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Lessons from neurolathyrism: A disease of the past & the future of *Lathyrus sativus (Khesari dal)*

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Neurolathyrism is past history in India since *Lathyrus sativus (khesari dal*) is no longer used as a staple. A consensus has evolved that *khesari dal* is harmless as part of a normal diet. L-ODAP (β-N-oxalyl-l-α-diamino propionic acid) the neurotoxic amino acid, from this pulse, is detoxified in humans but not in animals but still no laboratory animal is susceptible to it under acceptable feeding regimens. L-ODAP is an activator of protein kinase C and consequential crucial downstream effects such as stabilization of hypoxia inducible factor-1 (HIF-1) could be extremely conducive to humans under a variety of situations. ODAP is gradually finding a place in several patents for this reason. Homoarginine the second amino acid from *L. sativus* can be a better substrate for endogenous generation of nitric oxide, a crucial signaling molecule associated with the cardiovasculature and control of hypertension. These features could make *L. sativus* a prized commodity as a functional food for the general cardiovasculature and overcome hypoxic events and is set to change the entire perception of this pulse and neurolathyrism.

Key words Functional food - homoarginine - hypertension - hypoxia inducible factor - *khesari dal* - L-ODAP - neurolathyrism - nitric oxide

Neurolathyrism

The disease neurolathyrism is now history and may never be a challenge in the future in view of the vast public awareness and governmental and infrastructural capabilities. The present review highlights the recent thoughts on this disease and how a certain dignity can be restored to *Lathyrus sativus* (*Khesari dal* also known as grass pea) which indeed has been a saviour for vast populations under severe drought and famine situations in several countries around the world.

A causal relationship between the excessive consumption of *L. sativus* and neurolathyrism, an upper motor neuron disorder characterized by a spastic paraparesis of the lower limbs is well known since several decades[1]. Neurolathyrism has always surfaced only during extreme situations such as famine and drought when other food crops are in short supply resulting in an exclusive consumption of the pulse almost as a staple for prolonged periods lasting as long as 3-6 months. The disease affects only a very small percentage of the population[2] and in its absence large

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populations would have perished. In fact, the disease is generally seen among the poor labourers or a small section of the population. The last recorded episode of the disease is from Ethiopia during the 1995-1997 famine[2] and in India it has virtually disappeared during the last three decades despite its continued cultivation and consumption in several States[3]. Even during the recent Ethiopian famine the disease was well controlled by the timely introduction of food aid cereal supplementation[4]. What has become abundantly clear over the years is the fact that as part of a normal diet like most legumes *khesari dal* appears to be well tolerated and is evidenced by the virtual disappearance of the disease from India during the past three decades. In the entire history of nutrition science there has never been an instance wherein any legume other than *L. sativus* has been examined as a staple for toxicity. The present review is a critical appraisal of some of the more recent developments and emerging thoughts on this legume and its relevance to ground realities of human neurolathyrism and its future role in human health, nutrition and diet.

Experimental neurolathyrism

A causal relationship between the incidence of neurolathyrism and excessive exclusive consumption of *L. sativus* as a staple is undisputed although it affects only a small percentage of the population. Also its utility and safety as part of a cereal based diet or normal balanced diet like any other legume is unquestionable.

Despite innumerable attempts for nearly a century demonstrating neurolathyrism in a laboratory animal model has still remained a far cry. Even with the discovery in the early sixties of L-ODAP (β-N-oxalyl-L-α, β-diaminopropionic acid) as a neurotoxic amino acid in the seeds of *L. sativus* it still remains elusive[5]. Species differences in susceptibility or toxicity to orally administered doses of L-ODAP is still shrouded under mystery[6]. However, the discovery of L-ODAP did provide a breakthrough and fresh outlook of *L. sativus* toxicity. Neurotoxicity of L-ODAP however, is now fairly well established in many *in vitro* systems[7]. The early studies with adult monkeys established that ODAP when administered intrathecally results in a flaccid paralysis but the model was of limited application[8]. Another study conducted in 1977 with four adult monkeys given orally 1g/kg of L-ODAP for 27 days however, failed to establish any toxicity[9]. Spencer *et al*[10] later established that ODAP given orally to adult monkeys under a more complex feeding regimen produced a spastic paraplegia. Recently Kusama-Eguchi *et al*[11] have developed a rat model of neurolathyrism by repeated subcutaneous injection of L-ODAP to neonatal rats although the incidence rate of hind limb paraplegia was extremely low.

There are also other reports trying to establish the toxicity of *L. sativus* in other animals like horses, goats, swine and cattle with variable results[12]. However, most investigations with adult animals to date have always ended up as desperate attempts to establish the toxicity of *Lathyrus* by feeding large quantities of the pulse and or ODAP. The day old chick and C57BL/6J black mice are the only two animals that respond reproducibly to ODAP and hence have been used for several acute toxicological studies[13]. The results from all the chronic animal studies carried out to date with either ODAP or *L. sativus* can safely be surmised and, till date it has not been possible to demonstrate in any laboratory animal signs of paraplegia by mere oral feeding of *L. sativus* or even ODAP within acceptable limits and dosages.

