

Gastroprotective effect of *Canna edulis* Ker. ethanolic extract in piroxicam-induced rats

Dara Pranidya Tilarso^{1,2}, Moch. Saiful Bachri^{*1}, Wahyu Widyaningsih¹

¹Department Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl. Prof. Dr. Soepomo, S.H., Janturan, Yogyakarta, Indonesia

²Departement of Pharmacy, STIKes Karya Putra Bangsa
Jl. Raya Tulungagung-Blitar KM 4, Tulungagung, East Java, Indonesia

Submitted: 25-09-2020

Reviewed: 21-01-2021

Accepted: 29-03-2021

ABSTRACT

Medications derived from plants and pure natural ingredients have far lower side effects and risks than synthetic drugs. One side effect of piroxicam is irritation of the digestive tract. One of the therapeutic preventions to minimize peptic ulcers was utilizing *Canna edulis* Ker. This study aims to prove that ethanolic extract of *Canna edulis* Ker. can be used as an alternative to prevent piroxicam-induced peptic ulcers based on ulcer index parameters and the protection ratio. The rats were divided randomly into 6 groups consisting of 5 rats. The normal group was given food and water. The negative control group was given 0.5% CMC-Na. The extract groups were given various doses of ethanolic extract of *Canna edulis* Ker. (50, 100, and 200 mg/kg BW). The positive control was given sucralfate at 360 mg/kg BW dose. Rats were treated orally for 14 days. One hour after the treatment on the 14th day, all groups except group I were orally administered with piroxicam dissolved in 0.5% CMC-Na at 1.8 mg/kg BW dose. Twenty-four hours later, animals were sacrificed, dissected, and their stomach organs were removed to analyze its number of ulcers. Ulcer observation was formed by giving a score and protection ratio. The mean ulcer index value was 0.33 ± 0.58 for 50 mg/kg BW and 100 mg/kg BW ethanol extract treatment groups, while the 200 mg/kg BW group showed 0.67 ± 0.58 . The protection ratio was 83.33 ± 28.87 in 50 mg/kg BW and 100 mg/kg BW treatment groups, while 66.67 ± 28.87 was shown in the 200mg/kg BW group. *Canna edulis* Ker. ethanolic extract has the gastroprotective ability by decreasing the index of gastric ulcers and increasing the protection ratio to the stomach of piroxicam-induced rats.

Keywords: *Canna edulis* Ker., ethanolic extract, gastroprotective, piroxicam

***Corresponding author:**

Moch. Saiful Bachri

Department Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan

Jl. Prof. Dr. Soepomo, S.H., Janturan, Yogyakarta

Email: msaifulbachri@yahoo.co.id



INTRODUCTION

Medications derived from plants and pure natural ingredients have far lower side effects and risks than synthetic drugs. One side effect of piroxicam is irritation of the digestive tract (gastritis). The prevalence of gastritis with a high clinical impact is commonly caused by nonsteroidal anti-inflammatory drugs (NSAIDs). One of the therapeutic preventions to minimize peptic ulcers was utilizing *Canna edulis* Ker. Previous research had been done by utilizing simplicia powder, Canna starch, and glucomannan. The occurrence of gastrointestinal inflammation and hemorrhage can be reduced in the stomach of 450 mg/kg BW aspirin-induced rats; an 81.13% increase in the protection ratio at simplex powder and Canna starch and a 58.49% increase at glucomannan treatments (Kusbandari, 2013). The *Canna edulis* Ker. contained flavonoids, polyphenols, and proanthocyanidin (Mishra et al., 2012). Flavonoids are known as antiulcer and antioxidants in the digestive tract, with efficacious cytoprotective ingredients by protecting the gastric mucosa against ulcerogenic agents through the free radical destruction mechanism and increased mucus production (O'Leary et al., 2004). Some compounds that are also known to treat stomach ulcers are alkaloids. Alkaloids reduce gastric acid secretion, increase mucus and alkali secretion, and improve gastric mucosal blood flow to cure and prevent stomach ulcers against irritant agents/factors (Falcão et al., 2008). The content of efficacious compounds in *Canna edulis* Ker. will be more effective if used in extract form. The extraction carried out can attract the compound's desired components by separating one or more of the existing source components based on a compound's polarity (Mukhriani, 2014). The resulting extract will achieve the desired therapeutic target by its activity and as an alternative in the use of Canna tuber preparations to reduce the risk of patient non-compliance, which can cause the severity of peptic ulcers in patients given NSAIDs. This study aims to prove that *Canna edulis* Ker. ethanolic extract can be used as an alternative to prevent piroxicam-induced peptic ulcers based on ulcer index parameters and the ratio of protection in the stomach.

