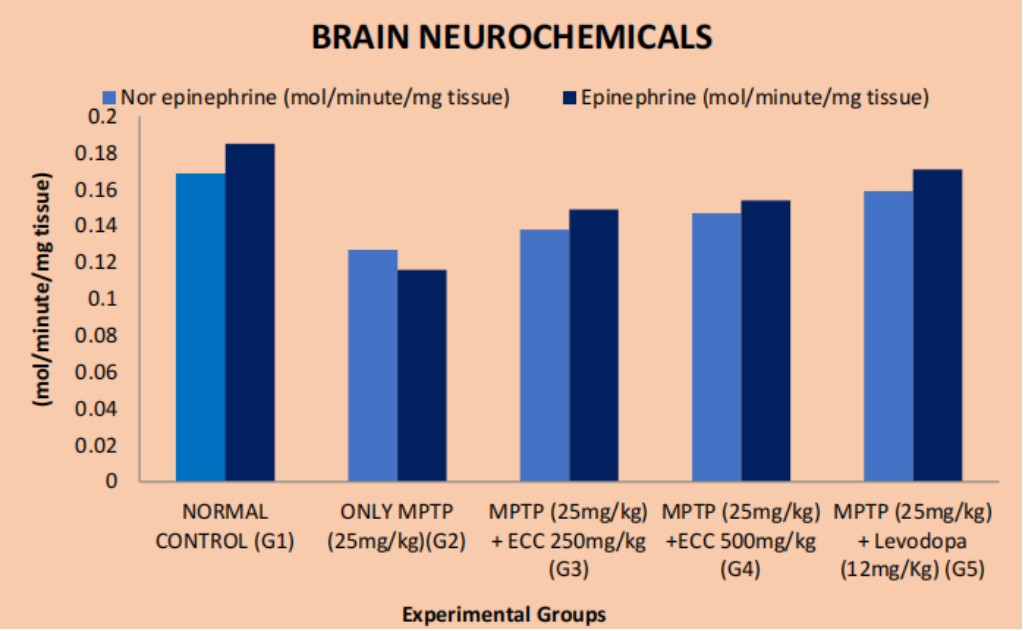
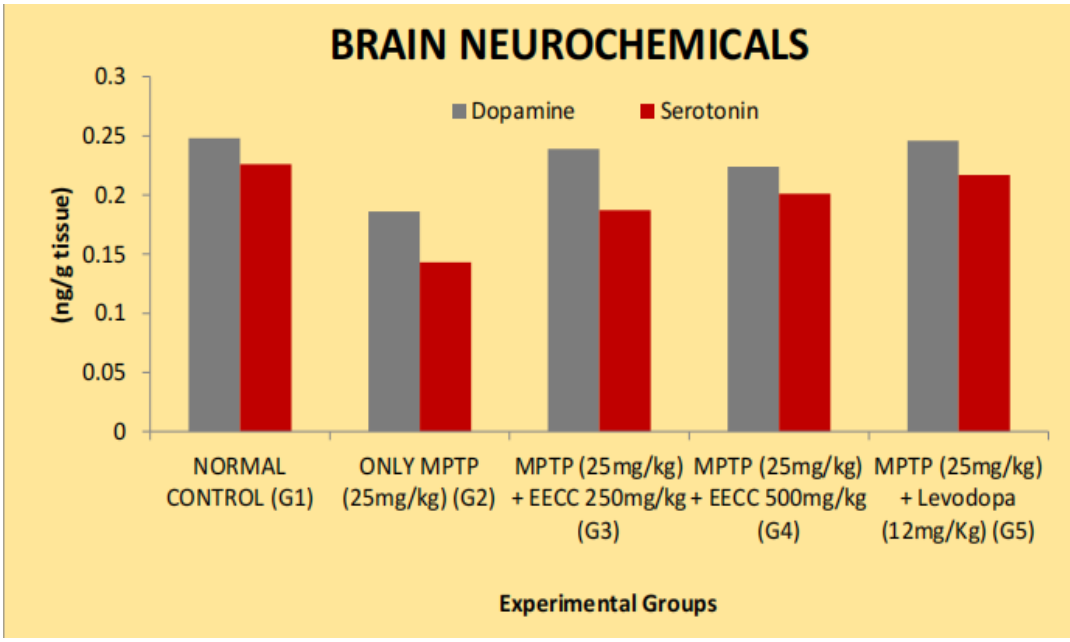


Figure 1.1, it is evident that the increase in brain Norepinephrine and Epinephrine levels was observed in MPTP induced Parkinson rats G2 (0.127±0.003 and 0.116±0.042 mol/minute/mgtissue), when compared to control G1 (0.168±0.029 and0.185±0.006 042

mol/minute/mgtissue) and Levodopa treated rats G5 (0.159±0.0029 and 0.171±0.0029 mol/minute/mgtissue). The decreased level in Parkinson rats was reversed significantly by administration of ethanolic leaves extract of *Crescentia cujete* at 250 mg/kg b.wt G3 (0.138±0.071and 0.149±0.0009 mol/minute/mgtissue) and ethanolic leaves extract of *Crescentia cujete* at 500 mg/kg b.wt G4 (0.147±0.0059 and 0.154±0.0049 mol/minute/mgtissue).

