

21. Popov, L. E., Holmer, L. E., Hughes, N. C., Pour, M. G. and Myrow, P. M., *Papers in Palaeontology*, 2015, pp. 1–55.
22. Bhargava, O. N., Kumar, G. and Gupta, S. S., *J. Geol. Soc. India*, 1982, **23**, 183–191.
23. Bhargava, O. N., Bhandari, A. K. and Sharma, R. K., *Bull. Indian Geol. Assoc.*, 1986, **19**, 65–68.
24. Bhargava, O. N. and Srikantia, S. V., *J. Geol. Soc. India*, 1985, **26**, 880–886.
25. Sudan, C. S., Sharma, U. K., Sahnai, A. K. and Shah, S. K., *J. Geol. Soc. India*, 2000, **55**, 649–654.
26. Sudan, C. S. and Sharma, U. K., *J. Palaeontol. Soc. India*, 2001, **46**, 161–171.
27. Parcha, S. K., Singh, B. P. and Singh, B. P., *Curr. Sci.*, 2005, **88**, 158–162.
28. Parcha, S. K. and Pandey, S., *J. Asian, Earth Sci.*, 2011, **42**, 1097–1116.
29. Virmani, N. and Singh, B. P., 7th Chand. Sci. Cong. Prog. Abst., 2012, pp. 72–73.
30. Virmani, N., Singh, Birendra, P., Bhargava, O. N., Kishore, N. and Gill, A., *Indian Coll. Micropalaeontol. Strat.*, 2013, 178–179.
31. Hughes, N. C., Sell, B. K., English, L., Myrow, P. M. and Singh, B. P., *J. Palaeontol. Soc. India*, 2013, **58**, 175–193.
32. Virmani, N., Singh, Birendra, P. and Gill, A., *J. Geol. Soc. India*, 2015, **85**, 557–566.
33. Srikantia, S. V., *Contemp. Geol. Res. India*, 1981, **1**, 31–48.
34. Bhargava, O. N. and Bassi, U. K., *Mem. Geol. Surv. India*, 1998, **124**, 1–212.
35. Kumar, G., Raina, B. K., Bhargava, O. N., Maithy, P. K. and Babu, R., *Geol. Mag.*, 1984, **121**, 211–219.
36. Pascoe, E. H., *A Manual of the Geology of India and Burma (3rd edition)*, Geological Survey of India, Calcutta, 1959, vol. 2, pp. 1–1343.
37. Myrow, P. M., Snell, K. E., Hughes, N. C., Paulsen, T. S., Heim, N. and Parcha, S. K., *Geol. Soc. Am. Bull.*, 2006, **118**, 491–510.
38. Bhargava, O. N., *J. Palaeontol. Soc. India*, 2008, **53**, 113–129.
39. Bhargava, O. N., *Mem. Geol. Soc. India*, 2011, **78**, 69–99.
40. Walcott, C. D., *Smithson Misc. Collect.*, 1920, **67**, 261–364.
41. Bengtson, S. and Missarzhevsky, V. V., In *Second International Symposium on the Cambrian System* (ed. Taylor, M. E.), US Geological Survey Open File Report, 1981, vol. 81, pp. 743–252.
42. Bengtson, S., Conway Morris, S., Cooper, B. J., Jell, P. A. and Runnegar, B. N., *Mem. Assoc. Aust. Palaeontol.*, 1990, **9**, 1–364.
43. Bengtson, S. and Hou, X., *Ann. Palaeontol. Pol.*, 2001, **46**, 1–22.
44. Qian, J. and Xiao, B., *Chin. Acad. Geol. Sci.*, 1984, **13**, 65–90.
45. Moore, J. L., Porter, S. M., Steiner, M. and Li, G., *J. Palaeontol.*, 2010, **84**, 1040–1060.
46. Smith, P. M., Brock, G. A. and Paterson, J. R., *Alcheringa*, 2014; DOI:10.1080/03115518.2014.951917
47. Skovsted, C. B., *J. Paleontol.*, 2006, **80**, 1087–1112.
48. Elicki, O., *Neues Jahrb. Geol. Palaentol., Abh. B*, 1994, **191**, 69–93.
49. Kouchinsky, A., Bengtson, S., Clausen, S., Gubanov, A. and Malinky, J. S., *Alch.*, 2011, **35**, 170–189.
50. Sarmiento, G. N., Remolar, D. F. and Concuoglu, M. C., *Colo. Palaeontol.*, 2001, **52**, 117–134.
51. Fernandez-Remolar, D. C., *Rev. Españ., Palaeontol.*, 2001, **16**, 39–61.
52. Landing, E., Geyer, G. and Bartowski, K. E., *J. Palaeontol.*, 2002, **76**, 287–305.
53. Clausen, S. and Alvaro, J. J., *Acta, Palaeontol., Pol.*, 2006, **51**, 223–238.
54. Topper, T. P., Brock, G. A., Skovsted, G. B. and Paterson, J. R., *Mem. Assoc. Aust. Palaeontol.*, 2009, **37**, 199–246.
55. Skovsted, C. B. and Peel, J. S., *Acta Palaeontol. Pol.*, 2007, **52**, 729–748.
56. Wrona, R., *Acta Palaeontol. Pol.*, 2004, **49**, 13–56.
57. Gravestock, D. I. et al., *Trans. Palaeontol. Inst. Russ. Acad. Sci.*, 2001, **282**, 1–344.
58. Paterson, J. R., Skovsted, C. B., Brock, G. A. and Jago, J. B., *Mem. Assoc. Aust. Palaeontol.*, 2007, **34**, 131–146.
59. Hughes, N. C., Peng, S., Bhargava, O. N., Ahluwalia, A. D., Walia, S., Myrow, P. and Parcha, S. K., *Geol. Mag.*, 2005, **142**, 57–80.

