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EFFECT OF POMESTEEN POWER ON THE HISTOMORPHOLOGY OF THE TESTES AND SOME HAEMATOLOGICAL INDICES IN ADULT ALBINO WISTAR RAT

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ABSTRACT
Effects of the pomesteen power (a forever living product) on the histomorphology of the testes and some hematological indices in adult male wistar rat were studied for 21 days. Thirty (30) adult male Wistar rats weighing 150-180g were divided into five groups with each group having six (n=6) rats. Group 1 served as control received 1ml of distilled water while the tests groups (groups 2-5) were administered by oral compulsion with 0.5ml/150g, 1.0ml/150g, 1.5ml/150g and 2.0ml/150g of pomesteen power drink respectively for 21 days . The result from the study indicates that the pomesteen supplement had no marked significant effects on the PCV, WBC, Eosinophil, and monocyte counts respectively; however, it influenced the alteration in neutrophil and Lymphocyte counts respectively. Morphological changes in the total body weights and organ weight of the testes were not statistically remarkable. Histological observation of the testes revealed spermatogenic series at different level of maturation with no marked pathology observed. The study concluded that the oral administration of pomesteen power has the ability to activate the immune system while an accurate dose dependent administration of the supplement has the tendency to boost the reproductive capacities in male and it is safe for consumptions, however functional studies are recommended to support these findings in subsequent studies.

KEYWORDS: Testes, Pomesteen power, histomorphology and haematology.

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INTRODUCTION

Pomesteen power is an antioxidant drink product produced by Forever living product company (An international family of companies that produces and market exclusive health and beauty product) which has as its major constituent mangosteen (*Garcinia mangostana*) and Pomegranate (*Punica granatum)* fruits.

Punica granatum is grown as a fruit crop plant, and as ornamental trees and shrubs in parks and gardens. Mature specimens can develop sculptural twisted bark multi-trunks and a distinctive overall form. Pomegranates are drought-tolerant, and can be grown in dry areas with either a Mediterranean winter rainfall climate or in summer rainfall climates. In wetter areas, they can be prone to root decay from fungal diseases. They are tolerant of moderate frost, down to about −10 °C (14 °F (LaRue, 1980). Insect pests of the pomegranate can include the pomegranate butterfly *Virachola isocrates* and the leaf-footed bug *Leptoglossus zonatus*. Pomegranate grows easily from seed, but is commonly propagated from 25–50 cm hardwood cuttings to avoid the genetic variation of seedlings. Air layering is also an option for propagation, but grafting fails (Aviram and Dornfeld, 2000).

In preliminary laboratory research and clinical trials, juice of the pomegranate may be effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation (Aviram *et al*.,2000). In mice, "oxidation of LDL by peritoneal macrophages was reduced by up to 90% after pomegranate juice consumption (Aviram *et al*., 2000). In a limited study of hypertensive patients, consumption of pomegranate juice for two weeks was shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme. Juice consumption may also inhibit viral infections while pomegranate extracts have antibacterial effects against dental plaque (Kaplan, Hayek, and Raz, 2011).

The purple mangosteen (*Garcinia mangostana*), colloquially known simply as mangosteen, is a tropical evergreen tree believed to have originated in the Sunda Islands and the Moluccas of Indonesia. Nevertheless, it also grows in tropical South American countries such as Colombia, where the tree has been introduced. The tree grows from 7 to 25 m (20–80 ft) tall. The fruit of the mangosteen is sweet and tangy, juicy, and somewhat fibrous, with an inedible, deep reddish-purple colored rind (exocarp) when ripe. In each fruit, the fragrant edible flesh that surrounds each seed is botanically endocarp, i.e., the inner layer of the ovary (Mabberley, 1997).

Various parts of the plant have a history of use in folk medicine, mostly in Southeast Asia. It is reputed to have possible anti-inflammatory properties, and may have been used to treat skin infections or wounds, dysentery or urinary tract infections (Obolskiy, Pischel, Siriwatanametanon, and Heinrich, 2009).

