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β -ODAP AS AN ANTI-NUTRITIONAL COMPONENT IN *LATHYRUS* SPECIES - A REVIEW

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ABSTRACT

Grass pea or *Lathyrus sativus* is commonly known as Kesari dal in India. An annual crop cultivated for mainly its dried seeds used as a pulse in human foods. It is also used as animal feed and fodder as well as green forage manure. An epidemiological association is very commonly known between kesari dal and lathyrism and the causative agent being β- N- oxalyl-L- α, β-diaminopropionic acid, well known as ODAP. In lathyrism, the paralytic effect occurs due to ODAP toxicity if the consumption of the pulse is more than 75% of the diet intake, whereas, it is rendered safe when taken at 5-30% of the total diet intake. The production of this valuable crop and the bright prospects of grass pea are handicapped by the stigma of its toxicity. Since there is not much data available on ODAP in *Lathyrus sativus* and that the pulse has excellent nutritional qualities, especially, protein apart from taste and acceptability. This review attempts to view on the various aspects of ODAP present in *Lathyrus sativus.* The toxin is identified as β-ODAP whose isolation, characterization was reported by many eminent scientists. The isolation of β-ODAP involved chromatography of the crude extracts of *Lathyrus sativus L*. on Dowex-50 H^+ column followed by elution with water. The initial fractions were discarded and later fractions containing β-ODAP were concentrated and then β-ODAP was precipitated using acetone. In India, several grass pea germplasm of *Lathyrus sativus L.* are present and, among them the edible seeds of plants grown in some States of Bihar, Chattisgarh, West Bengal, Odisha and Andhra Pradesh are most common for the local cuisine.

INTRODUCTION:

Lathyrus sativus has a long history in agriculture but its origin is not known, however its prime centre of origin is southwest and Central Asia (Smartt, 1990). An ancient Hindu treatise '*Bhava-prakasa'* depicted "the triputa pulse causes a man to become lame and it cripples and irritates nerves". Even Hippocrates was aware that certain peas were toxic to human beings. Neurolathyrism is an ancient disease known since the time of Hippocrates who wrote that continuous consumption of peas could lead to `impotence' in the legs (Cohn & Kislev, 1987). The toxicity of various species of *Lathyrus* like *L. sativus*, *L. odorotus*, *L. tuberosus* and *L. latifolius* are known since ancient times. The Encyclopedia of plants, London (1855) records the utility of *L. sativus* was forbidden by an edict of George, Duke of Wattenberg, 1671. In 1671, the Duke of Wurttemberg in Germany issued a decree that prohibited the baking and consumption of bread containing grass pea (Cohn & Streifer 1978). *Lathyrus sativus* - Kesari dal has immense potential as a food, feed, fodder as well as green manure. An epidemiological association exists between the intake of Kesari dal and a motor neural disease named “Lathyrism”-the paralysis of lower limbs in humans. The chief causative agent is the toxic principle identified to be ß –N-0xalye-L-α, ß-diaminopropionic acid (ODAP), well known as ß-oxalyl amino alanine (BOAA). This toxin is present in all parts of the plant (Campbell et al., 1994). The production of this valuable crop and the bright prospects of grass pea are handicapped by the stigma of its toxicity. The paralytic effect occurs because of ODAP toxicity in case the consumption of the seeds grass pea is taken as a staple food, that is, 75% of the diet intake-whereas, it is rendered safe when consumed at 5-30% of the total diet intake. Nevertheless, the presence of a toxic chemical in the seed, which causes paralysis of the limbs when consumed excessively, makes it a threatening crop (Tsegaye *et al*., 2007). Neurolathyrism is an upper motorneurone disease caused by over-consumption of the drought-tolerant grass pea, *Lathyrus sativus*, that contains the glutamate analogue neurotoxin β-N-oxalyl-α, β-diaminopropionic acid (β-ODAP), also known as β-oxalylaminoalanine (BOAA) (Spencer et al. 1986). Due to the presence of toxic principle in *L. sativus*, its cultivation was banned since 1954 under the Prevention of Food Adulteration Act (PFA, 1954). Later in February, 2016, the ban on *Lathyrus sativus* was rescinded. The cultivation of grass pea was banned due to its association with lathyrus in humans in Feb., 1961 as it is believed that Beta-N-oxalyl-aminoalanine, a neurotoxic amino acid in the legume, caused neurolathyrism or a gradual paralysis of the lower limbs and occurs mostly in adults who consume the pulse in large quantities. It is consumed as food, feed and fodder. The seeds are generally hulled and parched for being used as food. It is commonly consumed as soup, sauce, pancake-like unleavened bread, dal, flour, paste balls, fried and roasted seeds, immature pods and young vegetative parts as green vegetable and other such domestic cooked preparations. It is known that the toxin can be removed from the seeds by simply soaking them in hot water and discarding the latter or by consuming it after the removal of its coat. Thus, the toxin is detoxified upto 60% by the above-mentioned methods and to about 90% detoxification can be seen by other processes like fermentation. However an effective ban on the cultivation of *Lathyrus* is not possible in view of the lack of a suitable crop acceptable to the farmers. Thus, it is of interest whether *Lathyrus* itself can be suitably processed so as to free it of the toxin(s) and render it fit for human consumption. In this context, it is also important to estimate and know the nutritive quality of this pulse.