ACKNOWLEDGEMENTS. B.P.S. thanks UGC, New Delhi for providing the Startup grant (No. F.20-1/2012(BSR)/20-8(12)/2012 (BSR)). O.N.B. was supported by a grant from INSA, New Delhi. R.S.N. thanks UGC for JRF-fellowship and N.V. thanks DST, New Delhi for providing JRF-INSPIRE fellowship. B.P.S. thanks Dr Artem Kouchinsky (Sweden) for discussions on fossils images and help in identification. We thank Prof. Ashok Sahni (INSA) for encouragement to work on recovery of microfossils from the Cambrian of the Spiti region; Rajeev Patnaik (PU, Chandigarh) for SEM and Kunzang Tinley (Sagnum), for safe passage in the Parahio valley, Spiti Himalaya.

Received 4 November 2014; accepted 29 September 2015

BIRENDRA P. SINGH<sup>1</sup>\*  
O. N. BHARGAVA<sup>2</sup>  
K. P. JUYAL<sup>3</sup>  
R. S. NEGI<sup>1</sup>  
NANCY VIRMANI<sup>1</sup>  
C. A. SHARMA<sup>1</sup>  
AMAN GILL<sup>4</sup>

<sup>1</sup>Center of Advanced Geology,  
Department of Geology,  
Panjab University,  
Chandigarh 160 014, India

<sup>2</sup>103, Sect-7,  
Panchkula 134 109, India

<sup>3</sup>76-A Dharampur,  
Dehradun 248 001, India

<sup>4</sup>Consolidated Contractor International  
Company,  
Doha, Qatar

\*For correspondence.

e-mail: v\_ruh@rediffmail.com

## Litchi fruit contains methylene cyclopropyl-glycine

Earlier we had reported on annual seasonal outbreaks of hypoglycaemic encephalopathy in children in Muzaffarpur district, Bihar<sup>1,2</sup>. Until our studies in 2013 (ref. 1), this disease was misclassified by all others as acute viral encephalitis first and later as acute encephalitis syndrome, since no viral agent could be

detected after years of search<sup>3–6</sup>. Media had been calling it a mystery disease, as no definitive clinical diagnosis consistent with International Classification of Diseases had been made, in spite of investigations dating back from 1995 onwards by many investigators – local, national and international.

Hypoglycaemic encephalopathy occurs sporadically in children predisposed by inborn errors of metabolism, triggered mostly by long hours of no food intake<sup>7</sup>. In children not genetically so predisposed, an extrinsic toxin is necessary to cause the disease. The one well-known extrinsic toxin causing hypoglycaemic

encephalopathy (also called Jamaican vomiting disease) is methylene cyclopropyl-alanine (MCPA), also known as hypoglycin A, present in ackee fruits, popular in Jamaica<sup>8</sup>.

In the case of Muzaffarpur hypoglycaemic encephalopathy, strong epidemiological association exists with litchi fruits, as we have described earlier<sup>1,2</sup>. Jamaican ackee (*Blighia sapida*) and Asian litchi (*Litchi sinensis*) belong to the same family, Sapindaceae (soapberry)<sup>1</sup>. Many decades ago, phytotoxicology experts had described the presence of an analogue of hypoglycin A, methylene cyclopropyl-glycine (MCPG) in litchi seeds<sup>9</sup>. In experimental animals, both MCPA and MCPG cause hypoglycaemia and encephalopathy<sup>10,11</sup>. With all this information already available, we had postulated first and predicted later, that the extrinsic stimulant causing the Muzaffarpur hypoglycaemic encephalopathy is MCPG<sup>1,2</sup>.

