

**REPORT****Khesari Dal: A Food or a Dreadful Poison and Its Prevention and Detection by Chromatographic Method**

I. CHAKRABORTI\* and R.N. SALHAN†

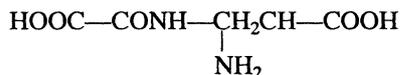
*Directorate General of Health Services, Ministry of Health and Family Welfare  
Nirman Bhawan, New Delhi - 110011, India*

Khesari pulse is cultivated in all parts of India which contains a toxic amino acid  $\beta$ -N-oxalyl amino-L-alanine (BOAA) which causes a deadly disease lathyrism, a crippling action on human beings.

**Key words:** Khesari dal, poison, detection, prevention, chromatography.

Over vast tracts, particularly in Central India, thousands of young population between the age group of 12 years to 45 years are suffering from a type of paralysis called lathyrism, a nervous disease that cripples man. This disease does not kill a man but makes the victim cripple throughout his life. It affects the youth community and comes suddenly without any warning symptoms. It is noticed that it affects young men more than young women. This disease is known to result from an excessive consumption of the Khesari pulse (*Lathyrus sativus*) which is a hard crop with wild growth and drought resistance and is cheaper than other conventional pulses. Though rich in proteins, the pulse has never been popular because of its harmful effect which is known since ancient times. Similarity in appearance with Bengal gram dal and Tur (Arhar) dal has prompted its use as an adulterant. It is a very common adulterant in Besan (Bengal gram flour). Khesari dal has therefore remained a poor man's food and an adulterant of more conventional pulses. Though the existence of the disease has been known for a long time, no effective steps have been undertaken to control it effectively<sup>1,2</sup>.

*Lathyrus sativus* is a native of southern Europe and West Asia but is now frequently grown in India, mainly in M.P., U.P., Bihar, Bengal, Maharashtra and Assam and being consumed excessively in M.P., Bihar and West Bengal. The toxic principle as isolated from Khesari dal is  $\beta$ -N-oxalyl- $\beta$ -diamino propionic acid (ODAP), which causes lathyrism, a kind of paralysis of legs in human beings and animals. ODAP is an amino acid with the formula given below:



\*Senior Chief Technical Officer, Dte G.H.S., Nirman Bhawan, New Delhi, India.

Address for correspondence: Anand Villa, SA-142, Shastri Nagar, Ghaziabad, India.

†Officer on special duty (Ex-Medical Superintendent, Safdarjung Hospital), Dte G.H.S., Nirman Bhawan, New Delhi, India.

The amino acid  $\beta$ -N-oxalyl amino-L-alanine (BOAA), which is highly acidic, forms diamino propionic acid and oxalic acid on hydrolysis.

Lathyrism has been reported in almost all developed or developing countries of the globe like Greece, France, Germany, Syria, Italy, Spain, Iran, Algeria, Ethiopia, Russia (South), Pakistan and Bangladesh, while it has been eradicated from European countries by a ban on the cultivation of *Lathyrus sativus*. But it is still crippling people in India, due to its adulteration in Besan (Bengal gram flour) and Arhar (pigeon pea flour) although its consumption is prohibited.

*Lathyrus sativus* (Khesari dal) is known by different local names in regional languages and as chickling vetch or white vetch in English. Rule 44A of Prevention of Food Adulteration Rules (1955) has been quoted below which also gives the equivalents of khesari gram in some of the Indian languages as follows:

RULE 44-A. No person in any State shall, with effect from such date as the State Government concerned may by notification in the Official Gazette specify in this behalf, sell or offer or expose for sale, or have in his possession for the purpose of sale, under any description or for use as an ingredient in the preparation of any article of food intended for sale:

- (a) Kesari dal (*Lathyrus sativus*) and its products.
- (b) Kesari dal flour (*Lathyrus sativus*) and its products.
- (c) A mixture of Kesari gram (*Lathyrus sativus*) and Bengal-gram (*Cicer arietinum*) or any other gram.
- (d) A mixture of Kesari dal (*Lathyrus sativus*) and Bengal-gram dal (*Cicer arietinum*) flour or any other dal.
- (e) A mixture of Kesari dal (*Lathyrus sativus*) flour and Bengal-gram (*Cicer arietinum*) flour or any other flour.