L-ODAP the neurotoxin from *L. sativus* seeds

(i) ODAP and excitotoxicity: ODAP is an unusual compound in that it is one of the most anionic amino acids known and is also a good metal chelator and some of its features especially, in *in vitro* systems may be related to this property[14]. Ever since its discovery more than 30 different chemical, biological and physiological activities of ODAP have been identified and only some are reviewed here.

One of the very early studies by Watkins *et al*[15] had identified ODAP as one of the most excitatory substance in spinal interneurons and Betz cells of cats. Several *in vitro* cell culture studies have established that L-ODAP is an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor agonist (glutamate receptor agonist) and this has been investigated extensively[16]. This excitotoxic theory has been widely held responsible for all the neurotoxic properties of ODAP and has been overhyped to some extent. Although this theory is a highly attractive proposition, but it cannot explain the species differences in susceptibility to ODAP. The non N-methyl-D-aspartate (NMDA) receptors being universal and common to all systems cannot be different between the black mice and white mice or between the chick and the Wistar rat. Also any receptor interaction has to exhibit significant specific binding but L-ODAP fails to show any significant specific binding to glutamate receptors[17]. Also, our own work shows that compounds which are much more potent than ODAP in electrophysiological studies are in fact nontoxic[18]. The excitotoxic theory however,

explains the neurotoxicity of several other compounds such as β-N-methyl-amino alanine (BMAA) the cycad neurotoxin[19].

(ii) Oxidative stress in neurolathyrism: Some studies suggest a free radical oxygen species (ROS) generation as a mechanism of ODAP toxicity in rats following its focal hippocampal application[20]. Also, *in vitro* studies with mouse brain slices treated with ODAP suggest an inhibition of mitochondrial complex I (NADH dehydrogenase)[21]. Although oxidative damage from ROS is a generally accepted mechanism of toxicity, some of the studies with ODAP have not been confirmed by other workers[22].

(iii) Inhibition of tyrosine amino transferase (TAT) by L-ODAP: Most mechanisms suggested for ODAP toxicity fall short of explaining the species differences in susceptibility to ODAP. In this context, the reported inhibition of TAT by ODAP both *in vivo* and *in vitro* suggests an alternative interesting mechanism of neurotoxicity[13]. TAT is the only enzyme identified so far that is substantially inhibited by L-ODAP. C57BL/6J black mice following ODAP treatment show a significant increase in brain DOPA and other chatecholamines[13]. BALB/c mice which normally do not respond to L-ODAP, become susceptible when predosed with tyrosine. It is likely that excessive generation of catecholamine metabolites like 6-OH DOPA which are well known neurotoxins might be the cause for neurodegeneration[23]. Further, 6-OH DOPA is also a non NMDA receptor agonist[24]. TAT inhibition by ODAP can thus explain the species difference in susceptibility to ODAP at least between black and white mice.

(iv) Metabolism of ODAP in humans and animals: The reasons for the very low incidence of neurolathyrism in cohorts subsisting on *khesari dal* have never been explained. Even within a family not everyone is susceptible to neurolathyrism. The fate of orally ingested ODAP from *L. sativus* had not been examined in detail. Since ODAP was not metabolized in experimental animals including primates[25], the same had been extended to humans also without any validation. A study carried out by us in healthy volunteers and subjects normally consuming *L.sativus* established that humans can almost quantitatively detoxify/metabolize orally ingested ODAP in sharp contrast to animals[25]. This established for the first time that humans have a unique ability to metabolize ODAP. This raises the question as to how humans can be susceptible to ODAP toxicity when it is being detoxified while all animals examined so far are not susceptible even when they lack the ability to metabolize/detoxify ODAP. This paradox raises the possibility that may be a few individuals in the population are unable to detoxify ODAP and they may be susceptible to neurolathyrism. However, in a small group of individuals no individual has been identified so far who exhibits this trait and calls for a study involving a larger group[26]. The precise biochemical mechanism of metabolism of ODAP in humans however, remains to be elucidated. Oxalate has been shown to be one of the end products in humans. It is likely that humans alone have a unique ability to completely oxidise ingested ODAP. This could also be the reason for not finding cases of neurolathyrism when *L. sativus* was being used by vast populations as part of their daily diet. A deficiency of sulphur amino acids has been proposed as a contributing factor in precipitating neurolathyrism[27].

Future of *L. sativus* (*khesari dal*) and emerging concepts

Several new concepts have emerged during the last three decades that will shape the future course for *L. sativus* and in a recent conference *L. sativus* had been rightly titled as the golden pulse of the future[28]. Almost all recent research publications emphasize that there is no neurolathyrism when the pulse is consumed as part of a normal diet and this is very true in the Indian context. No legume other than *L. sativus* has in fact ever served as a staple food. Some of the recent findings and developments with *L. sativus* provide some strategic approaches in this direction and will be briefly reviewed. Conventional plant breeding techniques for the development of low ODAP seeds of *L. sativus* made sufficient progress in the last few years but for various reasons this approach has remained an unfulfilled promise[29].