MATERIALS AND METHOD

Materials

Wistar strain female rats with 150-200 g bodyweight, 6-8 weeks old, healthy condition were obtained from LPPT Gadjah Mada University Yogyakarta. Canna bulbs were obtained from Canna tuber farmers purchased through traders at the Ngemplak Tulungagung market. Piroxicam used in this research is pure piroxicam obtained from PT. Indofarma. The carrier (solvent) for the test solution was a 0.5% CMC-Na solution obtained from the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Ahmad Dahlan. The rat feed used was AD II.

Methods

Plant determination

To ensure that the material used is *Canna edulis* Ker., plant determination was carried out in the Biology Laboratory, Biology Study Program, Universitas Ahmad Dahlan Yogyakarta.

Extract preparations from Canna tubers

The Canna tuber's skin was discarded or peeled and cleaned from impurities by washing using running water. The Canna tuber was cut into pieces with a thickness of 3-5 mm, then were put into the oven at 60-70°C. After drying, these pieces of tubers were pulverized using a blender. The Canna tuber powder was extracted by maceration using 96% ethanol solvent for three (3) days. Then the mixture was filtered with a Buchner funnel and thickened with a rotary evaporator. The viscous extract was dissolved in 0.5% CMC-Na at 50, 100, and 200 mg/kg doses for the gastroprotective study.

Ethanol-free test of ethanol extract

Ethanol extract of *Canna edulis* Ker. was added with H₂SO₄, then added again with CH₃COOH, and heated. The test results were declared ethanol-free (negative) if the ester odor could not be smelled (Zhang et al., 1990)

TLC content determination of ethanol extract

First, the ethanolic extract of *Canna edulis* Ker. was dissolved with 96% ethanol. After that, the GF 254 silica gel was activated in an oven for 30 minutes at a temperature of 140°C. Heating was done to remove water vapor which could interfere with the absorption of the eluted. At the same time, the mobile phase was first saturated in the chamber before being used to elute the silica because the mobile phase would elute perfectly at saturated chamber conditions. After the GF 254 silica gel was activated, the test solution was placed on the TLC plate of silica gel GF 254 using a capillary tube. The first test solution was a flavonoid standard, the Quercetin's standard solution, and the second was a sample (ethanolic extract of *Canna edulis* Ker.). Next, the silica gel plate was eluted with a mobile phase of n-butanol-acetate acid-water (BAW) with a ratio of 4: 1: 5. Finally, the results obtained were identified under UV lamps of 254 nm and 366 nm.

Grouping and treatment in experimental animals for peptic ulcers

This research began with submitting an ethical clearance request to the Committee of Research Ethics of Ahmad Dahlan University and passed the Ethical Clearance (ethic reference number: 011810128). The rats were maintained (at room temperature) on standard pellet diets and tap water. The rats were divided randomly into 6 groups consisting of 5 rats. The normal group was given food and water; the negative control was given 0.5% CMC-Na; the extract groups were given various doses of *Canna edulis* Ker. ethanolic extract (50, 100, and 200 mg/kg BW) and positive control group was given sucralfate at a 360 mg/kg BW dose. Rats were treated orally for 14 days. One hour after the treatment on the 14th day, all groups except the normal group were orally administered with piroxicam dissolved in 0.5% CMC-Na at a dose of 1.8 mg/kg. Twenty-four hours later, animals were sacrificed, dissected, and their stomach organs removed to analyze the stomach's number of ulcers (Pertiwi et al., 2016).