**Explanation:** The equivalents of Kesari gram in some of the Indian Languages are as follows:

1. Assamese: Khesari, Teora
2. Bengali: Khesari, Teora, Kassur, Batura,
3. Bihari: Khesari, Teora, Kassur, Batura
4. English: Chikling vetch
5. Gujarati: Lang.
6. Hindi: Khesari, Kessur, Kesari, Kassartiuri, Batura, Chapri, Dubia, Kansari, Kesori, Latri, Tinra, Tiuri, Kassor.
7. Kannada: Laki Bele, Kessari Bele.
8. Malayalam: Kesari, Lanki, Vattu.
9. Tamil: Muku.
10. Marathi: Lakheri, Batri, Lakhi, Lang, Mutra, Teora, Botroliki-dal, Lakh.
11. Oriya: Khesra, Khesari, Khesari dali.
12. Persian: Masang.
13. Punjabi: Kisari, Chural, Karas, Karil, Kasa, Kesari, Chapa
14. Sanskrit: Sandika, Triputi
15. Sindhi: Matter.
16. Telugu: Lamka.

*Lathyrus sativus* (Khesari dal) is a rabi crop sown in October or November in dark loamy soil or sandy loamy soil and harvested in late March or early April. Its cultivation does not require any irrigation; it is a drought-resistant hard crop and hence thrives even under adverse conditions when other crops fail to grow and, therefore, it works as a life-saving crop during famine for the poor community. The plant is a legume and the dal after dehusking resembles Channa dal (Bengal gram) or Tur (Arhar) dal.

Lathyrism occurs because the victims are very poor and landless labourers. Generally they work the whole day in the field and at the day's end the landlord in place of monetary payment (money wage) pays them in the form of Khesari gram to run their livelihood. The labourers are poor and do not have any cooking fuel. They prepare roti or chapati out of the khesari flour and take it with some salt. It is sweet, tasty and heavier than wheat or Bengal gram. So they get into the habit of liking it and eating it. Sometimes the whole crushed pulse is boiled in water and eaten as porridge with a little salt. In fact, labourers feel more satisfaction when he is given khesari dal, in preference to other cereals. When this pulse is eaten in relatively large quantities as staple food, the disease lathyrism occurs. This disease is most prevalent among the unskilled labourers who are more or less attached to the landowners in tribal areas and are at their mercy.

The warning signs of the disease may be apparent in many ways, usually a few days prior to onset of actual paralysis. The individual experiences a sudden agonising pain in the calf muscles at night, after he has been resting and the legs are stretched. The pain is accompanied by a spasmodic contraction of the calf muscles into the shape of a ball or lump, known locally as 'Lodakas'. A similar type of contraction occurs sometimes in the muscles at the back of the thighs. Along with the spasm there is a downward clenching of the toes and feet. The muscles remain in this contracted state for about 10 to 15 minutes, until the spasm passes off either with massage or by itself.

A week or two later, some of the individuals so affected develop the disease, while some others do not. Some of the latter remain in the 'latent stage' of the disease. They do not have the typical signs of physical disability. There is only a slight bending of the knees and awkwardness and difficulty in running or walking downhill. In other individuals, the spasm is infrequently repeated and ultimately stops after several months only when the patient develops the full fledged condition. If the individual stops eating *Lathyrus sativus* after the first muscle spasm, the disease is arrested and he does not develop lathyrism in its full form.

There are several stages of lathyrism.

