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EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF SEEDS OF *BUCHANANIA LANZAN* SPRENG. IN ANIMAL MODELS

AMIR MEHMOOD[1], IRFAN HAMID[2], ALI SHARIF[1], MUHAMMAD FURQAN AKHTAR[1], BUSHRA AKHTAR[3], AMMARA SALEEM[4], JAVED IQBAL[1], MARYAM SHABBIR[1] and SAJID ALI[1]*

[1]Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[2]Akson College of Health Sciences, Mirpur University of Science and Technology, AJK, Pakistan
[3]Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
[4]Department of Pharmacy, Government College University, Faisalabad, Pakistan

Abstract: The present study was designed to evaluate the anti-inflammatory, analgesic and antipyretic activities of the aqueous and ethanolic extracts of seeds of *Buchanania lanzan* Spreng. Albino mice were used as experimental animals to evaluate these activities. The study was performed in three phases; Phase-I for evaluation of anti-inflammatory activity, Phase-II for antipyretic and Phase-III for analgesic activities were evaluated. Carrageenan induced paw edema, brewer yeast induced pyrexia and acetic acid induced writhing methods were used to evaluate anti-inflammatory, antipyretic and analgesic activities, respectively. Tests were performed by dividing the animals in five groups. First group was negative control, second group was positive control, third, fourth and fifth groups were treated with 125, 250 and 500 mg/kg of extracts. respectively. The data were statistically analyzed using ANOVA where $p < 0.05$ were considered significant. The results suggested that seeds of *Buchanania lanzan* Spreng. possess anti-inflammatory, analgesic and antipyretic activity.

Keywords: *Buchanania lanzan* Spreng., analgesic, anti-inflammatory, antipyretic

Traditional system of medicines or herbal care is the original source for most of the medicines and is used worldwide. Medicinal plants possess a wide variety of chemical constituents which are helpful for curing different diseases. Recent discovery of medicinal plants have led to improvement of human health care systems. Plant based drugs are used as medicines either as a whole or their principle constituents are separated by different chemical techniques. This practice continues because of different biological advantages, pharmacological activities and fewer side effects associated with medicinal plants (1).

Inflammation is a primary defense mechanism that helps the body to protect itself against various types of diseases, allergens, chemicals and other toxic reactions. Inflammation is a process which involves a large variety of inflammatory mediators and prominent increase in localized leucocytes. Prostaglandins are the substances that modulate the cell and tissue response during inflammation. Their biosynthesis also continues during the cardiovascular disease, cancers and colonic adenomas. Although it is a mechanism of protection for the body, an uncontrolled inflammation and various mediators of inflammation can induce or maintain a disease and can even aggravate it (2). Plants are widely used for treating inflammation because the adverse effects produced by these plant based drugs are very few. In addition, they are easily affordable and lower in cost than the synthetic drugs (3).

Pyrexia or fever occurs as body's natural defense mechanism in response to any infection or disease. In pyrexia, an environment is created inside the body in which the infectious pathogens and damaged tissues are unable to survive (4). Normal body temperature is maintained and regulated by hypothalamus that maintains a balance between heat loss and production. Fever occurs due to some disturbances in this thermostat of body and as a result body's temperature rises. The temperature regulating mechanisms then works to normalize the body

* Corresponding author: e-mail: sajidalichishti@gmail.com; phone: 0092-333-4404880

temperature. These mechanisms include dilation of superficial blood vessels and sweating etc. When any infectious agent enters into the body through a break in its barriers, it will interact with immune cells and will promote the release of endogenous mediators like cytokines, prostaglandins and endothelins. In pre-optic area of anterior hypothalamus, PGE_2 play a vital role in fever induction (5).

Pain is a sensorial modality and sometimes it is the only feature for diagnosis of various diseases. Many forms of therapy are available for curing this condition, among them medicinal herbs are widely used (6). Pain is a sensation which is unpleasant and is localized to a part of body. In response to tissue injury visceral distensions, peripheral nociceptors and some other factors are stimulated, which develop pain. Perception of pain is a normal physiological response and it is mediated by the healthy nervous system (7).