Macroscopic observation of gastric ulcer

The stomach was isolated to determine the number and size of lesions/ulcers formed on the gastric mucosa. The stomach was opened surgically in the largest arch (major curvature) and was cleaned with 0.9% NaCl solution. Then, it was spread on the flat surface to observe the formed ulcers (Gusdinar et al., 2009). Ulcer observation was formed by giving: score 0 if the tissue has no pathological changes (normal); score 1 if there was 0-1% gastric mucosal bleeding; score 2 if there was 1-5% bleeding; score 3 if there was 5-10% bleeding; score 4 if there was a 10-15% bleeding; and a score of 5 if there was bleeding >15% of the gastric mucosal area. Meanwhile, the protection ratio was calculated based on the following formula:

$$\% \text{ protection ratio} = 100\% - \left[\frac{\text{UI treated control}}{\text{UI ulcer control}} \times 100\% \right] \text{ (Saptarini, 2011).}$$

Note: UI = ulcer index

Microscopic observation of gastric ulcer

The stomach that had been taken was then washed with 0.9% NaCl. Then, it was put in a pot, weighed, then was put in a 10% formalin solution. The histopathological examination was done based on work procedures applied in the Pathology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University. Histopathological quantitative analysis was carried out by scoring each preparation based on the level of damage formed. This scoring method is a modified histopathological scoring

method which was done by (Okatiranti, 2011). The results of observation histopathological can be seen in Table 1.

Table 1. Scoring of stomach damage on observation histopathological (Okatiranti, 2011)

Damage Rate	Score
There are no pathological changes	0
There is congestion, edema, and inflammatory cell infiltration	1
There is erosion, congestion, edema, and inflammatory cell infiltration	2
There is bleeding, edema, and inflammatory cell infiltration	3
There is erosion, bleeding, edema, and inflammatory cell infiltration	4

Data Analysis

ImageJ analysis was used to see the percentage of ulcer area produced in the stomach. Data were expressed as mean±standard deviation and statistically evaluated using one-way analysis of variance, followed by Kolmogorov-Smirnov and Levene tests using SPSS software version 24. $P < 0.05$ was considered significant.

RESULT AND DISCUSSION

The plant determination proved that the plant used in this research was *Canna edulis* Ker. The maceration used the extraction method because it was simply done by soaking, without heating, thus avoiding damage to the compound content (Dean, 2009). The solvent used in the extraction process was ethanol 96%, which was selected based on the safety and ease level when evaporating (Khoiriyah, 2014). Nevertheless, the maceration method's disadvantage is that it requires a lot of solvents and no less than perfect extraction, so stirring is essential to optimize the method.

The extraction results obtained in this study were tested for ethanol. This ethanol-free test was carried out to free the extract from ethanol to obtain a pure extract without any contamination. Ethanol could cause damage to the gastric mucosa, which would not cause false negatives in the treatment of the sample. The results of the ethanol-free test of *Canna edulis* Ker. ethanolic extract indicated that the extract was ethanol-free so that it can be concluded that an extract that can be used for the next stage was obtained. The results of the ethanol-free test can be seen in Table 2.

Table 2. Ethanol-free test from the ethanolic extract of *Canna edulis* Ker. (Zhang et al., 1990)

Identification	Procedures	Results
Ethanol-free test	Extract + H ₂ SO ₄ + CH ₃ COOH → heated	No smell of ester

In the experimental results of UV observation of 254 nm, brown spots were obtained in the sample solution with an R_f value of 0.87, which was different from the Quercetin standard solution, which shows a 0.75 R_f value. Different spots were found on 366 nm-UV observation; there were four (4) purple spots with R_f values of 0.15, 0.4, 0.68, and 0.87, and brown quercetin spots with an R_f value of 0.75. The results of TLC determination can be seen in Figure 1.

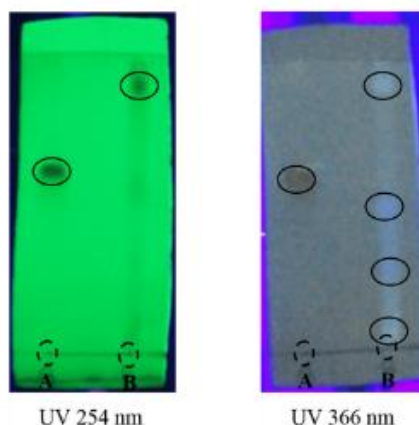


Figure 1. TLC content determination of *Canna edulis* Ker. extract

Note : (A) Quercetin standart

(B) Ethanolic extract of *Canna edulis* Ker.