*The first stage:* The largest number of victims, including most female subjects, are found in the first stage of the disease. It is characterised by a typical manner of walking with short steps and jerky movements, which is an important sign for diagnosis. Owing to varying degrees of stiffness in the muscles of the lower limbs, the heels do not completely rest on the ground while walking, and leave the ground earlier than the loss of the preceding step. Thus the patient walks on toes, with knees slightly bent and ankles extended, resulting in jerky movements and a kind of scissors or crossed gait. At this stage, the patient manages to walk without the aid of a stick. This, therefore, may be called the

Non-Stick Stage. The patient may remain in this condition for the rest of his life. Or, he may pass on to the next stage.

*The second stage:* A patient may go into the second stage directly after the onset. Here all the signs of the first stage are present to a greater degree. The muscular stiffness has increased. There is appreciable bending at knee-joints and raising of heels while walking, This makes it necessary to perform all walking by tilting the pelvis to such a degree that a stick is necessary to maintain balance. This is, therefore, sometimes referred to as the One Stick Stage.

*The third stage:* It is not very common for an individual to go into this stage directly after the onset. The number of patients in this stage are also less than in any of the previous stages. Muscular stiffness is marked and the walking is slow and clumsy owing to considerable bending at knee-joints and crossed gait. The muscular rigidity is so great that the patient needs two sticks for support and there is marked tilting of the pelvis sideways to maintain balance. This is sometimes called the Two Stick Stage.

*The final stage:* A patient may pass through more than one of the previous stages to reach this extreme stage of the disease. By this time the patient is unable to walk upright on account of considerable bending of the knees and extreme stiffness of the lower limbs. Ultimately the legs give way at the knee-joints. The patient is then reduced to crawling or shuffling along by taking his weight on his hands.

Besides many studies carried out to devise simple and practical procedures for the removal of the toxic factor  $\beta$ -N-oxalyl amino-L-alanine (BOAA) from Khesari dal, the following methods may be adopted:

- (a) Cook the pulse in excess of water and drain off the liquid.
- (b) Steep the dehusked seeds in hot water for two hours. This process yields a product that can be easily dried under natural conditions, ground to a flour and then baked into bread. The flour and bread so prepared are not markedly different in appearance and taste from those prepared from untreated seeds.
- (c) The degree of removal of undesirable factors can be increased by soaking the seeds in cold water overnight.

Though there are various methods available for detection of ODAP in admixture but less is reported regarding the quick chromatographic technique of its separation. Especially not much work has been reported for paper chromatography. On the other hand, some TLC methods have been reported in recent years but all these TLC methods are time consuming, tedious and unsuitable for routine analysis<sup>34</sup>. The present method, however, is quite appropriate from all other methods.

## EXPERIMENTAL

All the experiments were performed at room temperature (31°C) and at normal atmospheric pressure in presaturated chambers.

**Reagents and Chemicals:** All the solvents employed were of AR grade while ninhydrin was of BDH. The filter paper was Whatman No. 4 size 18 × 3 cm in rectangular chromatography chamber for ascending chromatography.

Spray reagent was prepared by making (a) 2% ninhydrin in butanol, (b) 10% glacial acetic acid and by mixing 5 parts of A and 5 parts of B. Weighed about 10 g pure *Lathyrus sativus* and ground it well. Admixed Bengal gram flour in 4 different concentrations, viz., 5%, 2%, 1%, 0.5%. Now all the six samples of admixed pulse as well as pure *Lathyrus sativus* and Bengal gram flour were defatted by extracting these with solvent ether. The same process was repeated 3–4 times. The defatted material was kept on a water bath until the etherial portion evaporated. Transferred into a 250 mL round-bottomed flask (fitted with water condenser) having 100 mL of 70% alcohol and refluxed the mixture for approximately 30 min after boiling started. Allowed to cool and settle, filtered by using Whatman No. 4 filter paper, until clear solution was obtained and concentrated it up to 4.5 mL. Spotted approximately 5  $\mu$ L filtrate with the help of a small capillary tube on chromatography paper No. 4 size 18  $\times$  32 cm and allowed it to develop in freshly prepared system-I: butanol : acetone : glacial acetic acid : water (35 : 35 : 10 : 20) until approximately 10 cm of solvent ran over the paper; removed the paper and dried in hot air oven followed by spraying with ninhydrin. Heated it in hot air oven for 2 min at 110°C.