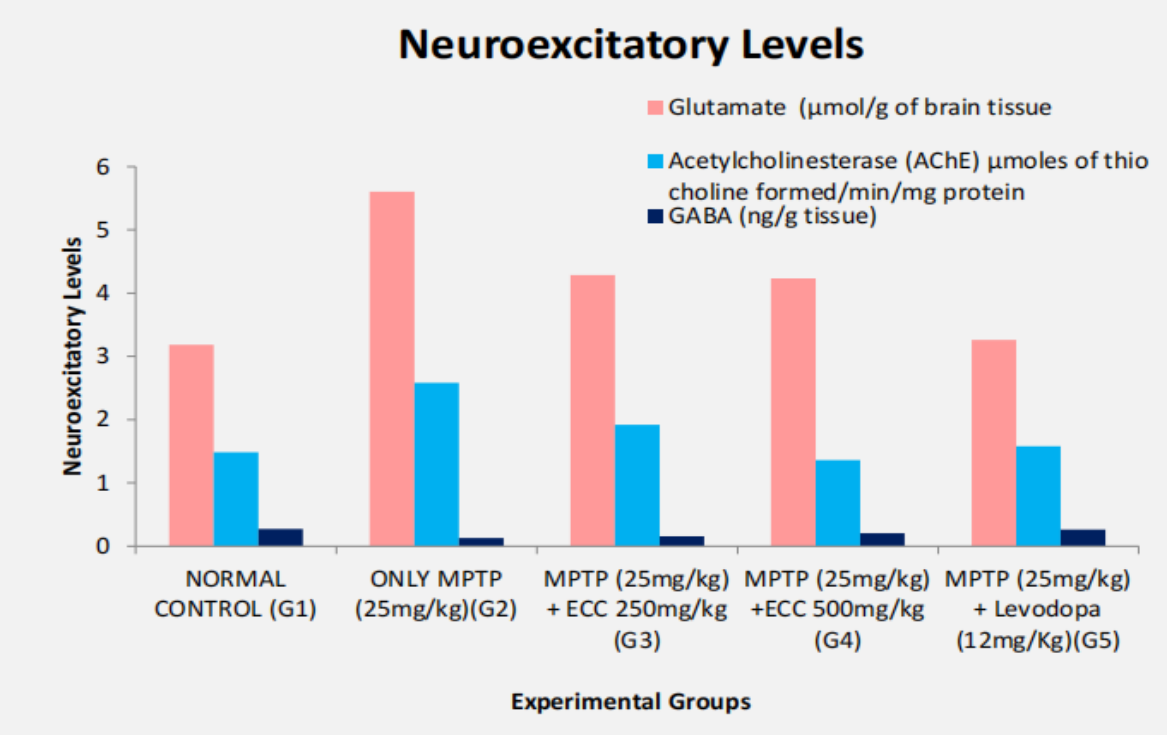
Fig 1.2: Effect of ethanolic leaves extract of *Crescentia cujete* on brain Dopamine and Serotonin level in MPTP treated mice



The dopamine system is believed to be involved in both physiological as well as the pathophysiology of basal ganglia disorders like PD. Despite excitatory glutamate and inhibitory GABA, dopamine has proved to be a regulatory neurotransmitter in basal ganglia (Morningstar, *et al* 2014)

Figure 1.2, depicts decreased levels of Dopamine and Serotonin hormone in MPTP induced Parkinson group G2 (0.186±0.042 and0.143±0.004 ng/g tissue), when compared to normal control G1 (0.248±0.006 and 0.226±0.009 ng/g tissue) and Levodopa treated rats G5(0.246±0.0034 and0.217±0.010 ng/g tissue) The levels in MPTP induced Parkinson rats (G3&G4) was improved significantly by the administration of ethanolic leaves extract of *Crescentia cujete* at 250 mg/kg bw (0.239±0.0009 and 0.187±0.006 ng/g tissue) and ethanolic leaves extract of *Crescentia cujete* at 500 mg/kg bw (0.224±0.0049 and0.201±0.0035 ng/g tissue).