The TLC test determination results of this study showed that the ethanolic extract of *Canna* tuber did not contain Quercetin based on the resulting Rf value and the fluorescent difference at 254 nm-UV and 366 nm-UV. Dark spots occurred at [Figure 1A](#) (Quercetin standart) and fluorescence at [Figure 1B](#) (ethanolic extract of *Canna edulis* Ker.). It was suspected that the compounds contained in the *Canna* tuber extract were other flavonoid compounds that were more polar.

Analysis was carried out on six groups of test animals for 14 days. Observation of test animals was carried out on the 15th day by giving piroxicam as an ulcer-forming agent, which had been previously fasted for 24 hours to reduce the stomach contents to facilitate observation. It had been known before that NSAIDs could cause damage to the gastroduodenal mucosa through several mechanisms, including topical irritant effects on the epithelial and mucosal barrier, inhibiting prostaglandin synthesis, reducing blood flow to the gastric mucosa, and inhibiting superficial repair. Piroxicam is a potent NSAID and has gastrointestinal side effects like other NSAIDs ([Wallace and Vong, 2008](#)).

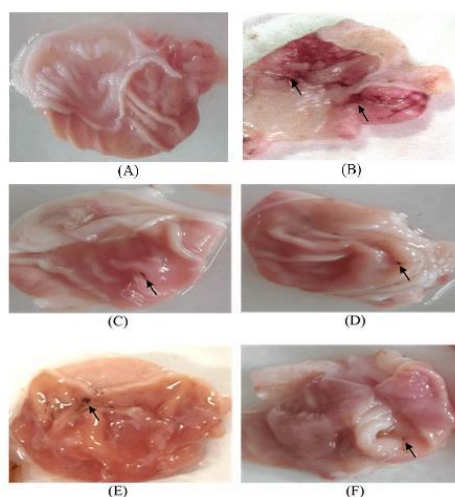


Figure 2. Macroscopic picture of gastric damage in piroxicam-induced rats (A) Normal (B) Negative control (C) 50 mg/kg BW extract (D) 100 mg/kg BW extract (E) 200 mg/kg BW extract (F) Positive control (Sucralfate). Note: Black arrows indicate the presence of lesions and hyperemia

Based on the evaluation results in [Figure 2](#), it can be seen the level of gastric damage in each treatment group. Gastric features were obtained using ImageJ to determine the percentage of abdominal area that had increased bleeding by looking at dark spots compared to the normal group. Observations were made three (3) times with different subjects to avoid subjective assessments. The results of ImageJ analysis can be seen in [Table 3](#).

Table 3. Percentage of abdominal area with bleeding

Group	Dose (mg/kg BW)	$\bar{X} \pm SD$ (%)
Normal	-	$0.00 \pm 0.00^*$
Negative (piroxicam)	1.8	1.81 ± 0.11
Extract	50	$0.48 \pm 0.10^*$
	100	$0.46 \pm 0.27^*$
	200	$0.61 \pm 0.16^*$
	360	$0.28 \pm 0.13^*$
Positive (Sucralfate)	360	$0.28 \pm 0.13^*$

* sig <0.05, there is a significant difference with negative control

Macroscopic analysis of the percentage of abdominal area that had increased hemorrhage was followed by area scoring. The obtained results showed the ulcer index and the ratio of protection in the stomach of piroxicam-induced rats. The results of ulcer index scoring and protection ratio can be observed in [Table 4](#).

Table 4. Mean values of ulcer index score and protection ratio in piroxicam-induced rats treated with the ethanolic extract of *Canna edulis* Ker

Group	Dose (mg/kg BW)	Ulcer Index Score	Protection Ratio
Normal	-	$0 \pm 0^*$	$100 \pm 0^*$
Negative (Piroxicam)	1.8	2 ± 0	$0 \pm 0^{**}$
Extract	50	$0.33 \pm 0.58^*$	$83.33 \pm 28.87^*$
	100	$0.33 \pm 0.58^*$	$83.33 \pm 28.87^*$
	200	$0.67 \pm 0.58^*$	$66.67 \pm 28.87^*$
Positive (Sucralfate)	360	$0 \pm 0^*$	100 ± 0