Thus the last missing piece of the puzzle of the Muzaffarpur hypoglycaemic encephalopathy was the question of the presence of MCPG in litchi fruits. The likelihood of children swallowing litchi seeds is extremely low. However, children eat litchi fruits, available in plenty during the harvest season<sup>3</sup>. One of us (T.J.J.) has interviewed knowledgeable local people and learnt two interesting facts. The majority of affected children belong to the families of litchi harvesting labourers who camp in litchi orchards for harvesting the fruits early in the morning, before sunrise. Second, only fruits in bunches are saleable, but the many single fruits that fall to the ground have no commercial value and are free for children to pick up and hoard for eating later. A previous study found strong association of litchi consumption by children with the disease<sup>3</sup>.

Therefore, we focused our attention to the chemical investigation of litchi fruits for the presence of MCPG. We present a

preliminary report of the detection of MCPG in Muzaffarpur litchis.

Litchi seeds and semi-ripe and fully ripe fruits were collected in Muzaffarpur and stored frozen until testing. Dry seed powder and fruit pulp were macerated in ethanol and the slurry filtered and concentrated by ethanol evaporation. The concentrates were washed in water to remove carbohydrates. The aqueous mixtures were centrifuged and the supernatants analysed for MCPG by LC-MS/MS and the data were compared with synthetic MCPG procured from AKos (GmbH, Germany).

LC-MS/MS analysis showed a peak corresponding to standard MCPG in seeds, ripe and semi-ripe litchi pulp, indicating the presence of MCPG in all the three samples. Based on the product ion scan (MS2) of MCPG, product ions qualified for monitoring were 109.9 and 82.0 (Figure 1). Following optimization of multiple reaction monitoring mass spectrometry conditions based on better baseline and sensitivity, the precursor ion  $\rightarrow$  product of  $m/z$  128.0  $\rightarrow$  82.0 was used for quantitation purpose. Table 1 shows the result of quantification of MCPG in the samples. Litchi seeds contain 1.8  $\mu\text{g/g}$  MCPG, while the ripe and semi-ripe pulp show a value of 0.187 and 0.566  $\mu\text{g/g}$  (fresh wt) MCPG respectively. The ripe litchi pulp has the least

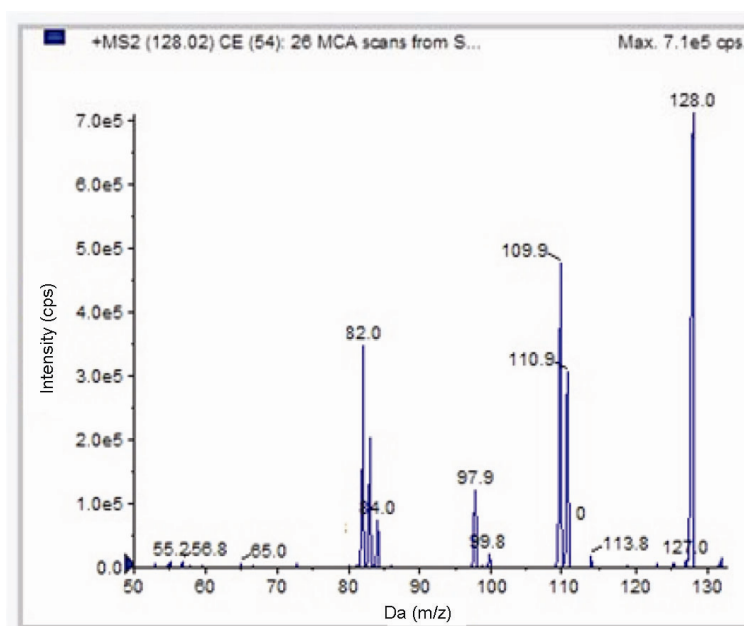
concentration of MCPG which is less by a factor of 3.0 and 9.6 compared to semi-ripe pulp and litchi seeds respectively. Earlier studies have shown the presence of MCPG in litchi seeds at a concentration of 0.8  $\mu\text{g/g}$  fresh seeds<sup>9</sup>. Since these seeds were fresh and had 50% water content as reported<sup>9</sup>, the concentration of MCPG in these seeds would be 1.6  $\mu\text{g/g}$  on dry weight basis. Thus, the concentration of MCPG (1.8  $\mu\text{g/g}$  dry wt) found in the litchi seeds in the present study is almost similar to that reported earlier<sup>9</sup>.

