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EFFECTS OF ORAL ADMINISTRATION OF ETHANOLIC LEAF EXTRACT OF *MUCANA PURIENS* OBTAINED FROM AFIKPO ON SERUM BIOCHEMICAL PARAMETERS AND ELECTROLYTES IN ALBINO WISTAR RATS

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ABSTRACT

The effects of oral administration of ethanolic leaf extract of *Mucana puriens* obtained from Afikpo, Ebonyi State, Nigeria for 28 days on some serum biochemical parameters and electrolytes of albino wistar rats were investigated using standard protocols. Thirty-two (32) albino rats comprising 16 males and 16 females were used for the study. The rats were divided into four groups namely A, B, C and D. Each group was made up of 4 male and 4 female rats separated but given similar treatment. Group A served as the control and were treated with normal saline (0.4ml/100g body weight). Each of groups B, C, and D were administered through oral intubation 250 mg/kg body weight, 500 mg/kg body weight and 750 mg/kg body weight of extract respectively. The result of acute toxicity test obtained showed a lethal dose (LD_{50}) of greater than 5000mg/kg body weight of the extract. Results indicated that administered doses of 250, 500 and 750mg/kg body weight of the leaf extract showed significant ($P < 0.05$) increase in body weight, serum total protein and albumin and significant decrease in serum total bilirubin, uric acid, urea and creatinine in a dose dependent manner. Non-significant ($P > 0.05$) increase was observed in serum Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+} and inorganic P at all doses except for Ca^{2+} and inorganic P whose increase was significant at a dose of 750mg/kg body weight of extract. These results are indications that the extract is biochemically safe and could have beneficial health effects.

KEYWORDS: *Mucana puriens*, Biochemical parameters, Electrolytes, Health, Afikpo.

INTRODUCTION

The increasing use of plants in ethno-medicine as valuable sources of therapeutic agents is attracting the interest of numerous researchers worldwide (Sofowora, 1993). Reports have shown that many underutilized plant materials have some medicinal activities (Gbile and Adesina, 1989; Odebiyi and Sofowora, 1993). The treatment and prevention of diseases in Nigeria as in many other countries of the world have for a long time been handled by traditional healers (Ebomoyi *et al*., 2004). These plants with medicinal properties are believed to be important sources of new chemical substances with potential therapeutic effects (Farnsworth, 1989) and as such, many of them have been found and put into use by traditional healers in the management of many ailments for many years (Sofowora, 1993; Ogunka-Nnoka *et al*., 2012; Ahmad *et al*., 2015). However, the problems associated with the use of these plant materials as medicines are that in many cases no definite doses are prescribed, often resulting in overdoses (Sule and Mohammed, 2006; Malomo *et al*., 2006; Enechi and Ozougwu, 2014).

Mucuna pruriens is a tropical legume known as velvet bean or cowitch and by other common names, found in

Africa, India and the Caribbean. The plant is an annual, climbing shrub with long vines that can reach over 15 m in length (Katzenschlager *et al*., 2004; Akpanabiatu *et al*., 2005). *Mucuna pruriens* is underutilized and infamous due to the extreme itchiness produced on contact, particularly with the young foliage and the seed pods. The leaves and seeds have been reported to exhibit significant aphrodisiac, antispasmodic, anticataleptic, antiepileptic, anti-diabetic, antimicrobial, anti-inflammatory, pain-relieving and fever-reducing activities from various clinical researches with animal models (Amin *et al*., 1996; Hussain and Manyam, 1997; Manyam *et al*., 2004; Sathiyanaryanan and Arulmozhi, 2007; Majekodunmi *et al* 2011; Champasingh *et al*., 2011; Lampariello *et al*., 2012). The leaves are consumed for their nutritional value and are also used in folk medicine as a therapy for various diseases such as diabetes, arthritis, dysentery, infertility, obesity and cardiovascular disorders and for maintenance of

homeostasis (Nadkaru, 2001; Bishop *et al*., 2010; Ram and Mohammad, 2011).1

Therefore, this research work is aimed at determining the effects of aqueous leaf extract of *Mucuna pruriens* on selected serum biochemical parameters and electrolytes in albino wistar rats in order to generate data on their safety and wholesomeness for use in medicinal applications.