* sig <0.05, there is a significant difference with negative controls

Based on [Table 4](#), it can be concluded that the entire treatment group of *Canna edulis* Ker. ethanolic extract showed a decrease in the ulcer index's mean value of the rats' stomachs compared to the negative control. However, the ulcer index's mean value of each treatment group of *Canna edulis* Ker. ethanolic extract showed a difference, where the lowest ulcer index value (0.33 ± 0.58) was found at 50 mg/kg BW and 100 mg/kg BW doses, while 200 mg/kg BW dose showed 0.67 ± 0.58 . The assessment of the ulcer index's mean values will affect the protection ratio. The calculation of protection ratios using the formula by ([Saptarini, 2011](#)) shows the existence of protection ratios in *Canna edulis* Ker. ethanolic extract treatment groups.

The averaged protection ratio results based on [Table 4](#) were: the largest protection ratio was 83.33 ± 28.87 , shown by the 50 mg/kg and 100 mg/kg BW treatment groups, while extract 200 mg/kg BW had an average value of the percent protection ratio of 66.67 ± 28.87 . The analysis continued with a statistical test to see the differences that occurred in each treatment group. Mann-Whitney test results showed that the sucralfate treatment group showed no significant difference with the 50 mg/kg and 100 mg/kg BW treatment group of *Canna edulis* Ker. ethanolic extract with a significance value of 0.317 (sig>0.05), along with 200 mg/kg BW ethanolic extract treatment group with a significance value of 0.114 (sig>0.05).

Based on the results of macroscopic observations made, it can be concluded that the *Canna edulis* Ker. ethanolic extract treatment group has a gastroprotective effect as indicated by a reduction in the abdominal area that had been bleeding, the decrease in ulcer index, and an increase in protection ratio percentage.

The microscopic analysis was carried out to observe microscopic gastric damage by performing histopathological examinations on rats' stomachs. Sliced preparations of gastric tissue were made for each treatment group for the histopathological examination. The examination results were qualitatively and quantitatively stated. The qualitative analysis can be seen in [Figure 3](#).

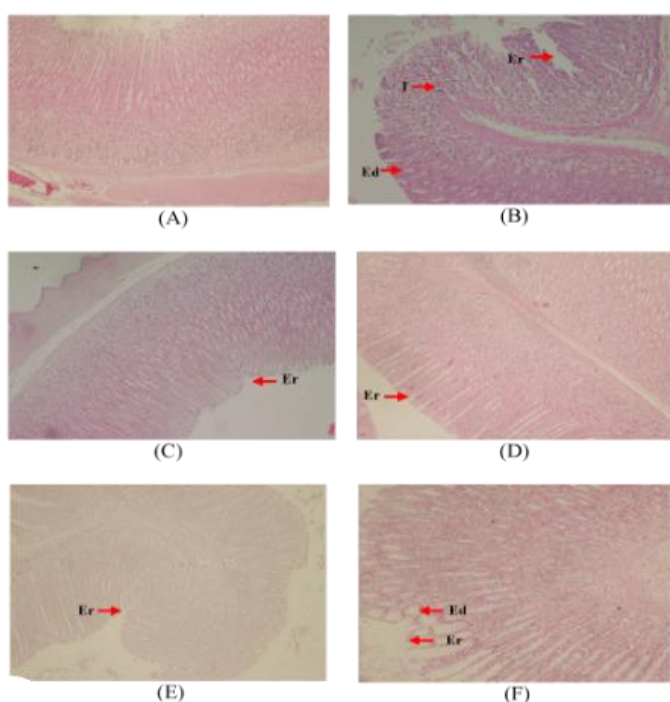


Figure 3. Histopathological Stomach Damage (A) Normal; (B) Negative control; (C) 50 mg/kg BW extract; (D) 100 mg/kg BW extract; (E) 200 mg/kgBW extract; (F) Positive control (Sucralfate). Note: Edema (Ed), Erosion (Er); Inflammatory cell infiltration (I). Hematoxylin eosin staining, 100x magnification

Quantitative analysis was performed by scoring each preparation. The scoring results of observing the gastric damage level in each treatment group are presented in [Table 5](#).