Fig 1.3: Effect of ethanolic leaves extract of *Crescentia cujete* on Glutamate, Acetylcholinesterase and GABA levels



MPTP- treated rats showed Figure 1.3 increased glutamate level and decreased GABA level at the end of the study G2 (on day 14) as compared with normal control rats G1. These observations may support the hypothesis that many of the signs and symptoms of advanced stages of PD may be driven by an increase in glutamate and a decrease in GABA in the striatum (Carlsson and Carlsson 1990). ECC (250 mg/kg and 500 mg/kg) G3 & G4 and levodopa treated G5 significantly and dose-dependently reversed the alteration in glutamate and GABA levels as compared with MPTP control G2

From the result, MPTP group showed a very significant increase in AChE activity with 2.582±0.033 when compared to control group 1.486±0.089. The rats treated with Levodopa showed a moderate decrease in AChE activity with 1.581±0.087 when compared to control group whereas a significant decrease in AChE activity is also observed in the groups treated with ECC 250 and ECC 500 mg/kg b.w. with 1.918±0.020 and 1.361±0.036.

Fig 1.4: Effect of ethanolic leaves extract of *Crescentia cujete* on MAO A and B activities in MPTP induced parkinsonism mice

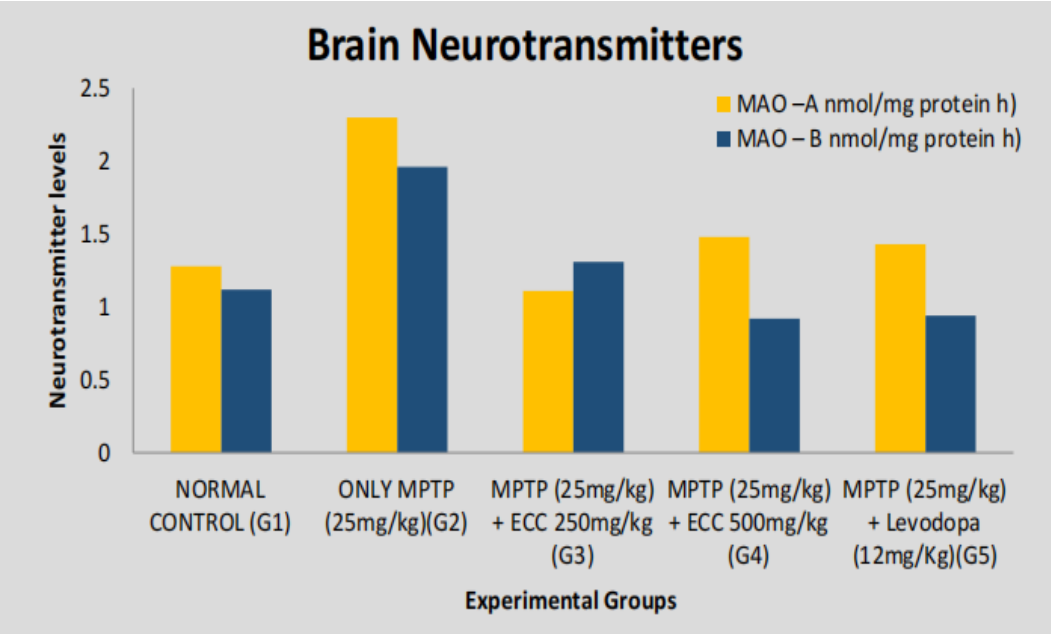


Fig 1.4, it is evident that the increase in MAO-A and B activities in MPTP induced parkinsonism mice (2.30±0.32 &1.96±0.25 nmol/mg protein h) when compared to control (1.28±0.004 &1.12±0.06 nmol/mg protein h) and levodopa treated rats (1.43±0.06 & 0.94±0.09 nmol/mg protein h). The increased level in parkinsonism rats was reversed significantly by the administration of ethanolic leaves extract of *Crescentia cujete* at 250 mg/kg b.wt (1.11±0.21 &1.31±0.02 nmol/mg protein h) and ethanolic leaves extract of *Crescentia cujete* at 500 mg/kg b.wt (1.48±0.03 & 0.92±0.09 nmol/mg protein h).

Consequently, the extract is likely to protect the dopaminergic neurons against these enzyme inhibitions. Our study suggests that ECC possesses the ability to reduce MAO-A activity in combination with an anxiolytic effect and an anti-depressant effect in non-lesioned mice (Tanasawet *et al* 2017).

CONCLUSION

The present *in vivo* studies postulated that chronic administration of MPTP can produce morphological abnormalities enabling visualization of any changes on the brain slices. It confirms ethanolic leaves extract of *Crescentia cujete* offered a defense to the dopaminergic neurons by preventing the neurochemical changes induced by MPTP. Thus, the C*rescentia cujete* leaves extract at the dose of 250 & 500 mg/kg b.wt p.o. showed significant neuroprotective activity.

CONFLICT OF INTEREST

Author has no conflict of interest

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