Nutritional Importance of grass pea:

Grass pea is the most drought tolerant legume producing the cheapest protein, but containing a neuro-excitatory amino acid β-ODAP and can give rise to excito-toxicity under certain conditions of prolonged overconsumption, malnutrition and oxidative stress. This same neuro-excitatory amino acid β-ODAP is also identified in seeds and roots of Ginseng (*Panax* ginseng) (Kuo et al. 2003). In Chinese traditional medicine Ginseng root is considered a longevity promoting substance. The haemostatic compound ‘‘dencichine’’ extracted from Ginseng is identical to β-ODAP and has been patented as herbal medicine (Lan et al. 2011). Grass pea is valued for its high protein content (26-32 %), high degree of adaptability under extreme conditions, disease resistance and low input requirement for its cultivation. Despite its tolerance to drought, grass pea is not affected by excessive rainfall and can be grown on land subjected to flooding, including very poor soils and heavy clays (Urga *et al*., 2005). Grass pea has high potential in the provision of high levels of protein, carbohydrates, and minerals for humans. The seeds of *L. sativus* contain 31% protein, 41% carbohydrate, 17% total dietary fiber (2% soluble and 15% insoluble), 2% fat and 2% ash, on a dry matter basis (Akalu, 1998; Aletor, 1994). Grass pea, which contains significantly rich amounts of protein about 20-32% (Castell et al. 1994), is usually cheaper than other pulses; but it is known to cause lathyrism among rural populations. The disease affects the poor sections of a community, especially under conditions of acute food shortage when grass pea, it forms a major part of the diet (Latif et al. 1976). The main limitation is the presence of various anti-nutritional factors and the neurotoxin β-ODAP, which could greatly undermine the potentials (Urga *et a*l., 2005), and is an important food crop in Asia and the Middle East where the whole seed is used in soups and ground to make unleavened bread. In India, Pakistan, Bangladesh and Nepal the most common use of grass pea is a *dhal* (a soup-like dish). In India, the grains are sometimes boiled whole, but are most often processed through a *dhal* mill to obtain split *dhal*. *Dhal* is the most common method of retailing the crop in the Indian subcontinent (Tsegaye *et al*., 2007). In Canada uses would be for high protein livestock feed and as a green manure crop or cover crop. Grass pea is nutritious, rich in protein (28-32 %) and contains good quantities of essential amino acids except the sulfur containing amino acids. In addition to being important source of protein and calories, grass pea is rich in minerals. The seeds have a higher concentration of magnesium and phosphorus followed by calcium (Urga *et al*., 2005). The chemical composition of grass pea may vary according to varieties/ genotype, geographical region of their growing and maturity and environmental factors (soil fertility, nitrogen nutrition, temperature, and water stress and soil pH). There appears to be few studies on the nutritional aspects of grass pea.