Table 5. Stomach damage score based on mean value total histopathological observation scoring in piroxicam-induced rats treated with *Canna edulis* Ker. ethanolic extract

Group	Dose (mg/kg BW)	$\bar{X} \pm SD$
Normal		$0.33 \pm 0.47^*$
Negative (Piroxicam)	1.8	1.67 ± 0.47
Extract	50	$0.33 \pm 0.47^*$
	100	$0.33 \pm 0.47^*$
	200	$0.33 \pm 0.47^*$
	360	$1.00 \pm 0.00^{**}$
Positive (Sucralfate)		

* sig <0.05, there was a significant difference with negative controls

The qualitative test results of the histopathological imaging showed that all treatment groups showed pathological changes in the stomach. The microscopic image of the stomach is said to be normal if there were no signs of ulcers. In the normal group that was only given food and drink, it showed a picture of mild damage (mild infiltration) with a score of 0.33 ± 0.47 , which was significantly different from the negative control with a significance value of 0.009 (sig <0.05). This might be due to external variables that cannot be controlled. During this study, it was suspected that the rats experienced severe stress due to the 15-day treatment period and other unknown reasons. Thus, the rats' stomachs acid increased excessively. Another reason might be due to the initial condition of the rats' stomachs that had been damaged.

This study's observations showed that inflammatory cell infiltration was found in almost all *Canna edulis* Ker. ethanolic extract treatment groups. The mean score of histopathological observations showed a value of 0.33 ± 0.47 , significantly different from the negative control with a significance value of 0.009 (sig <0.05). The most dominant cell infiltration was in the mucosal layer. It was suspected that the lymphocyte infiltration is not due to pathological abnormalities. Several lymphocytes, plasma cells, neutrophils, and mast cells are commonly found in the mucosal layer under normal conditions. This is because the migration of inflammatory cells is a general response reaction to toxic substances that enter the body and is a pathophysiological reaction against harmful agents (Bain, 1991).

The histopathological imaging was carried out to strengthen the quantitative analysis results of ulcer index and protection ratio observations. The histopathological images presented that the *Canna edulis* Ker. ethanolic extract provided conditions for gastric repair by decreasing the ulcer index value and increasing the protection ratio. The most severe gastric damage occurred in the positive treatment group that was given sucralfate. This was different from the treatment group of *Canna tuber* ethanolic extract, which is a natural product. The body is relatively easier to digest drugs from natural ingredients in traditional medicine than synthetic materials because of their natural properties.

In general, the therapeutic effect will increase with increasing doses. However, it was not the case for *Canna edulis* Ker. ethanolic extract treatment groups. The description of macroscopic observations in the stomach of piroxicam-induced rats that showed a gastroprotective effect did not depend on increasing the dose (dose-dependent). The decrease in the ulcer index and the increase in the protection ratio did not increase linearly with increasing doses at extract 50 mg/kg BW and 100 mg/kg BW. This is because of the compounds contained in *Canna edulis* Ker. plants do not work independently to produce a cumulative effect (not parallel) (Kingsbury, 2001). Especially in the treatment dose of 200 mg/kg BW, the ulcer index reduction and an increase in the protection ratio are not as effective as in other doses. This allegedly happened because of the saturation factor of the extract at specific doses. Several components of active compounds in plants are simultaneously involved in therapy, thus enabling these active compound components to have a synergistic or antagonistic effect (Syahrir et al., 2016). Thus, the synergistic effect that occurs can increase concentration to the saturation point.

According to previous studies, *Canna edulis* Ker's protective mechanism was due to its flavonoids contents and cytoprotective effects. Flavonoids work by protecting the gastric mucosa against ulcerogenic agents through the free radical destruction mechanism, increased mucus production, and anti-secretory system (O'Leary et al., 2004). Alkaloids in *Canna tuber* ethanolic extract also work by a mechanism to reduce gastric acid secretion, increase mucus and alkaline secretion, and improve gastric mucosal blood flow to help healing and prevention of gastric ulcer against irritant agents/factors (Falcão et al., 2008). Tannin compounds are also known to have a gastroprotective effect that inhibits gastric secretion and local gastric mucosa protection (Ezaki et al., 1985).

CONCLUSION

Canna edulis Ker. ethanolic extract has the gastroprotective ability by decreasing the index of gastric ulcers and increasing the ratio of protection to the stomach of piroxicam-induced rats.

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