Different experimental models are used for evaluating anti-inflammatory, analgesic and antipyretic activities of herbal plants. For studying anti-inflammatory activity, a large number of experimental models have been developed. These models are basically of two types; acute inflammatory model and chronic inflammatory model. Acute models are developed to study and evaluate the drugs which are involved in erythema, leucocyte migration and chemotaxis, changes in vascular permeability, phagocytosis-polymorphonuclear leucocytes, measurement of local pain and rat paw edema (8). Acute-inflammatory models are carrageenan induced paw edema, xylene

Table 1. Experimental design.

Groups	Drug	Dose	Route	No. of animals
Group 1 (negative control)	Normal saline	1 mL/kg	Oral	5
Group 2 (positive control)	Indomethacin	10 mg/kg	Oral	5
Group 3	Plant extract	125 mg/kg	Oral	5
Group 4	Plant extract	250 mg/kg	Oral	5
Group 5	Plant extract	500 mg/kg	Oral	5

Table 2. Anti-inflammatory activity of *Buchanania lanzan* Spreng. (aqueous extract).

Groups	Average paw volume (mm)		
	1 h	2 h	3 h
Normal saline	3.70 ± 0.01	3.76 ± 0.01	3.97 ± 0.01
Indomethacin	2.93 ± 0.03	2.13 ± 0.01	1.91 ± 0.01
125 mg/kg	3.41 ± 0.02	3.16 ± 0.05	2.86 ± 0.05
250 mg/kg	3.37 ± 0.02	2.94 ± 0.01	2.48 ± 0.04
500 mg/kg	3.25 ± 0.41	2.71 ± 0.01	2.39 ± 0.08

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Table 3. Percentage inhibition of carrageenan induced paw edema (aqueous extract).

Groups	Inhibition of carrageenan induced paw edema (%)			Average inhibition
	1 h	2 h	3 h	(%)
Indomethacin	20.8	43	51.8	38.2
125 mg/kg	7.8	15.9	27.9	17.2
250 mg/kg	8.9	21.8	37.5	22.7
500 mg/kg	12.1	27.9	39.7	26.5

Table 4. Anti-inflammatory activity of *Buchanania lanzan* Spreng. (ethanolic extract).

Groups	Mean values of paw edema (mm) ± SEM		
	1 h	2 h	3 h
Normal saline	3.72 ± 0.01	3.88 ± 0.01	3.98 ± 0.01
Indomethacin	2.70 ± 0.07	2.10 ± 0.01	1.94 ± 0.01
125 mg/kg	3.21± 0.01	2.84 ± 0.01	2.78 ± 0.01
250 mg/kg	3.11 ± 0.01	2.52 ± 0.01	2.38 ± 0.01
500 mg/kg	2.93 ± 0.01	2.48 ± 0.01	2.14 ± 0.04

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Table 5. Percentage inhibition of carrageenan induced paw edema (ethanolic extract).

Groups	Inhibition of carrageenan induced paw edema (%)			Average inhibition
	1 h	2 h	3 h	(%)
Indomethacin	7.4	47.0	51.2	41.8
125 mg/kg	13.0	26.0	30.0	23.0
250 mg/kg	16.0	35.0	40.0	30.3
500 mg/kg	21.0	36.0	46.0	34.3

induced ear edema, egg albumin induced edema, arachidonic acid induced ear edema and croton oil induced edema in mice/rats. Chronic models are cotton pellet induced granuloma and adjuvant arthritis in mice/rats (9). Analgesic activity is determined in two ways; central analgesic activity and peripheral analgesic activity. Peripheral analgesic activity is evaluated by acetic acid induced writhing and hot plate method (10). Antipyretic activity is studied by brewer's yeast induced pyrexia, vaccines induced pyrexia and D-amphetamine induced pyrexia (11).