Table - Composition of four samples of grass pea seeds

Component	Range
Water (%)	7.5-8.2
Starch (%)	48.0-52.3
Protein (%)	25.6-28.4
Acid detergent fibre (%)	4.3-7.3
Ash (%)	2.9-4.6
Fat (%)	0.58-0.8
Calcium (mg/kg)	0.07-0.12
Phosphorus (mg/kg)	0.37-0.49
Lysine (mg/kg)	18.4-20.4
Threonine (mg/kg)	10.2-11.5
Methionine (mg/kg)	2.5-2.8

Cysteine (mg/kg) --------------3.8-4.3

Source: Rotter *et al*. (1991) The major problem in this endeavor is the high variability of the ODAP biosynthesis. ODAP levels are highly variable and depend on variety, growth location, soil, fertilisation, plant part and age (Gurung et al., 2011; Jiao et al., 2006; Jiao et al., 2011). Environmental factors such as drought, zinc deficiency, iron oversupply and the presence of heavy metals in the soil can considerably increase the level of ODAP in the seeds grown in farmers' fields as compared to more optimal experimental fields. The presence of cadmium in the soil can increase the ODAP level up to six-fold (Lambein *et al*., 2007). Food preparation is also an important factor. Toxic amino acids are readily soluble in water and can be leached. Bacterial (lactic acid) and fungal (tempeh) fermentation is useful to reduce ODAP content. Moist heat (boiling, steaming) denatures protease inhibitors which otherwise add to the toxic effect of raw grass pea through depletion of protective sulfur amino acids. Processing can detoxify grass pea seeds. Steeping and boiling decreased ODAP levels by 90% (Padmajaprasad et al., 1997), while extrusion reduced ODAP levels by 46% (Ramachandran et al., 2004). The food processing methods including soaking, germination, decortications, fermentation and cooking greatly influence the nutritive values of legumes. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and improves palatability, which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al*., 2006). Effect of different processing techniques (Extrusion, fermentation, germination and autoclaving) on the nutritive value of grass pea had also studied (Ramachandran, and Ray, 2008). Different traditional processing methods including roasting, boiling, preparation of sauce and unleavened bread food samples were collected and assayed for β-ODAP levels (Teklehaimanot et al., 1993). The effect of soaking time and soaking solution on the nutritional quality of grass pea seeds were investigated (Urga and Gebretsadik 1993). The effect of cooking, roasting, autoclaving and fermentation on the content of β-ODAP in the whole seeds and flour of grass pea were determined at different levels of temperature, time, pH, degree of soaking and moisture content (Akalu *et al*., 1998). Boiling in water or repeated steeping in hot water and discarding the extracts can detoxify the seeds. Roasting of seeds, at 140ºc for 15 to 20 minutes, result in 80 to 90% destruction of the neurotoxins. Some people soak the seeds overnight and decant the water before cooking. This eliminates about 90% of the toxin. Toxic amino acids are readily soluble in water and can be leached. Fermentation is useful to reduce β-ODAP content. Moist heat (boiling, steaming) denatures protein inhibitors, which otherwise add to the toxic effect of raw grass pea through depletion of protective sulfur amino acid (Rao SLN, 2001). It is a well-known fact that the legume Khesari (*Lathyrus sativus*) causes lathyrism, a disease characterised by paralysis of the lower limbs in human beings. The toxic constituent is an amino acid identified as B-Oxalyl-Amino L-Alanine (BOAA). It has been reported that if the legume is boiled for two hours and the water is then decanted, almost 85% of the toxic amino acid is eliminated. Therefore, this investigation constitutes an effort to prevent the loss of other nutrients, simultaneously to the elimination of toxicity. Lathyrism or neurolathyrism is neurological disease of humans and domestic animals, caused by eating certain legumes of the genus *Lathyrus*. Many *Lathyrus* species are implicated in a paralysis of humans and animals known as lathyrism, which has different causes and symptoms depending on the *Lathyrus* species involved (Hanbury et al., 2000).

