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Luminescence dating of Neolithic pottery in North East India

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Imprecise chronological data have long been affecting archaeological studies in Assam and Meghalaya, North East India. Relative dating methods have been used to study the antiquity of stone tools and ceramics found in the archaeological sites of these two areas. Both the areas are important as the eastern Asiatic Neolithic complex of double-shouldered celts and cord-marked pottery was first reported in India from Daojali Hading, Assam (1961), Garo Hills, Meghalaya has the highest concentration of prehistoric sites found in North East India. Optically stimulated luminescence dating offered an excellent opportunity for dating the ceramic samples recovered during the first excavations in 1961 (Daojali Hading in Dima Hasao district Assam) and 1999 (Gawak Abri, Garo Hills), as the method provided a direct age estimate of the time of last exposure of quartz or feldspar minerals to light or heat, and the purity of the etched quartz (i.e. any feldspar contaminations) can be confirmed by infra-red stimulated luminescence technique. Date obtained from Daojali Hading is 2.7 ± 0.3 ka (LD1728) and that from Gawak Abri is 2.3 ± 0.2 ka (LD1727).

Keywords: Cord marked, dating, hypothesis, neolithic, pottery.

NEOLITHIC sites have been reported from all the eight states of North East India. Excavated Neolithic sites from the region are Daojali Hading¹ and Saru Taro² from Assam; Gawak Abri³ and Law Nongthroh⁴ from Meghalaya; Napachik and Nongpok Keithelmanbi from Manipur^{5,6}; Parsi Parlo from Arunachal Pradesh⁷ and Ranyak Khen from Nagaland⁸. Daojali Hading, excavated in 1961, is the first stratified Neolithic site discovered in NE India (Figure 1). This site has put NE India in the Neolithic map of the world because of the recovery of the double-shouldered celt and cord-marked pottery which till then was considered as a character of the Neolithic of 'East Asia'¹. After this discovery, the boundary of the East Asiatic 'Cord ware' Neolithic culture was extended to include NE India^{9–11}.

For the first time in India, the shouldered celts were put into a stratigraphical context and the presence of quadrangular adzes and square-shouldered celts at the site suggest that the culture belonged to a Late Neolithic phase, the date of which is most probably linked to the late Neolithic cultures of southwest China and Southeast Asia. As there were no absolute dates, these observations

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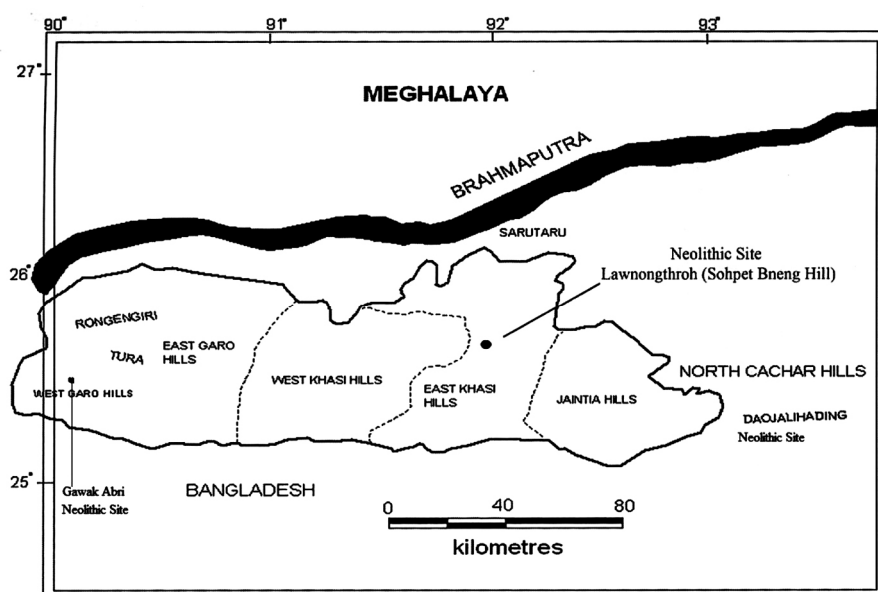


Figure 1. Map of the study area.