Testes, also called Testicle, in animals, the organ that produces sperm, (the male reproductive cell. In humans the testis is either of the paired, oval-shaped organs that produce sperm and the male hormones, the androgens. They are contained within the scrotal sac, which is located directly behind the penis and in front of the anus. Each testis weighs about 25 grams (0.875 ounce) and is 4 to 5 centimetres (1.6 to 2.0 inches) long and 2 to 3 centimetres (0.8 to 1.2 inches) in diameter. Each is covered by a fibrous capsule called the tunica albuginea and is divided by partitions of fibrous tissue from the tunica albuginea into 200 to 400 wedge-shaped sections, or lobes. Within each lobe are 3 to 10 coiled tubules, called seminiferous tubules, which produce the sperm cells. Both the partitions between lobes and the seminiferous tubules converge in one area near the anal side of each testis to form what is called the mediastinum testis. Sperm cells produced in the seminiferous tubules migrate, by short contractions of the tubules, to the mediastinum testis; they are then transported through a complex network of canals (rete testis and efferent ductules) to the epididymis) for temporary storage. The epididymis partially surrounds the top and anal side of each testis.

This present study was carried out to investigate the effect of pomesteen power 0n the Histomorphology of the testes and its effects on some hematological indices.

MATERIALS AND METHODS

Adult male albino Wister rats was obtained and acclimatized at the animal house of the Faculty of Basic Medical Sciences, Delta State University Abraka. The animal were allowed free water and rat feed for the period of two (2) weeks acclimazation. The animals were of comparable age of 10-12 weeks old and were allowed free access to standard chow diet and tap water ad libitum. All rats were housed under standard conditions in a room in groups per cage at a temperature of 21-24^{0}C.

Experimental Design

A total of thirty (30) Male Albino Wister rats were used for the experiment.
CONTROL = control (n = 6)
GROUP 1 =feed+water+ 0.5ml pomesteen power treated rats (n = 6)
GROUP 2 = feed+water+1.0ml pomesteen power treated rats (n = 6)
GROUP 3 = feed+water+1.5ml pomesteen power treated rats (n = 6)
GROUP 4 =feed+water+ 2.0ml pomesteen power treated rats (n = 6)

The animals were housed in a cage of five compartments with six rats to one compartment.

Animal Treatment

The animals were acclimatized for two (2) weeks upon which they were fed with rat feeds and water. They were administered the pomesteen power for 21 days. The dosage was determined from the suggested dose by the manufacturer and administered orally through a cannula. The rats were scarified after 21days by cervical dislocation method. They were dissected and their testes were harvested.

The total body weight of the rat was recorded at an interval 1 week while the organ weights were recorded using the electronic weighing balances.

Histopathological Analysis
The testes tissue specimens separated for Histopathological examination were fixed in Bouin's solution and then embedded in paraffin. Paraffin sections of 5cm thick and, stained with hematoxylin and eosin (H & E) were obtained. Histopathological changes on tissue sections were examined with a light microscope.

Collection and Analysis of Blood
The blood was obtained from rats by means of cardiac puncture and 5ml syringe was used to collect blood and transferred to anti-coagulant ETDA bottles in order to prevent blood from clotting. The blood collected was tested for white blood cell count (WBC).Packed cell volume (PCV) and blood differentials (neutrophils, lymphocytes, eosinophils and monocytes)

Haematological Tests Procedure
White Blood Cell (WBC)
Turk's solution of 1.0% glacial acetic acid was used as the diluents. The 1:20 dilution was then charged on an improved Neuber chamber and counted. Values were expressed in X 109 mg/dl.

Packed Cell Volume (PCV)
Microhaematocrit method was used. The sample was collected into a heparinized capillary tube and spun at 300rpm for 10 minutes. The resultant product consisting of packed cells, Buffy coat and plasma was read with the reader and the values expressed in percentage volume.

Blood Differentials
The test for blood differentials included the Neutrophils, Lymphocytes, Eosinophils and Monocytes, the Leishman stain method was used.

Photomicrography
The stained tissue images were captured using a digital microscopic eyepiece "SCOPETEX"DCM 500, 5.0 mega pixel connected to a computer.