Anti-nutritional and toxic factors in grass pea:

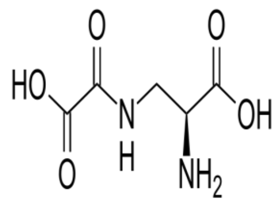
Anti-nutrients have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Francis *et al*., 2001). In common with those of other grain legumes, grass pea seeds contain a variety of anti-nutritional factors. The most frequently occurring anti-nutritional substances in grass pea are protease and amylase inhibitors, lectins, tannins, saponins, alkaloids, phytates, and lathyrogens (Ramachandran and Ray, 2008). The main anti nutritional factors occurring in grass pea include protease inhibitors (trypsin inhibitors), phytic acid, tannins, and β-ODAP. It produces protein energy malnutrition and lathyrism appears frequently. One of the reasons is due to the presence of anti-nutritional factors, which inhibits the digestibility of food and the bioavailability of essential minerals and trace elements. The other more serious reason is that when the grass pea, if consumed by the people as staple food for 3-4 months it causes lathyrism (Malek et al., 1995). Overconsumption of grass pea for an extended period of time can cause spastic paraparesis of the legs in up to 6% of the population, affecting mainly the young males (Strickland GT, 1988). Since grass pea is deficient in cysteine and methionine, and consumption of cereals richer in these amino acids and condiments rich in antioxidants seem to be protective factors (Getahun, 2005), malnutrition and oxidative stress have to be considered as contributing factors in the etiology of neurolathyrism, together with the ingestion of the neurotoxin. The other more serious reason is that when the grass pea, if consumed by the people as staple food for 3-4 months it causes lathyrism (Malek et al., 1995). Overconsumption of grass pea for an extended period of time can cause spastic paraparesis of the legs in up to 6% of the population, affecting mainly the young males (Strickland GT, 1988). Moderate daily consumption of grass pea like other legumes has no deleterious effects, and some authors even mention beneficial effects for human health (Rao SLN, 2011). ODAP is also an ANF and is almost unique to the Lathyrus genus. There are only a small number of published studies of levels and activities of ANFs, other than ODAP, in *L. sativus* (Latif et al., 1975; Deshpande and Campbell, 1992; Aletor et al., 1994; Urga et al., 1995; Srivastava and Khokhar, 1996; Wang et al., 1998)

Lathyrogens

Lathyrogens are toxic compounds found in certain *Lathyrus* plant species, including the flat-podded vetch *(L. cicera),* Spanish vetch *(L, clymenum),* and the *L. sativus*. Lathyrogens include -amino propionitrile and the neurotoxic amino acid-N-oxalyl-L-,-diaminopropionic acid. Consumption of lathyrogens in humans causes a disease called lathyrism; the toxicity symptoms including skeletal lesions, retarded sexual development and paralysis (Tacon, 1995).

β- ODAP:

(Murti *et.al.*, 1964 and Rao *et al.*, 1964) independently isolated the neurotoxic amino acid, β-N-oxalyl-L-α, β-diaminopropionic acid (ODAP) or β-N-Oxalylaino-L-alanin (BOAA), which is suspected culprit of neurolathyrism. 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 (Rao et al., 1964). 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 (Lambein et al., 2007). In the case of *Lathyrus sativus*, the seeds and vegetative parts contain a neurotoxic non-protein amino acid, the ß-oxalyl-L-a,ß-diaminopropionic acid (ODAP or BOAA) (Neurochem Int. 2002).