(i) L-ODAP an activator of protein kinase C: Over the years, L-ODAP has been shown to affect a number of metabolic events, but the discovery of the activation of specific protein kinases C (PKC) in the chick brain has opened up exciting possibilities which have a bearing on human health[30]. While PKC activation could result in activation of AMPA receptors some of the downstream effects of PKC activation could have beneficial effects[31]. *In vitro* studies with human neuroblastoma cells show that L-ODAP but not D-ODAP results in nuclear translocation (stabilization) of HIF-1 (hypoxia inducible factor-1)[30]. Since HIF-1 stabilization is the primary adaptive response to lowered oxygen concentrations, the ability of ODAP to affect this opens up an exciting applications for ODAP (and *L. sativus*)

to overcome hypoxic events[32,33]. Most PKC activators are also apoptic and there is a constant search for non apoptotic PKC activators because of their potential therapeutic benefit for a variety of neurological disorders. In general PKC activators possess potent neurotrophic and neuroprotective properties[34]. L-ODAP holds great promise in these applications.

(ii) Homoarginine - the forgotten amino acid from L. sativus: The non protein amino acid L-homoarginine (HA) was the first to be characterized from *L. sativus* seeds even before ODAP, but had been ignored over the years since it did not possess any toxic attributes[35]. HA is now recognized as a normal metabolite in humans and its role in human health is becoming increasingly important. The concentrations of HA in blood increases nearly three-fold during the final semesters of pregnancy[36]. During the last decade, nitric oxide (NO) has emerged as one of the most important signaling molecules both in the cardiovascular system and cerebral metabolism. The health benefits of NO are now universally recognized. Though arginine was initially recognized as the physiological substrate for NO generation, it is now recognized that it can be generated from HA also. HA is in fact, a better substrate for NOS since it results in a sustained NO generation[37]. This makes *L. sativus* a valuable natural dietary source of HA and hence a prized commodity as a functional food. HA in *L. sativus* has certain advantages over arginine since it is a poor substrate for arginase and hence stays in circulation for longer duration and contributes to a healthier cardiovasculature[38]. Following a *L. sativus* meal urinary nitrate level, the ultimate end product of NO metabolism is higher even after 14 h (unpublished data). NO has both direct and indirect beneficial as well as deleterious effects. This skepticism has however, disappeared over the years and the benefits of NO to the vasculature and overall performance is now clinically established beyond doubt[38]. Low circulating HA is now identified as a strong risk factor for sudden cardiac death[39,40]. Several commercial formulations and performance boosting products recommend a daily intake of 8-10 g of arginine. *L. sativus* could thus become an important functional food. As a 'functional food' it could have a vasodilator effect, and may prevent prehypertension from progressing to full-blown hypertension, a major risk factor for heart attacks and strokes.

The importance of HA in human health and wellbeing is growing beyond the boundaries of being a substrate for NO generation. HA is a weak inhibitor of alkaline phosphatase[41]. A recent study shows that in female patients of walking disabilities HA deficiency is associated with increased bone turnover and low bone mineral density (BMD)[42]. HA levels are also inversely correlated with beta crosslaps and osteocalcin[42]. These effects on bone health and metabolism are likely the result of a weak inhibitory effect of HA on alkaline phosphatse.These studies raise the interesting proposition that *L. sativus* as a functional food might contribute to the bone health of the elderly. Arginine is a known insulin secretagogue in the pancreas[43]. However, arginine has a short half life due to the pancreatic arginase. But pancreatic arginase does not act on HA and thus HA may act as a better insulin secretagouge.We have also shown that HA is a good inhibitor of glycine uptake at the synapse (unpublished data) and may thus benefit LTP effects.

Neurolathyrism and *L. sativus* (*khesari dal*) an introspection

This short review is a quick summary down the lane of the past five decades of research on neurolathyrism. Much has been learnt on this "killer pulse" and the dreaded disease and crucial lessons have emerged. We are finally able to tell that *L. sativus* is safe if consumed as part of a normal diet. We have now come to realize that *L. sativus* has tremendous potential as a functional food for a healthy heart and cardiovasculature. ODAP which is also called as dencichine, has already received a US patent for application as a hemostatic agent and recently a Chinese patent also, and more applications of a therapeutic nature are certain to follow in the near future[44-46]. Some States in India like Maharashtra have already lifted the ban on sale of *khesari dal.* A systematic survey of *L. sativus* consuming cohorts by task force teams avoiding a reflex hammer approach should be able to recommend an acceptable daily limit of *khesari dal* for consumption and also identify its health benefits as part of a normal diet. Meanwhile, *khesari dal* can be recommended as a functional food since we have the necessary biochemical, epidemiological and clinical basis for that and this would be the way to move forward. We should be able to exploit fully the nontoxic features of ODAP, the potentials of homoarginine and the agro economic traits of *L. sativus* which would change our entire perception of this disease and remove some of the stigma associated with this pulse. The presence of both homoarginine and ODAP in *L. sativus* makes it a potentially important functional food of great value to human health in areas of cardiovascular physiology, hypoxia and nutrition as well[44]. Once this is realized *L. sativus* will no longer serve as a staple and will be universally accepted as a health food on many counts.

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