Data Analysis
The results were expressed as Mean ± SD;

RESULTS
Morphological Studies
Table 1: Effect of Pomesteen Power on Body Weight

Groups	Day 1 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Control	183.3±39.33	194.17±38.5	190.83±41.40	210±21.79
0.5ml	165±5.48	187.5±15.41	179.17±12	198±10.41
1.0ml	160±0	175±4.47	175.83±3.76	186.7±5.77
1.5ml	151.67±4.08	175±4.48	171.67±5.16	176.7±25.16
2.0ml	148.3±4.10	163.3±12.5	167.5±5.24	181.7±2.89

All values are expressed as Mean ± SD

Table 2: Effect of Pomesteen power *on the* Testesticular weight of Wistar Rat after 21 Days

PARAMETER	Control	0.5ml	1.0ml	1.5ml	2.0ml
Organ Weight (g)	1.8 ± 0.26	2.33 ± 0.23	2.33±0.32	1.8±0.44	2.13±0.42

All Values are presented as Mean±SD

HAEMATOLOGICAL INDICES RESULT

Table 3: Effect of Pomesteen power on Hematological Parameters after 21 Days

PCV	45±3	51±7.8	47.3 ± 5.13	47.6 ± 2.51	47.6±3
WBC	5500 ± 498	4000 ± 346.4	4233.3± 351.2	5970±35.5	5316 ± 189.3
Neutrophils	64.3 ± 4.5	47 ± 9.5	27.35 ± 9.54	23 ± 2.63	36 ± 27
Lymphocytes	32 ± 5.6	47.3 ± 10.96	69.7 ± 6.67	73.7 ± 3.05	61.3± 3.05
Eosinophils	1.7 ± 0.0	1 ± 0.0	1. 0 ± 0.0	1.0 ± 0.00	1.0 ± 0.0
Monocytes	2.0 ± 1.0	1.0 ± 0.0	1.3 ± 0.57	1.0 ± 0.0	2.0 ± 1.0

HISTOPATHOLOGICAL RESULT

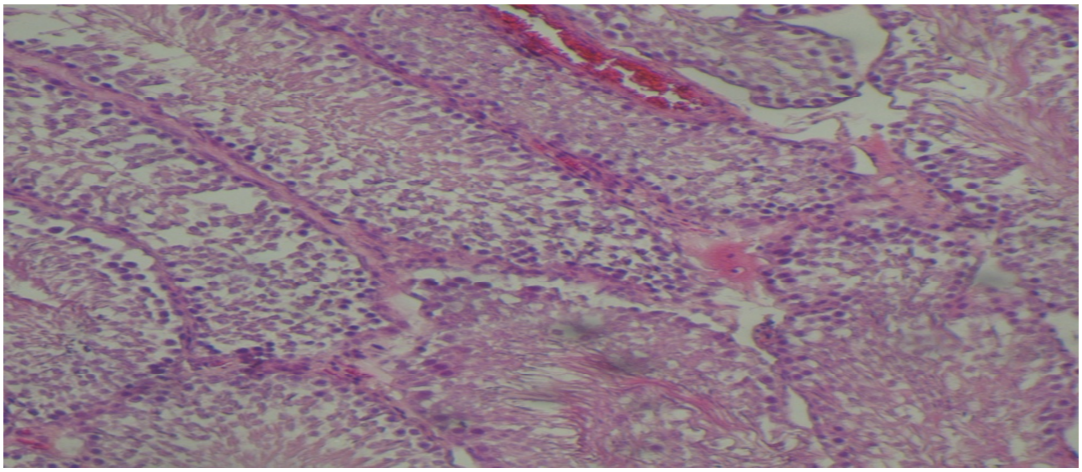

Fig. 1: H & E X 100 Control testes showing seminiferous tubules with spermatogenic series at different level of maturation. The germ cells, sertoli cells leydig cells and the general architeure are normal.