Buchnania lanzan Spreng. (family Anacardiaceae) is commonly known as Char, chironji, Almondette Tree, Murkali, Nurkale. It is an indigenous Hill Plant, Plain Land commonly found throughout the greater part of India in dry deciduous forests up to an altitude of 1200 m. The decoction of its bark is useful in abdomen disease, cough and bronchitis. Fruit is used to treat nervous debility, cardiac debility, constipation, fever, ulcer, oligospermia and general debility (12). The parts of plant like roots, leaves, fruits, seeds and gum are used for various medicinal applications like cure for blood disorder, fever, ulcer, burning sensation of body, diarrhea, dysentery, asthma and snakebite (13). Aqueous and ethanolic extracts of seeds of Char were used to evaluate anti-inflammatory, analgesic and anti-pyretic activity.

The present study was undertaken to assess the anti-inflammatory, antipyretic and analgesic activity of aqueous and ethanolic extracts of seeds of *Buchnania lanzan* Spreng.

MATERIAL AND METHOD

Collection of plant material

Seeds of *Buchnania lanzan* Spreng. were purchased from local market of Lahore, Pakistan. It was identified and authenticated by Prof. Dr. Zaheerudin, Botany Department of Government College University, Lahore, Pakistan. The seeds were preserved in herbarium of Government College University, Lahore, Pakistan.

Washing, drying, grinding and storage of plant

The seeds were washed with distilled water to remove dirt particles and contamination. Washed seeds were dried at room temperature under shade for appropriate time period. Fully dried seeds were then grinded with mortar and pestle until a coarse powder was obtained. The powder was passed through sieve #20 and stored in air tight, labeled containers till further processing.

Extract preparation

Aqueous and ethanolic extracts of *Buchnania lanzan* Spreng. seed's powder were prepared by triple maceration process. For the preparation of aqueous extract, 500 g of powdered material was soaked in 1000 mL of distilled water in an amber

colored glass container for 3 days. After stated time, the solvent was removed using muslin cloth and the remaining plant material was again soaked in 500 mL of distilled water and whole process was repeated thrice. Ethanolic extract was prepared by the same method using ethanol as solvent.

Drying of extracts

The extracts were filtered through Whatman filter paper and then dried in rotary vacuum evaporator until a semi-solid mass was obtained. This semi-solid mass was spread in Perti dishes to completely dry the extract in oven. The dried extracts were stored in refrigerator at 4°C until further processing.

Grouping of animals

Albino male mice were obtained from Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The weight of each mouse ranged in 20-25 g. The animals were of the same breed and batch. The mice were divided into 5 groups with each group having five mice. They were kept in polycarbonate cages under controlled temperature (22-25°C) and had free access to feed and water.

Pharmacological evaluation

Pharmacological evaluation of the seed extracts of *Buchanania lanzan* Spreng. was done in three phases. In phase-I anti-inflammatory activity was evaluated. In phase-II analgesic activity and in phase-III anti-pyretic activity were evaluated. The treatment strategy for animal groups is given in Table 1.

Phase-I - Evaluation of anti-inflammatory activity

Inflammation was induced by injecting 1% carrageenan solution (dose of 0.05 mL) to the right hind paw of mice. Test drug and standard drug were given 1 h before the administration of carrageenan in respective groups. In negative control group, the mice were treated with normal saline and in positive control, indomethacin was given. A mark was made at the paw of each mouse up to the ankle joint. Paw volume was measured up to ankle joint in drug treated and drug untreated groups before and after administration at a time interval of 0, 1, 2 and 3 h (14).

Table 6. Brewer's yeast induced antipyretic activity (aqueous extract).

Groups	Mean values of temperature (°F) ± SEM				
	Before drug administration		After drug administration		
	Normal temp.	18 h	1 h	2 h	3 h
Normal saline	97.51 ± 0.01	102.95 ± 0.03	102.14 ± 0.02	102.78 ± 0.04	102.98 ± 0.01
Indomethacin	97.58 ± 0.09	102.71 ± 0.03	101.34 ± 0.04	100.31 ± 0.09	97.02 ± 0.01
125 mg/kg	97.96 ± 0.02	102.38 ± 0.08	101.86 ± 0.01	99.73 ± 0.01	99.04 ± 0.01
250 mg/kg	96.85 ± 0.03	102.74 ± 0.02	101.43 ± 0.01	99.34 ± 0.01	98.55 ± 0.02
500 mg/kg	97.20 ± 0.10	102.12 ± 0.02	101.14 ± 0.03	99.03 ± 0.03	98.19 ± 0.19

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Table 7. Brewer's yeast induced antipyretic activity (ethanolic extract).