MATERIALS AND METHODS

Plant material

Mucuna pruriens sample was collected from Akpoha in the month of May 2013 and was authenticated with the help of Mrs Stella Eberechukwu Obasi of the Plants and Environmental Biology Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana where a voucher specimen has been deposited.

Extraction

Fresh leaves of *Mucuna pruriens* (500g) were air died for seven days under atmospheric conditions and pulverized to fine powder using milling grinder (Thomas Wiley Model 4). The pulverized material was macerated in 70% ethanol to give an extract that was filtered. The filtrate was concentrated using a rotary evaporator to yield viscous slurry with a percentage recovery of 9.4%.

Animal Treatment

Thirty-two (32) albino rats of both sexes comprising 16 males and 16 females and weighing 150-200 grams were used for the study. The animals were housed in the Animal House, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, Nigeria. The animals were fed on standard feeds (Grower pellets of Royal feeds, Enugu, Nigeria) and allowed access to water *ad libitum*. The "Principle of laboratory animal care "(NIH publication No 85- 23)'' guideline and procedures were followed in this study (NIH publication reserved 1985).

The animals were randomized into experimental and control groups and were kept in polypropylene cages. The rats were divided into four groups namely A, B, C and D. Each group was made up of 4 male and 4 female rats separated but given similar treatment. Group A served as the control and were treated with normal saline (0.4ml/100g body weight). Each of groups B, C, and D were administered through oral intubation 250 mg/kg body weight, 500 mg/kg body weight and 750 mg/kg body weight of the plants extract respectively. All administrations were orally by gastric intubation. After twenty eight days of treatment, the animals in each group were sacrificed by chloroform anesthesia. Blood was collected by cardiac puncture and allowed to clot, centrifuged at 3000 rpm for 15mins and the serum aspirated.

Chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade.

Acute Toxicity Test

This was done using the Lorke's method (Lorke, 1983). A total of 20 albino wistar rats weighing between 150 – 200g were fasted overnight although with access to tap water. They were divided into five groups of four rats each comprising two males and two females. Four groups were given different doses of the extract in the following order: 200, 500, 2000, 5000mg/kg body weight of the rats while the fifth group (control) received only normal saline. The rats were observed for 12 hours for any lethality or signs of overt toxicity.

Determination of Serum Biochemical Parameters and Electrolytes

Serum total proteins, creatinine, urea, and uric acid assay was carried out by the method of Tiez (1995), total serum bilirubin was done by the method described by Jendrassik and Grof (1938) and serum albumin levels was examined as described by Grant (1987). Serum concentrations of potassium and sodium ions were determined using flame photometry according to AOAC (1984) while serum concentrations of calcium, magnesium, phosphorus and chloride ions were determined using the method described by Tietz (1995).

RESULTS

Oral administration of the ethanolic leaf extract of *Mucuna pruriens* for acute toxicity study produced no gross behavioral and/or physical changes in lethality at doses up to 5000mg/kg body weight. The results of biochemical parameters of Albino wistar rats orally administered with graded doses of ethanolic leaf extract of *Mucana puriens* are shown in Figures 1 to 6. The results indicated a dose dependent increase in serum total protein (Figure 1) and serum albumin (Figure 6) with 250mg/kg and 750 mg/kg body weight producing the lowest and highest serum proteins and albumins respectively. Serum total bilirubin (Figure 2), uric acid (Figure 3), urea (Figure 4) and creatinine (Figure 5) decreased significantly at $P < 0.05$ as the dose increased from 250mg/kg body weight to 750mg/kg body weight of the leaf extract.

The results of effects of ethanolic extract of *Mucuna pruriens* leaf on serum electrolytes of albino wistar rats are shown in Table 1. The results show non-significant ($P > 0.05$) dose dependent increase in Na, K, Cl, Mg, Ca and inorganic Phosphorus. The increase was however significant ($P < 0.05$) at a dosage of 750mg/kg body weight for all the serum electrolytes analyzed.