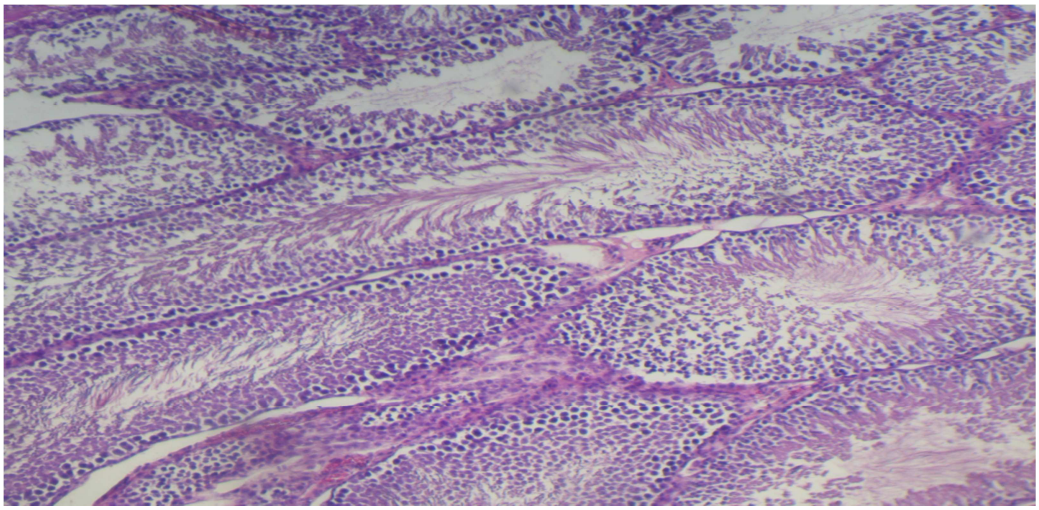

Fig.2 H&E X100 Testes of animals feed with 0.5ml/150g of pomesteen power.

Micrograph shows seminiferous tubules well outlined with spermatogenic cells appearing essentially normal. The Interstitium is free any collection.

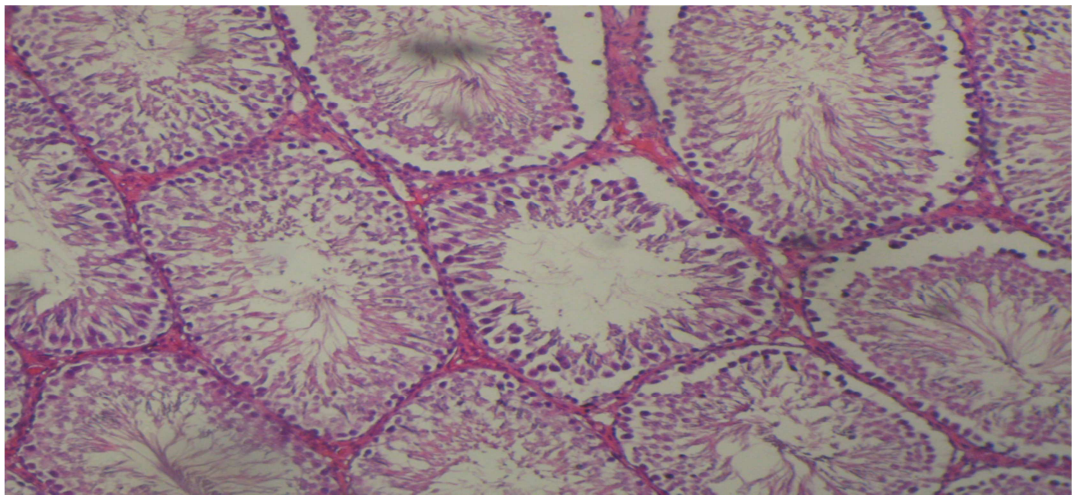

Fig.3 H&E X100 Testes of animals feed with 1ml/150g of pomeesteen power 21 days

Micrograph of shows numerous seminiferous tubules with lumen filled with germ cells at varying levels of maturity. Seminiferous tubules well outlined and free of inflammatory cells