Groups	Mean values of temperature (°F) ± SEM				
	Before drug administration		After drug administration		
	Normal temp.	18 h	1 h	2 h	3 h
Normal saline	97.52 ± 0.07	102.83 ± 0.27	102.04 ± 0.01	102.84 ± 0.01	102.96 ± 0.01
Indomethacin	97.72 ± 0.04	102.73 ± 0.03	101.50 ± 0.05	100.37 ± 0.03	97.23 ± 0.04
125 mg/kg	97.26 ± 0.02	102.54 ± 0.01	101.96 ± 0.01	100.73 ± 0.01	99.74 ± 0.01
250 mg/kg	97.15 ± 0.03	102.32 ± 0.02	101.44 ± 0.01	100.34 ± 0.01	99.56 ± 0.02
500 mg/kg	96.94 ± 0.03	102.34 ± 0.02	101.64 ± 0.03	100.03 ± 0.03	99.12 ± 0.12

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Table 8. Acetic acid induced analgesic activity of *Buchanania lanzan* Spreng. (aqueous extract).

Groups	Drug	Dose	Number of writhing (mean) ± SEM	Inhibition of writhing (%)
Group 1	Normal saline	1 mL/kg	66.8 ± 0.44	N/A
Group 2	Indomethacin	10 mg/kg	17.6 ± 0.54	73.65
Group 3	Test extract	125 mg/kg	44.4 ± 0.54	33.53
Group 4	Test extract	250 mg/kg	36.0 ± 0.04	46.12
Group 5	Test extract	500 mg/kg	29.4 ± 0.54	55.99

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Table 9. Acetic acid induced analgesic activity of *Buchanania lanzan* Spreng. (ethanolic extract).

Groups	Drug	Dose	Number of writhing (mean) ± SEM	Inhibition of writhing (%)
Group 1	Normal saline	1 mL/kg	65.0 ± 0.70	N/A
Group 2	Indomethacin	10 mg/kg	16.8 ± 0.83	74.15
Group 3	Test extract	125 mg/kg	40.0 ± 0.70	38.46
Group 4	Test extract	250 mg/kg	32.4 ± 0.54	49.37
Group 5	Test extract	500 mg/kg	25.6 ± 0.54	60.00

Note: All specified doses showed a statistically significant ($p < 0.05$) effect.

Quantification of anti-inflammatory activity

The anti-inflammatory activity of the seed extract of *Buchanania lanzan* Spreng. was quantified by measuring the paw edema (in mm) using digital Vernier caliper. The results were expressed in terms of mean values of paw edema of each group ± SEM.

Reduction in paw edema was measured by the percentage inhibition of the positive control and extract treated groups as compared to the negative control. The percentage inhibition was calculated using the following formula:

Percentage inhibition = (Control – Treated) / (Control) × 100

Phase-II - Evaluation of antipyretic activity

Brewer's yeast solution was used to induce fever in mice by subcutaneous administration of solution below the nape of the neck. Initial temperature was recorded by using digital clinical thermometer. After 18 h of administration, animal which showed a mean rise of 0.3-0.5^{o}C body temperature were selected. Animals were treated with normal saline, standard drug and drug extracts as per experiment design. Temperature of mice was recorded after an interval of 1, 2 and 3 h post dosing (15).

Quantification of antipyretic activity

Antipyretic activity was quantified by checking the decrease in body temperature at different time intervals. The results were calculated in F ± SEM for all groups.

Phase-III - Evaluation of analgesic activity

Acetic acid induced writhing method was used to evaluate analgesic activity of *Buchanania lanzan* Spreng. seed extract. The mice were treated with intraperitoneal injection of 1% acetic acid solution (0.1 mL) and number of writhing movements was counted for 20 min (16).