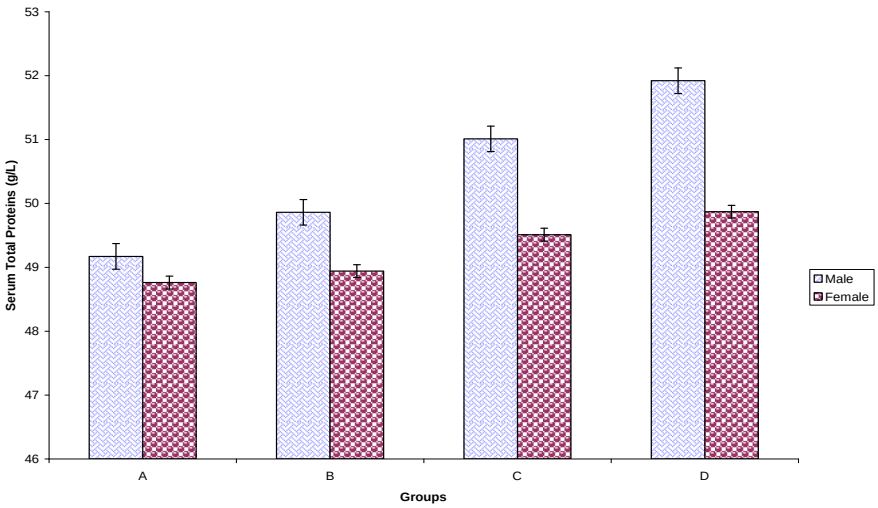

Figure 1: Effects of Ethanolic Extract of Mucuna pruriens on Serum Total Proteins (g/L) of Wistar Rats

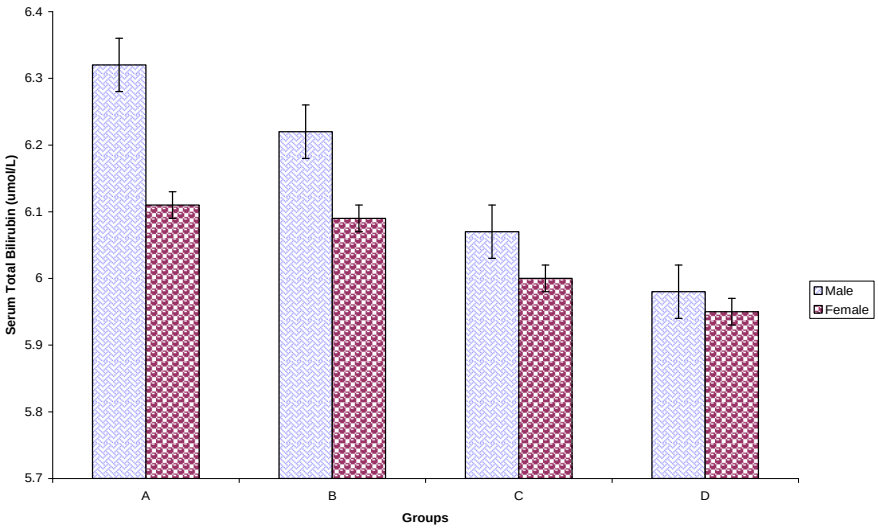

Figure 2: Effects of Ethanolic Extract of Mucuna pruriens on Serum Total Bilirubin (umol/L) of Wistar Rats