Table 1. Optically stimulated luminescence dating of pottery samples

Lab no.	Sample no.	Depth (cm)	U (ppm)	Th (ppm)	Potassium K (%)	Equivalent dose (Gy)	Dose rate (Gy/ka)	Age (ka)
LD1727	Gawak Abri	72	5.83 ± 0.06	22.52 ± 0.22	2.18 ± 0.02	12.0 ± 0.8	5.2 ± 0.1	2.3 ± 0.2
LD1728	Daojali Hading	105	2.41 ± 0.02	32.52 ± 0.3	2.23 ± 0.02	13.9 ± 1.5	5.1 ± 0.1	2.7 ± 0.3

remained hypothetical. Recently, using optically stimulated luminescence (OSL) dating technique, the site could be dated to 2.7 ± 0.3 ka (LD1728) and the hypothesis now stands confirmed.

Gawak Abri is a site in the Ganol–Rongram river valley of West Garo Hills, Meghalaya. Neolithic tools, mainly ground and polished celts, were first reported from Garo Hills in 1931 by G. D. Walker and systematic studies have been undertaken since then by various workers^{1–10}. During the latter half of the last century, the presence of tools belonging to this assemblage upon stretches of bare ground on the hill slopes has been reported, where they are usually exposed when the areas are cleared for shifting cultivation. In 1999, during exploration of the area, a trial trench was undertaken in Gawak Abri³ for ascertaining the stratigraphy of the Neolithic tools. With the Neolithic tools, pottery was also recovered from the site. This pottery was dated using OSL technique as 2.3 ± 0.2 ka (LD1727).

Both these dates could be obtained only because of the development of the OSL dating technique in India (Table 1). There has been a gap in the study of research materials of the Neolithic period in Assam and Meghalaya and only recently, we have started further analysis on these materials in detail. Datable carbon material was recovered in very small quantities from both the sites and was

not sufficient for conventional radiocarbon dating available then. Dating these sites is a major breakthrough, as Daojali Hading is a type site and the Gawak Abri date is the first date to come from the prehistoric sites of Garo Hills.

The dates were obtained from ‘vintage samples’, i.e. using samples collected during the excavations in 1961 (Daojali Hading) and 1999 (Gawak Abri). This has been done on an experimental basis, and a comparison with ¹⁴C dates from the area has also been provided to highlight the reliability of the technique. In the laboratory, under subdued red-light conditions, the pottery samples were crushed and transferred to a beaker after removing light-exposed materials from the surface. The samples were then treated for removing carbonate and organic matter using 1 N HCl and 30% H₂O₂ respectively, and later sieved to obtain 90–125 μm size fractions¹². From the separated size fractions (90–125 μm), quartz grains (density 2.65 g/cm³) were extracted by density separation using high-density liquid (sodium polytungstate solution). The extracted quartz grains were etched for 80 min in hydrofluoric acid (40%) to remove the outer layer (the HF treatment also removes any feldspar contamination) and subsequently treated with HCl and washed in distilled water and re-sieved. The purity of the etched quartz grains (i.e. any feldspar contamination) was confirmed by infrared stimulated luminescence (IRSL) technique.