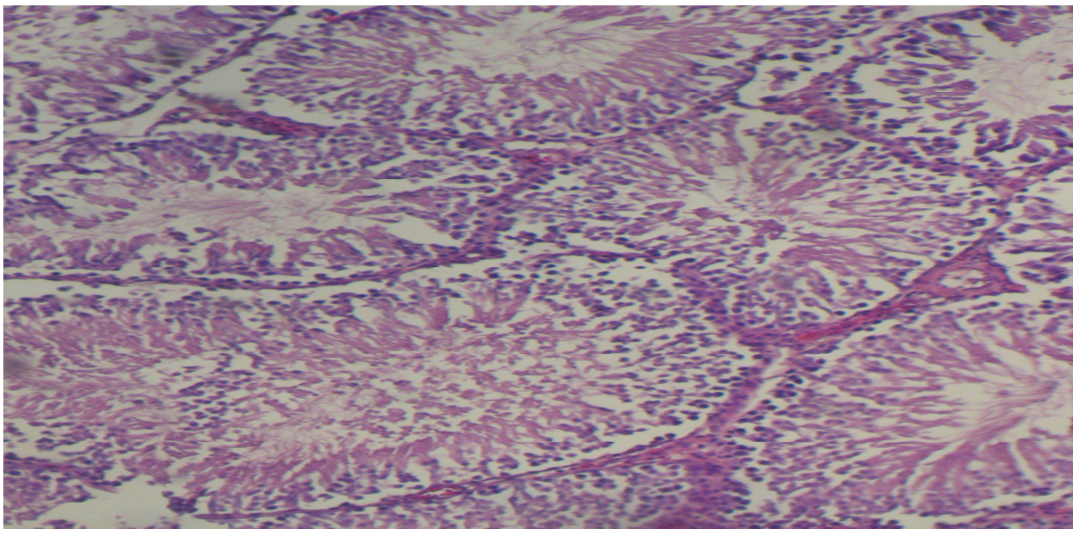

Fig.4 H&E X100 Testes of animals feed with 1.5ml/150g of pomesteen power 21days

Micrograph shows seminiferous tubules containing spermatogenic series at varying level of maturation, Interstitium is free of any collection and congestion.

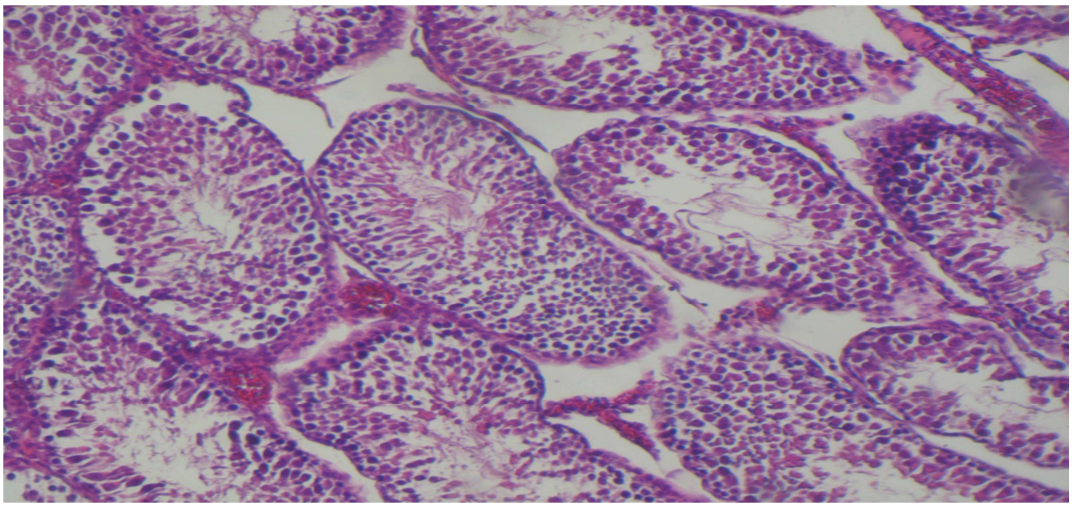

Fig 5: H&E X100 Testes of animals feed with 2ml/150g of pomeesteen power 21 days

Micrograph shows seminiferous tubules with spermatogenic series at different level of maturation. The germ cells, sertoli cells leydig cells and the general architeure are normal.