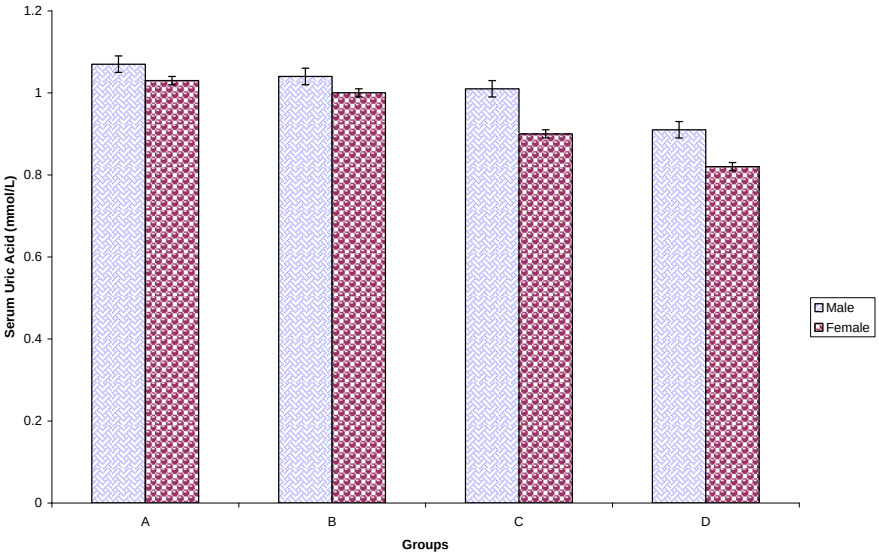

Figure 3: Effects of Ethanolic Extract of Mucuna Pruriens on Serum Uric Acid (mmol/L) of Wistar Rats

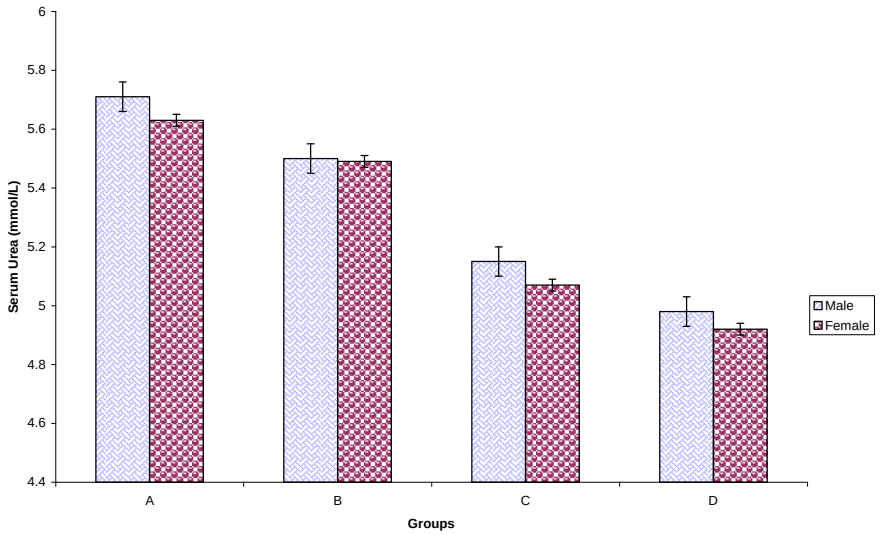

Figure 4: Effects of Ethanolic Extract of Mucuna Pruriens on Serum Urea (mmol/L) of Wistar Rats

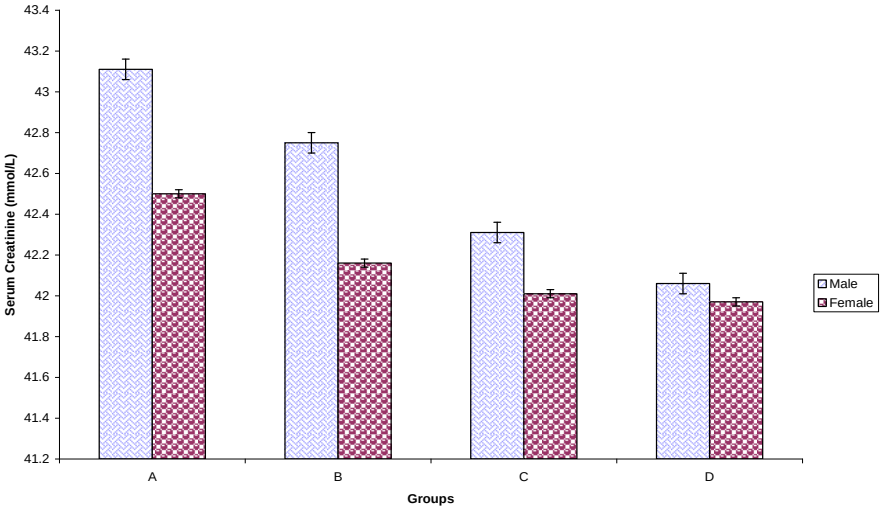

Figure 5: Effects of Ethanolic Extract of Mucuna pruriens on Serum Creatinine (mmol/L) of Wistar Rats