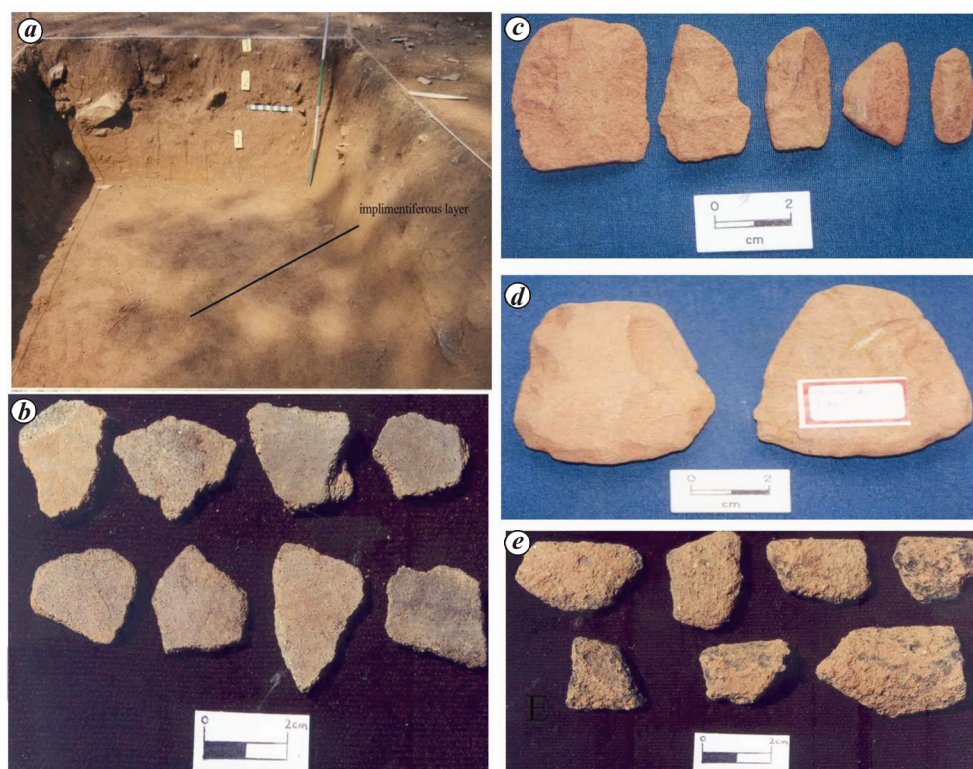


Figure 2. Gawak Abri. *a*, Implimentiferous layers; *b*, Fine fabric potsherds; *c*, Probably utilized flakes; *d*, Celts; *e*, Coarse fabric potsherds.

The etched quartz grains were then fixed at the centre of stainless steel discs (i.e. about 3 mm diameter mono layer of samples in 10 mm diameter steel discs) using silicon oil (adhesive agent) to determine the radiation energy received by the samples after burial (i.e. paleo dose or equivalent dose). The single aliquot regeneration protocol¹³ was used for equivalent dose (De) determination. OSL measurements were carried out in an automated Riso TL/DA-20 reader equipped with blue light-emitting diodes. The equivalent dose (De) was calculated using the initial integral (0.8 sec) of the OSL (using Dullers Analysis software).

For estimation of annual dose rate, concentration of uranium, thorium and potassium in the pottery and surrounding sediments needs to be measured. In the present case, no surrounding sediments were available and hence only the chemical concentration of U, Th and K in the pottery sample was measured and Grun's software was used for cosmic rays. The concentration of uranium, thorium and potassium in the pottery was measured using ICP-MS (inductively coupled plasma mass spectrometry). Grun's software was used for dose rate (including cosmic ray contribution) and age calculation

$$\text{Age} = \frac{\text{Equivalent dose (Gy)}}{\text{Dose rate (Gy/ka)}}$$

Gawak Abri is a single cultural site located in the uplands of the Ganol-Rongram Valley. The pottery was collected