(Carboxycarbonyl)amino]alanine

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 (Lambein et al., 1994).

Part of the plant	Toxin in mg/100g seeds
Embryo	400
Cotyledons	126
Seed coat	81
Stem	64
Leaf	60
Pod	24
Root	14

In a survey of over 100 accessions from various countries, the ODAP content in grass pea seeds varied from 0.2 to 7.2 g/kg DM (Deshpande et al., 1992). Other studies in Ethiopia reported ODAP content in seeds vary from 5.4 to 8.9 g/kg DM (Urga et al., 2005) or from 2.0 to 4.5 g/kg DM. The green parts and the straw contain lower concentrations of ODAP: 1.9 to 3.4 and 1.3 to 2.1 g/kg DM respectively (Denekew et al., 2009).

Efforts on Detoxification

Recent research suggests that sulfur amino acids have a protective effect against the toxicity of ODAP (Sriram K et al., 1998). Food preparation is also an important factor. Toxic amino acids are readily soluble in water and can be leached. Bacterial (lactic acid) and fungal (tempeh) fermentation is useful to reduce ODAP content. Moist heat (boiling, steaming) denatures protease inhibitors which otherwise add to the toxic effect of raw grass pea through depletion of protective sulfur amino acids. Processing can detoxify grass pea seeds. Steeping and boiling decreased ODAP levels by 90% (Padmajaprasad et al., 1997), while extrusion reduced ODAP levels by 46% (Ramachandran et al., 2004). It was found that water soaking of the seed could lower β-ODAP content but not sufficiently for continuous safe human consumption. Physical and chemical treatments have also been used in the detoxification to induce some mutants. The mutants were not widely used as their characters were unstable or β-ODAP content was not sufficiently low. Further efforts would be a necessary for further improvements (Institute of Tropical Medicine, 2008).

Content of ODAP (mg /100gm of Lathyrus sativus seeds) (Teklehaimanot et al., 1993): **Raw:** 421.18±7.82