Quantification of analgesic activity

The reduction in number of writhing after administering positive control and drug extracts with respect to negative control group was quantified by calculating percentage inhibition using following formula:

Percentage inhibition = $(N_c - N_t) / (N_c) \times 100$

where, N_c is mean number of writhing in control group, N_t is mean number of writhing in treated group

Statistical analysis

For statistical analysis, the data were evaluated using Statistical Package of Social Sciences (SPSS). The results were expressed as the mean ± SEM and analysis of variance (ANOVA) was applied to the data.

RESULTS AND DISCUSSION

In previous literature, phytochemical investigation revealed the presence of new glycoside, myricetin-3-rhamnoside-3-galactoside (17). The component fatty acids of the seed fat were found to be myristic, palmitic, stearic, oleic and linoleic acids. The moisture, crude protein, pentosan and water soluble mucilage contents of seeds were reported as 5.2, 6.95, 3.8 and 2.8 percent, respectively. The mucilage showed absence of protein and pentosan (18).

The results of anti-inflammatory effect of aqueous and ethanolic extract of *Buchanania lanzan* Spreng. on carrageenan-induced edema in paw of mice are presented in Tables 2 and 4, respectively, whereas percentage inhibition by aqueous and ethanolic extract is depicted in Tables 3 and 5, respectively. Edema development due to inflammation is a biphasic event in which the initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of protease, prostaglandins and lysosomes. Most of the anti-inflammatory drugs are clinically effective in second phase of inflammation (19). The data represented that the ethanolic extract at dose of 500 mg/kg showed significant reduction ($p < 0.01$) in edema and faster rate of inhibition as compared to other doses. However, the aqueous and ethanolic extracts showed moderate reduction in edema when compared with the extract of standard drug (Fig. 1). The carrageenan induced paw edema model is known to be sensitive to the effect of NSAIDs which primarily inhibits cyclooxygenase involved in syn-

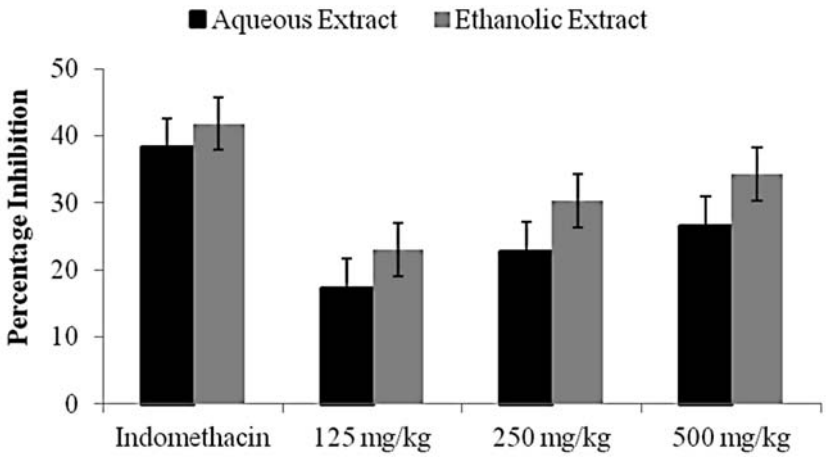

Figure 1. Percentage inhibition of carrageenan induced paw edema by standard drug, aqueous and ethanolic extract of *Buchanania lanzan* Spreng.

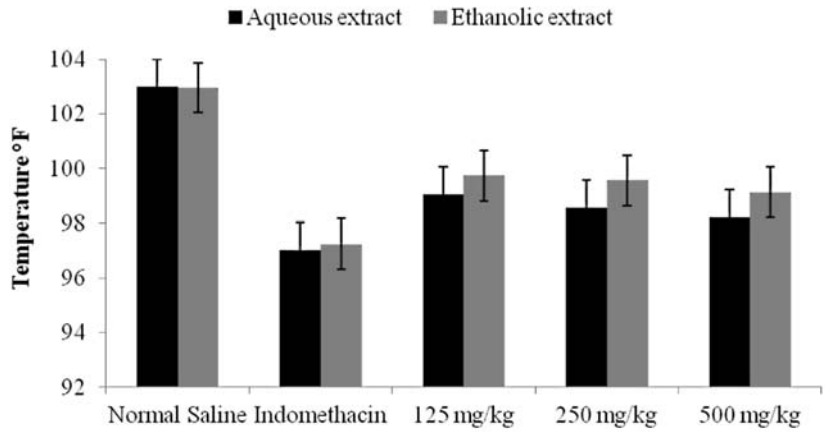

Figure 2. Percentage inhibition of Brewer's yeast induced pyrexia by standard drug, aqueous and ethanolic extract of *Buchanania lanzan* Spreng.