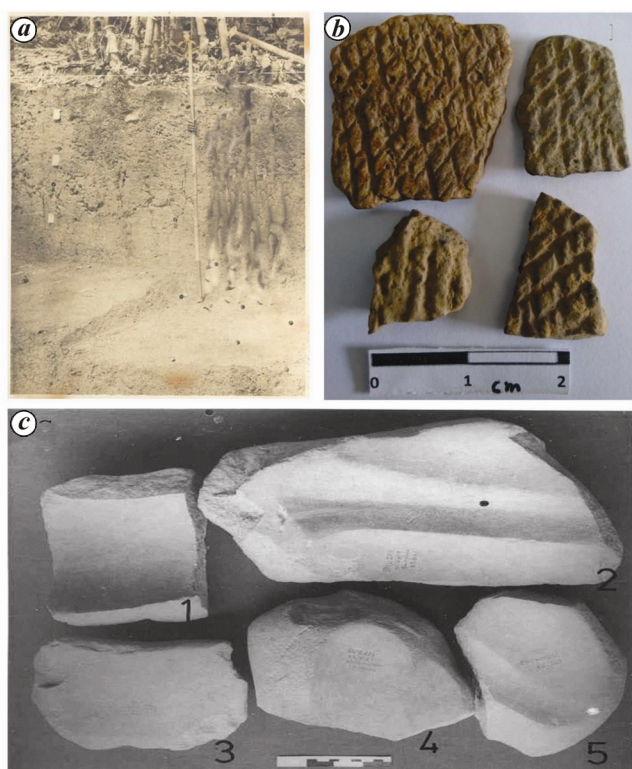
from a 2/2 m trial trench (Figure 2 *a*) on the northern side of the mound to ascertain the stratigraphy of the site during exploration. The implimentiferous layer is composed of highly acidic sandy alluvium of almost 1 m and is capped by a deposit of 1½ m. The lithic assemblage consisted of ground and polished celts (Figure 2 *d*), short axes, chipped celts and a high percentage of micro-sized flakes, some of which were probably used (Figure 2 *c*). The pottery consisted of two varieties (Figure 2 *b* and *e*). One was very coarse black pottery and the other used for dating was also of coarse variety, but comparatively finer with a thin grey slip³.

Daojali Hading consists of a low ridge (1500 ft above sea level) of Tertiary sandstone oriented in the northeast-southeast direction. It is situated at 25°26'N and 93°10'E (Survey Map Sheet No. 83 C Haflong)¹. Four trenches (Figure 3 *a*) were dug at four different locations of the site with the multiple objectives of determining the stratigraphy and ascertaining the context of the Neolithic artifacts (Figure 3 *b* and *c*). Artifacts were recovered from both the eastern and western faces of the ridge.

These dates agree well with the radiocarbon dates from the site of Law Nongthroh (2930 ± 30 BP; 07/B1 Beta No. 406664) in the east Khasi Hills district of Meghalaya. This site is the nearest Neolithic settlement to Daojali Hading with similar cultural characteristics. Cord-marked pottery and double-shouldered celts were found with mullers, pounders, thrashers, polishers, etc. in both the sites. Pottery, in the three sites Daojali Hading, Law

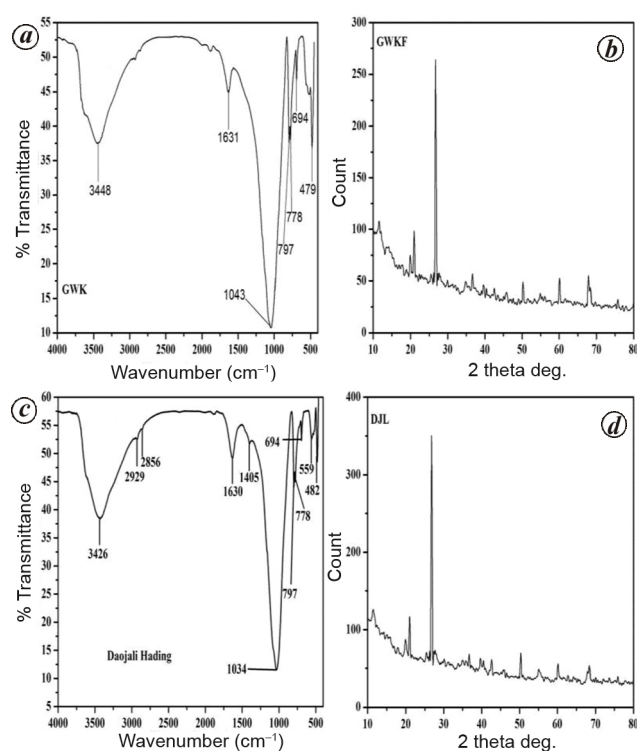
Table 2. Infrared peaks and tentative assignments of Daojali Hading (DJL) and Gawak Abri (GWK)