In conclusion, we have found MCPG, a hypoglycin, that we call hypoglycin G, in litchi fruits, both semi-ripe and fully ripe. The presence of MCPG in litchi seeds had been known for a long time, but there are no reports of MCPG in litchi fruits. Our finding provides the much needed evidence for biological plausibility that litchi consumption by undernourished children, especially after prolonged fasting, triggers the hypoglycaemic encephalopathy that manifests during the early hours of the morning (4–8 am)<sup>1,2</sup>.

In view of our observations and conclusions, the Government of Bihar has already introduced some interventions. In 2015, the local health workers have campaigned in the litchi-cultivating communities to teach the locals that no child should go to bed at night without eating a

**Table 1.** Methylene cyclopropyl-glycine (MCPG) content in litchi seeds, semi-ripe and ripe fruit pulp

Fruit part taken for analysis	MCPG content
Litchi seeds	1.80 $\mu\text{g/g}$ dry wt
Litchi semi-ripe pulp	0.57 $\mu\text{g/g}$ fresh wt
Litchi ripe pulp	0.18 $\mu\text{g/g}$ fresh wt



**Figure 1.** Product ion (MS 2) spectra of Methylene cyclopropyl-glycine (MCPG). MCPG shows a molecular ion peak at 128  $m/z$ , while two major fragmented peaks were also observed at 109.9 and 82  $m/z$  respectively.

cooked meal and for parents to restrict children eating litchis in the evening to none or very few. Unfortunately, no one is monitoring the outcome of these interventions. All primary health centre medical officers have been taught to draw blood for glucose measurement and immediately infuse 10% dextrose, instead of sending the children with encephalopathy to the two designated referral hospitals in the district headquarters, which may involve hours of delay that results in risk to life or full brain recovery<sup>2</sup>. Thus, both preventive and curative interventions are already in place to save the lives of many children.

5. Sahni, G. S., *Indian Pediatr.*, 2012, **49**, 502–503.
6. Sahni, G. S., *Ann. Trop. Med. Public Health*, 2013, **6**, 89–95.
7. John, T. J., *Indian Pediatr.*, 2003, **40**, 863–869.
8. Paireu, J. *et al.*, *Emerg. Infect. Dis.*, 2012, **18**, 1817–1824.
9. Gray, D. O. and Fowden, L., *Biochem. J.*, 1962, **82**, 385–389.
10. Melde, K., Buettner, H., Boschert, W., Wolf, H. P. O. and Ghisla, S., *Biochem. J.*, 1989, **259**, 921–924.
11. Melde, K., Jackson, S., Bartlett, K., Sherratt, H. S. A. and Ghisla, S., *Biochem. J.*, 1991, **274**, 395–400.

ACKNOWLEDGEMENTS. We thank the Director, CSIR-IITR, Lucknow for encouragement and support; and S. K. Purushottam, CSIR-IITR, Lucknow, Dr Gyan Bhushan, Civil Surgeon, Muzaffarpur and Dr Arun Shah, Kejariwal Maternity and Child Hospital, Muzaffarpur, for collection of litchi seeds, semi-ripe and ripe litchi fruits. Financial assistance from CSIR-INDEPTH BSC-0111 and Child Health Foundation is acknowl-

edged. The manuscript is CSIR-IITR communication #3354.

Received 29 October 2015; accepted 30 November 2015

MUKUL DAS<sup>1,\*</sup>  
SOMYA ASTHANA<sup>1</sup>  
SHEELENDRA P. SINGH<sup>2</sup>  
SUMITA DIXIT<sup>1</sup>  
ANURAG TRIPATHI<sup>1</sup>  
T. JACOB JOHN<sup>3</sup>

<sup>1</sup>*Food Toxicology Laboratory,  
Food, Drug and Chemical Toxicology  
Group, and*

<sup>2</sup>*Analytical Chemistry Laboratory,  
Regulatory Toxicology Group,  
CSIR-Indian Institute of Toxicology  
Research,*

*Mahatma Gandhi Marg, P. O. Box 80,  
Lucknow 226 001, India*

<sup>3</sup>*439, Civil Supplies Godown Lane,  
Kamalakashipuram,  
Vellore 632 002, India*

*\*For correspondence.*

*e-mail: mukul@iitr.res.in*

1. John, T. J. and Das, M., *Curr. Sci.*, 2014, **106**, 1184–1185.
2. Shah, A. and John, T. J., *Curr. Sci.*, 2014, **107**, 570–571.
3. Samuel, P. P., Muniraj, M., Thenmozhi, V. and Tyagi, B. K., *Indian J. Med. Res.*, 2013, **137**, 991–992.
4. Dinesh, D. N. *et al.*, *Int. J. Curr. Microbiol. Appl. Sci.*, 2013, **2**, 531–538.