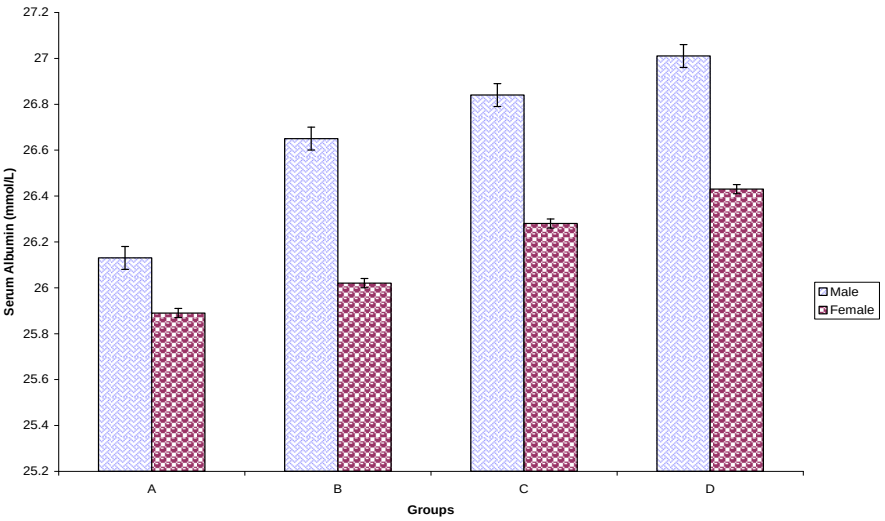

Figure 6: Effects of Ethanolic Extract of Mucuna pruriens on Serum Albumin (mmol/L) of Wistar Rats

Table 1: Effects of Ethanolic Extract of *Mucuna pruriens* leaf on Serum Electrolytes of Wistar Rats.

Groups	Dose	Electrolytes											
		Na^+ (mmol/l)		K^+ (mmol/l)		Cl^- (mmol/l)		Mg^{2+} (mmol/l)		Ca^{2+} (mmol/l)		InorganicP (mmol/l)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
A	Control	$148.62^{a} \pm 1.36$	$148.96^{a} \pm 0.67$	$5.12^{a} \pm 0.92$	$5.15^{a} \pm 1.31$	$111.80^{a} \pm 2.16$	$109.70^{a} \pm 0.97$	$0.94^{a} \pm 0.06$	$0.92^{a} \pm 0.03$	$2.41^{a} \pm 0.34$	$2.36^{a} \pm 1.07$	$4.20^{a} \pm 1.33$	$4.12^{a} \pm 0.89$
B	250mg/kg	$148.65^{a} \pm 0.97$	$148.98^{a} \pm 1.22$	$5.27^{a} \pm 1.03$	$5.22^{a} \pm 0.37$	$111.97^{a} \pm 1.78$	$109.87^{a} \pm 1.18$	$0.96^{a} \pm 0.11$	$0.93^{a} \pm 0.07$	$2.59^{ab} \pm 0.19$	$2.41^{ab} \pm 0.93$	$4.23^{a} \pm 0.97$	$4.21^{a} \pm 0.73$
C	500mg/kg	$148.81^{a} \pm 1.15$	$149.03^{a} \pm 1.33$	$5.25^{a} \pm 0.84$	$5.23^{a} \pm 1.05$	$111.93^{a} \pm 1.67$	$109.90^{ab} \pm 1.31$	$1.02^{a} \pm 0.08$	$0.98^{a} \pm 0.03$	$2.76^{bc} \pm 0.21$	$2.48^{bc} \pm 1.12$	$4.29^{ab} \pm 1.05$	$4.29^{ab} \pm 1.13$
D	750mg/kg	$148.93^{ab} \pm 1.32$	$149.01^{a} \pm 1.56$	$5.34^{ab} \pm 0.79$	$5.31^{ab} \pm 0.91$	$112.02^{ab} \pm 1.25$	$109.94^{b} \pm 0.68$	$1.05^{a} \pm 0.05$	$1.01^{a} \pm 0.17$	$2.81^{c} \pm 0.15$	$2.54^{c} \pm 0.67$	$4.34^{b} \pm 1.08$	$4.31^{b} \pm 0.69$

Values are mean of five (n=5) replicates ± standard deviation.
Values with different superscript alphabet are statistically significant at $P < 0.05$ using Ducan Multiple Range test.