Frequency cm^{-1}	As received state	Tentative vibrational assignments
DJL		
3426	Medium	O–H stretching of absorbed water molecule
1630	Very weak	H–O–H bending of water
1034	Very strong	Si–O–Si (kaolinite)
797, 694	Very weak	Si–O of quartz
559	Very weak	Fe_2O_3
482	Very weak	Si–O–Si bending (microcline)
GWK		
3448	Medium	O–H stretching of absorbed water molecule
1631	Very weak	H–O–H bending of water
1043	Very strong	Si–O–Si (kaolinite)
797, 778, 693	Very weak	Si–O of quartz
479	Very weak	Si–O–Si bending (microcline)

**Figure 3.** Daojali Hading. *a*, Implimentiferous layers; *b*, Cord-impressed potsherds; *c*, Mullers.

Nongthroh and Gawak Abri was made using red kaolin clay (Figure 4 *b* and *d*) and the firing temperature ranged between 500°C and 700°C (Figure 4 *a* and *c*; Table 2).

Daojali Hading and Law Nongthroh have a richer tool kit indicating agriculture and food processing, while the Gawak Abri tool kit with pottery is suitable for incipient agriculture and foraging only. This may be because Daojali Hading and Law Nongthroh are located on low ridges, close to a perennial river with ample ground available for cultivation, while ample wild food resources

**Figure 4.** *a*, FTIR peaks of Gawak Abri; *b*, Powder XRD pattern of Gawak Abri; *c*, FTIR peaks of Daojali Hading; *d*, Powder XRD pattern of Daojali Hading.

were available to the Gawak Abri inhabitants during all the seasons as they had access to the uplands, the valley and the flood plain, and topographical variation allowed growth of resources in particular patches during particular seasons³.

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Antibacterial activity of protease hydrolysates isolated from *Silybum marianum*

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In this study, the antibacterial activity of protease hydrolysates from *Silybum marianum* protein isolates (SMPIs) was investigated. Neutral protease, papain, pepsin and alkaline protease were used as experimental enzymes, while *Escherichia coli*, *Staphylococcus aureus*, *Sarcina lutea* and *Bacillus subtilis* were the bacterial indicators. The results showed that neutral protease, papain and pepsin hydrolysates exerted inhibitory effects on the four types of bacteria tested. However, alkaline protease hydrolysates of SMPI showed stimulatory effects on replication of the four bacteria tested. The antibacterial mechanism of SMPI hydrolysates was studied using scanning electron microscopy, and the results showed effective inhibition of *E. coli* (Gram-negative, *G*⁻) and *S. aureus* (Gram-positive, *G*⁺). It is speculated that the underlying mechanism of SMPI hydrolysates may involve injury to *E. coli* and *S. aureus* cell membranes. Currently, no similar studies have been conducted on the antibacterial activity of SMPI.

Keywords: Antibacterial activity, antibacterial mechanism, proteolysis, *Silybum marianum*.

SILYBUM MARIANUM (L.) Gaertn, also known as milk thistle, is an annual or biennial herbaceous plant belonging to the composite family. Originally from the Mediterranean, it is now distributed worldwide^{1,2}. From ancient times, *S. marianum* has been considered a folkloric hepatoprotective herb. Its constituents include silymarin, milk thistle oil³ and protein that are known to have high medicinal value^{4,5}.

Literature reports specify that *S. marianum* is mainly used for extraction of silymarin⁶ for clinical and pharmaceutical applications^{7–9} while a large portion of its protein content remains in the spent meal, which is used as forage or simply discarded after the extraction process. At the end of the last century, Chen, Wang¹⁰ and Zhu¹¹ studied *S. marianum* protein and confirmed that it contains all the amino acids, and suggested it to be a potential

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