DISCUSSION OF RESULTS

The present study focused on the effect of Pomesteen power on morphological parameters, hematology and histological status of the testes. The result presented in table 1 showed that there was no significant difference in body weight variation of Albino Wistar rats dosed various concentrations of Pomesteen power. However, weight gain observed across group could be associated with maturity of animals.

The results for the hematological indices showed no significant difference ($P>0.05$) on the packed cell volume, WBC count, eosinophil and monocyte counts of rats dosed various concentrations of pomesteen power, when compared to control after 21 days respectively.

Although, there was an increment in PCV which was concentration dependent, the WBC also experienced a concentration dependent reduction after the 21 day respectively. In another development, there was a significant difference ($P< 0.05$) in the Lymphocyte and Neutrophilic count, of albino Wistar rats dosed various concentrations of pomesteen power, these variations which led to a non-concentration dependent increase in the lymphocyte count and reduction in the neutrophilic count of all dosed group.

Lymphocytes have been known to be made up of two special cells which include the T cells (thymus cells) and B cells (bursa-derived cells). These cells are the major cellular components of the adaptive immune response and are involved in cell-mediated immunity, whereas B cells are primarily responsible for humoral immunity (relating to antibodies). Neutrophils on the other hand, are normally found in the blood stream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure, and some cancers, neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation (Kumar, Abas and Fausto , 2004).

The administration of pomesteen power supplement with standard dosage coupled swith non- availability of adequate scientific studies on their safety has raised concerns on reproductive health and safety. Alteration in weight is an indication of impairment in the normal functioning of the organs. Organ-body weight ratio may indicate organ swelling, atrophy or hypertrophy (Amresh *et al*., 2008). The reduction in the testes and body weight ratios following the administration of the different doses of pomesteen power supplement may be attributed to atrophy. This submission is supported by the observed increase in the body and absolute organ weights of the animals.

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal (Ashafa *et al.*, 2009). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu *et al.*, 2007). The various haematological parameters investigated in this study are useful indices that can be employed to assess the toxic potentials of pomesteen power supplement in living systems. Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies (Olson *et al*., 2000).

The non-significant effect of the pomesteen power supplement on PCV could mean that the balance between the rate of production and destruction of blood corpuscles (erythropoiesis) was not affected negatively. PCV are associated with the total population of red blood cells. Therefore, the absence of observable significant effect of the pomesteen power supplement on these parameters may be an indication that neither the incorporation of hemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered (Adebayo *et al*., 2005).

Lymphocytes are the main effector cells of the immune system (Yakubu *et al*., 2007;). Therefore, the observed increase in the level of lymphocytes in our study may suggest stimulation of the immune system of the animals. Monocytes have been shown to increase in cases of infection; hence the reduction in monocytes during the day 21 could imply that there was little or no infection caused by the pomesteen power supplement at that point.

This study has shown that despite the report of the beneficial effects of Pomesteen power, or prolonged administration of the Pomesteen power could be helpful in boosting the body systems. The histopathological findings observed in the tissues that were sampled were observed to be dose related. From the results of our study, in the male testis of all treated groups, there was no much adverse histopathological change; rather there were marked proliferation of spermatogenic cell lines, transformation from primodial cells to spermatids and further to spermatozoa at a high rate.

The seminiferous tubules were moderately enlarged, the central lumen inclusive. There was increased vascularity of interstitial and leydig cells which secrete the male hormone-testosterone. A comparison of the histological changes of testis following a dose of administration shows that there are no adverse lesions on the testes.

Histology provides conclusive evidence from internal cell characteristics that other techniques fail to highlight. The probable reason for the observed histological effects may be due to phytoestrogenic constituents in Pomesteen power which may interact with steroids sex hormone metabolism and also the hypothalamic-pituitary-gonadal axis (HPA) of the female reproductive tract. Phytoestrogenic activity is due to such bioactive secondary metabolites as isoflavonoid, sterols, lignans and essential oils. This may also be observed in the testes because of low estrogenic activity in males.

CONCLUSION

The present study has shown that the oral administration of pomesteen power has the ability to activate the immune system. The study also showed that the administration of pomesteen power supplement could help boost the reproductive capacity in males due to its antioxidant property.

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