DISCUSSION

The results of acute toxicity study revealed no gross behavioral and/or physical changes in lethality at doses up to 5000mg/kg body weight. This implies that the doses used in this study were toxicologically safe. The concentrations of total proteins, bilirubin and albumin in the serum are indicative of the integrity of the liver and the type of damage (Yakubu *et al*., 2005). The fact that the total protein and albumin levels were increased and total bilirubin decreased by the extract suggest that they may be important sources of proteins and amino acids and that the secretory function of the liver was not impaired. Albumin is used as an indicator of liver impairment via reduced absorption or protein loss (Sacher and McPherson, 2000). This results of this study revealed that *Mucana puriens* leaf extract may not have impairing effect on the liver. This could be attributed to the presence of vital nutrients such as essential amino acid (e.g. methonine and cysteine) in the extract (Ramachandram, 1980; Oliveira *et al*, 1999) that may have boosting effect on the total proteins and albumin level (Ekam *et al*., 2012).

Reports have shown that renal function indices are usually required to assess the normal functionality of different parts of the nephrons (Abolaji *et al*., 2007). Thus, serum levels of urea, uric acid and creatinne could serve as bio-indicators of the effect of a compound/plant extract on the tubular and/or glomerular part of the kidney. Therefore, the significant decrease produced by the extract of *M. puriens* at almost all the doses investigated on the renal function indices in this study may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected.

The integrity of glomeruli and regulation of osmotic pressure are indicated by the serum levels of any of albumin, total bilirubin, and globulin or a mixture of the biomolecules, respectively. They can also be used to assess the synthetic ability of the liver as well as damage to the hepatocytes (Ganong, 2001). Low albumin content in the serum suggests chronic damage to the liver. The increase in the serum albumin content observed in this study is an indication of increased synthetic function of the liver. High serum albumin as obtained in this study could not occur during liver damage or infection and or when albumin is lost continuously from the body (Naganna, 1989).

Bilirubin is an important catabolic product of blood with biological and diagnostic values (Chowdhury, 1989). Hepatocytes convert bilirubin to a polar form via phase II mechanism of biotransformation by adding glucuronic acid or sulfate molecules to it in a process referred to as conjugation. This reaction increases its solubility in water and thus enhances the ease with which bilirubin becomes excreted in the bile (Murray *et al*., 2000). The results suggest an increase in the levels of conjugated bilirubin in a dose dependent manner which may increase the ease with which bilirubin becomes excreted in the bile, and this may explain the dose dependent decrease in the level of total bilirubin observed in this study. Creatinine, urea, and uric acid are major catabolic products of muscle, protein, and purine metabolism, respectively. The observed decrease in creatinine, urea and uric acid level in this study indicate non-impairment in renal function (Cameroon *et al*., 1998).

Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. They comprise the single most important factor in the transfer and movement of water and electrolytes between the three divisions of the extracellular and intracellular compartments (Zalvia *et al*., 1991). Reports have shown that electrolytes homeostasis could be achieved through intake of certain foods (Bishop *et al*., 2010). The extract did not significantly alter the electrolytes and the slight increase is an indication that there are effective in the maintenance of both extracellular and intracellular fluids (Murray *et al*., 2000).

CONCLUSION

The study showed a lethal dose (LD_{50}) of greater than 5000mg/kg body weight of the extract. Results indicated that administered doses of 250, 500 and 750mg/kg body weight of the leaf extract showed significant ($P < 0.05$) increase in body weight, serum total protein and albumin and significant decrease in serum total bilirubin, uric acid, urea and creatinine in a dose dependent manner. Non-significant ($P > 0.05$) increase was observed in

serum Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+} and inorganic P at all doses except for Ca^{2+} and inorganic P whose increase was significant at a dose of 750mg/kg body weight of extract. These results are indications that the extract is biochemically safe and could have beneficial health effects.

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