Wet roasted: 370.5±3.90

Boiled: 260.0±7.80
Soaked: 222.3±24.70

Physico-chemical and functional properties of starch and fiber in raw and processed grass pea seeds were evaluated (Akalu *et al.*, 1998). The nutritional and anti-nutritional factors of twenty-five grass pea germplasm accessions were analyzed (Urga *et al*., 1995). The proximate composition, mineral contents and anti-nutritional components of local grass pea land-races collected from farmers' fields in different regions had been evaluated (Urga *et al*., 2005). When grass pea is processed, the protein inhibitor and other anti nutritional factors, which inhibit the protein digestibility and chelate the mono, di and trivalent metal ions and form insoluble complexes will be degraded to a smaller molecular form and release the protein and the essential elements. The food processing methods including soaking, germination, decortications, fermentation and cooking greatly influence the nutritive values of legumes. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and improves palatability, which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al*., 2006). Effect of different processing techniques (Extrusion, fermentation, germination and autoclaving) on the nutritive value of grass pea had also studied (Ramachandran, and Ray. 2008). Different traditional processing methods including roasting, boiling, preparation of sauce and unleavened bread food samples were collected and assayed for β-ODAP levels (Teklehaimanot et al., 1993). The effect of soaking time and soaking solution on the nutritional quality of grass pea seeds were investigated (Urga and Gebretsadik 1993). The effect of cooking, roasting, autoclaving and fermentation on the content of β-ODAP in the whole seeds and flour of grass pea were determined at different levels of temperature, time, pH, degree of soaking and moisture content (Akalu *et al*., 1998). Boiling in water or repeated steeping in hot water and discarding the extracts can detoxify the seeds. Roasting of seeds, at 140^{o}c for 15 to 20 minutes, result in 80 to 90 % destruction of the neurotoxins. Some people soak the seeds overnight and decant the water before cooking. This eliminates about 90% of the toxin. Toxic amino acids are readily soluble in water and can be leached. Fermentation is useful to reduce ODAP content. Moist heat (boiling, steaming) denatures protein inhibitors, which other wise add to the toxic effect of raw grass pea through depletion of protective sulfur amino acid (Rao SLN, 2001). It is a well-known fact that the legume Khesari (*Lathyrus sativus*) causes lathyrism, a disease characterised by paralysis of the lower limbs in human beings. The toxic constituent is an amino acid identified as B-Oxalyl-Amino L-Alanine (BOAA). It has been reported that if the legume is boiled for two hours and the water is then decanted, almost 85% of the toxic amino acid is eliminated. Therefore, this investigation constitutes an effort to prevent the loss of other nutrients, simultaneously to the elimination of toxicity. As has been observed, as much as half the protein content, as well as 80.36% total sugars, 63.13% reducing sugars, 86.05% amino acids, and all thiamine, riboflavin and niacin are lost from dhal (dehulled, separated cotyledons), while the respective losses from the whole seeds are 47.25%, 45.73%, 74.69% and 80.00%, and all vitamins, in just a one-hour treatment. The losses of the toxic amino acid from dhal and whole seeds are 71.46% and 68.74%, respectively. The data for losses occurring in the two-hour and three-hour treatment are also described. Small scale efforts have been made on improving the methods for reducing b-ODAP during culinary preparation of grass pea but until now these methods could not prevent the occurrence of these diseases (Teklehaimanot et al., 2005). In relation to β-ODAP, sauce making is the best, followed by boiling and roasting. Traditional processing methods especially boiling and preparing sauce significantly decreased the β-ODAP. However, Teklehaimanot et al. (1993) suggested that it would not be easy to set a standard of β-ODAP content that could be safe for consumption, because there is a large range of variability in β-ODAP concentration in germplasm collection of grass pea. Due to the absence of a well defined toxic level of β–ODAP, we can perhaps rely on the very low levels given in homeopathy to protect against paralysis of the legs (Lambein, 2000).The survey by Getahun et al., 2003) suggested that any addition of spices or condiments to grass pea can make it less toxic. The condiments added to the gravy form may improve the micronutrient balance, and its complex processing may wash out the water-soluble toxin. It was also reported that use of cereal and grass-pea flour mixtures reduced the risk of paralysis if they contained more than a third cereal. The addition of wheat and maize into grass-pea preparations could compensate for the deficiency of methionine and cysteine, as well as diluting the concentration of toxin (Getahun et al., 2003). The initial connection between Zn and neurolathyrism stemmed from the incidence of the disease in soils low or depleted in plant available Zn (Mannan and Rahim, 1988). It has been suggested that Zinc deficiency in the soil leads to a greater expression of the neurotoxin in the seeds, thus increasing the toxic hazards from consuming this food (Lambein et al., 2001). In addition, evidence showed Zn deficiency in the body to be implicated in motor-neuron disease (De Belleroche 1987; Rao SLN, 2001) and that β-ODAP may be a carrier for Zn (Lambien *et al.,* 1994); even though no physiological role of β-ODAP has been identified. Such medical evidence led (Lambien *et al.* 1994) to hypothesize that Zn deficiency can make an individual who consumes grass pea in considerable quantities more susceptible to the toxic action of β-ODAP or can lower the threshold for β-ODAP toxicity (Rao SLN, 2001). The decrease in β-ODAP with added Zn that we observed may be due to a chelating effect of the Zn on β-ODAP reducing its mobility to the grain. Adequate Zn nutrition for acceptable yields of grass pea, the Zn, either from the soil or applied as a fertilizer, may have the additional benefit of partially reducing β-ODAP levels in grass pea and thus making consumption of the crop safer for humans. The major drawback to widespread use of *Lathyrus sativus* as a dietary protein source is the inherent presence of the potent neurotoxin ß-ODAP. The present study indicates a high variability in ß-ODAP levels by area of cultivation. Our results further evidence that the *Lathyrus sativus* seeds are characterized as high neurotoxic varieties in which the level of ß-ODAP is quite variable, between 0.518 to 1.001 mg/100 g seed weight. Although the concentrations of ß-ODAP reported in this study are higher than the results previously reported, it must be emphasized that ß-ODAP levels, even within the same species, may be influenced by environment and agronomic practices. Several methods for the determination of β-ODAP in grass pea were carried out in different laboratories mainly in connection with development of low β-ODAP varieties and for large scale cultivation. Traditionally, different processing methods including roasting, boiling, preparing unleavened bread, sauce and fermentation of grass pea were evaluated for the content of β-ODAP (Teklehaimanot *et al.,* 1993; Akalu *et al*., 1998; Hanbury *et al*., 2000) summarized the result of different researchers of the raw grass pea content of β-ODAP (mg/100g) as 160 to 250 (Spain), 70 to 750 (Syria), 40 to 760 (Australia), 450 to 1400 (Bangladesh), 280 to 1500 (India), 180 to 520 (Chile), and 80 to 990 (China). Other researchers also reported different values: Rotter *et al.* (1991) (130 to 270mg/100g), Urga *et al* (2005)