## RESULTS AND DISCUSSION

Bluish violet coloured spots were visible on the chromatogram immediately but an extra purple coloured spot was also observed at about  $R_f$  0.58 as assumed for ODAP while such types of spot were not observed in Bengal gram flour or pigeon pea flour (*Cajanus cajan*)<sup>5-9</sup>. Most of the edible pulses like pea (*Pisum sativus*), black gram (*Phaseolus mungo*) were also examined by this method but only *Lathyrus sativus* gave the spot for ODAP at  $R_f$  0.58 which may be clearly understood with the help of Table-1.

TABLE-1

No. of Sample	% of <i>Lathyrus sativus</i>	$R_f$ *	Average
1.	100	0.59	
2.	5	0.58	
3.	2	0.59	
4.	1	0.58	0.58
5.	0.5	0.58	
6.	0	No spot observed	
7.	ODAP	0.58	

\*Each result is mean of five replicates.

Hence it is concluded that the above described method can be used as a quick routine method for the detection and identification of Khesari in other pulses.

In conclusion, it is emphasized that the following five factors should be stressed in the Education Programme to generate awareness among the common people:

- (a) The cause of the disease and the danger of eating large quantities of *Lathyrus* pulse.
- (b) The fact that *Lathyrus* pulse can safely form not more than a quarter of the total amount of cereals and pulses eaten per day.
- (c) The danger of paying wages in terms of *Lathyrus sativus*. Strict measures against the payment of wages in terms of *Lathyrus sativus* must be enforced.
- (d) Recognition of early symptoms like muscle spasm and the immediate withdrawal of the pulse from the diet. This also applies to persons in the early stages of the disease.
- (e) The need for growing alternative crops in place of *Lathyrus sativus*.
- (f) The extension of Nutrition Programme to areas where lathyrism is endemic should be seriously considered.

### REFERENCES

1. Wealth of India, Raw materials, Vol. VL, p. 37 (1962).
2. D.S. Chadda, Prevention of Food Adulteration Act, 1954.
3. Anonymous, *J. Chromatography*, **8**, D2 (1970)
4. I.M. Hais, K. Meack, Paper Chromatography: A Comprehensive Treatise, Public House Czechoslov. Academy of Sciences. Prague, p. 437 (1953).
5. C. Rukmini, *Indian J. Biochemistry*, **5**, 182 (1963)
6. V.L. Nagarjuna, V.S. Mohan, *Indian J. Med. Res.*, **55**, 9 (1967).
7. D.K. Das, Debi Mukherjee, Arti Saha and J.V. Mathew, *Res. & Ind.*, **19**, 2, 72, 6 (1974).
8. J. Gasparic and J. Churcek, A Hand Book of Paper Chromatography, Elis-Horwood Ltd., p. 176 (1978).
9. I. Chakraborti, *Acta Cienc. Indica*, **18C**, 31 (1992).

(Received: 13 September 2001)

AJC-2551

## 7th Rio Symposium on Atomic Spectroscopy

FLORIANÓPOLIS, SP, BRAZIL

7-12 APRIL 2002

Contact:

Prof. A.J. Curtius

Departamento de Química

Universidade Federal de Santa Catarina-UDSC

88040-900 Florianópolis, SC, Brazil

Tel: (+55-48) 331-9219

Fax: (+55-48) 331-9711

E-mail: [curtius@gmc.ufsc.br](mailto:curtius@gmc.ufsc.br)

URL: <http://www.gmc.ufsc.br/riosymposium>