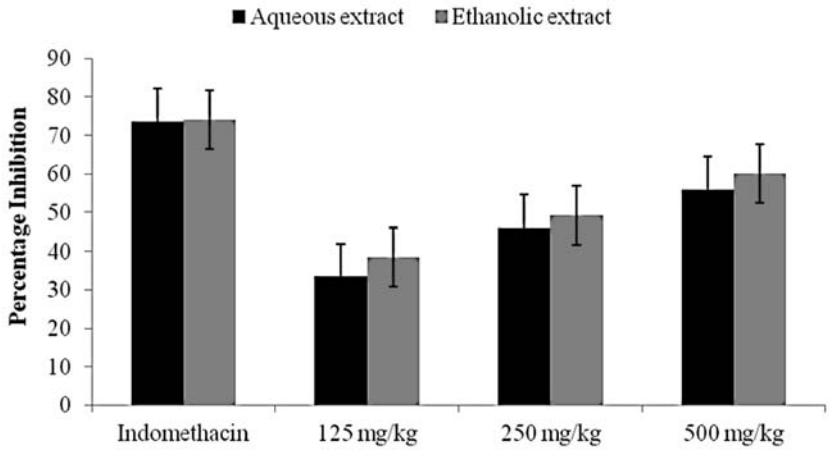

Figure 3. Percentage inhibition of acetic acid induced pain by standard drug, aqueous and ethanolic extract of *Buchanania lanzan* Spreng.

thesis of prostaglandins (20). Therefore, it can be reasonably concluded that the inhibitory effect of aqueous and ethanolic extract of *Buchanania lanzan* Spreng. on inflammation in mice may be due to the inhibition of cyclooxygenase enzyme.

The antipyretic activity of aqueous and ethanolic extract is given in Tables 6 and 7, respectively. An increase in temperature was evident after 18 h of Brewer's yeast administration. After the administration of indomethacin and drug extracts, a significant decrease in temperature was noted. The extracts showed dose dependant decrease in temperature with increasing potency from 125 to 500 mg/kg. The aqueous extract of seed at dose of 500 mg/kg had evident antipyretic activity but it was moderate as compared to the positive control after 3 h of drug administration. Therefore, aqueous extract proved to contain more antipyretic activity as compared to ethanolic extract but lesser than standard antipyretic drug (Fig. 2).

The peripheral analgesic activity of drug extract was tested by acetic acid induced writhing test. Acetic acid induced writhing test is a standard test to check the pain sensitivity of opiates and non-opiates analgesics. The pain sensation is due to abdominal constriction due to irritation of peritoneal cavity caused by acetic acid. Prolonged irritation leads to an increase in level of prostaglandins (PGE_2 and $PGF_{2\alpha}$) biosynthesis and lipoxygenase products in peritoneal fluids. The increase in these products enhances the level of free arachidonic acid which subsequently develops inflammatory pain by increasing capillary permeability in peritoneal cavity of abdomen (21). The reference drug used was indomethacin which is non-opiate analgesic. This NSAID involves blockade of production of prostaglandins by inhibition of both COX-1 and COX-2. The aqueous and ethanolic seed extract produced a significant decrease in the writhing counts (Tables 8 and 9). Minimum number of writhing was observed with 500 mg/kg ethanolic extract. The percentage inhibition of pain induced by acetic acid was dose dependent i.e., with an increase in dose of aqueous and ethanolic extract the number of writhing in groups decreased in contrast to negative control group (Fig. 3). Analgesic activity of aqueous and ethanolic seed extract indicates that peripherally active analgesic property might be present ($p < 0.01$).

CONCLUSION

The result of biological screening on the fractions from aqueous and ethanolic extract of *Buchanania lanzan* Spreng. confirms consistent activities in anti-inflammatory, antipyretic and analgesic tests.

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