(618.29 to 1001.49mg/100g), Urga *et al.* (1995) (172 to 354mg/100g) and Grela et al. (2001) (94.8mg/100g) of β-ODAP. The variability in _-ODAP content can be attributed to the different germplasm collection of grass pea and might be influenced by the environment, growing conditions and locality (Campbell, 1997). The reason for this reduction could be leaching of β-ODAP with the water used for soaking and cooking (Akalu *et al.*, 1998). Even though, it was not significantly different from the raw, higher levels of β-ODAP were observed in the unleavened bread (*kitta*) and similar result was reported by Teklehaimanot *et al*. (1993). Compound β1 is a water- soluble amino acid present in the ODAP that can be leached from seed by soaking in water (Mohan et al., 1966; Tekele-Haimanot et al., 1993). Steeping grass pea in a large volume of cold water for 3 min leached out approximately 30% of 1, with greater losers when hot water was employed (Tekele-Haimanot et al., 1993). Similarly, steeping dehusked seed in hot water for several hours and boiling the seed in water removed 70– 80% of the neurotoxin (Moslehuddin and colleagues 1987) also found that washing seed partially removed (Padmaja prasad and associates (1997) reported that boiling grain and discarding the water reduced 1 level up to 90%. Boiling has widely been used in the preparation of *L. sativus* seed as dahl, in bread-making and in vegetable preparations (Kay, 1979). Even though large number of population used to cultivate and consume *L. sativus* because of biophysical factors such as drought, low soil fertility, and water-logging pose severe constraints to production, in addition to many socio-economic factors (poverty, limited health care, etc). (Oliver, 1997). 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. (Surya S. Singh and S.L.N. Rao, 2013). The seeds of grass pea are also reported to contain many anti-nutritional compounds such as tannins, phytic acid, trypsin inhibitors, saponins and oligosaccharides (Ramachandran and Ray, 2008). The anti-nutritional factors like ODAP, phytic acid and tannins were estimated in the processed samples of *L. sativus* collected from different States of India. β-ODAP is the neurotoxic amino acid in the legume, which is the main causative factor the neurolathyrism are a gradual paralysis of lower limbs in adults who consume large quantities of it (Dwivedi MP, Prasad BG., 1964; Getahun et al., 1999; Hanbury et al., 2000; Smulikowska et al., 2008).

CONCLUSION

Finally, it should be stressed that excessive consumption of grass pea is the single most predisposing factor of neurolathyrism. Moreover, it was found that causation of the disease is poorly understood both by the population and by health workers working in neurolathyrism-prone areas (Getahun et al., 2002), wherein the main culprit being β-ODAP present in all the *Lathyrus* species.

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