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# Effect of ionizing radiation on antinutritional features of velvet bean seeds (*Mucuna pruriens*)

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**Abstract**

Impact of gamma irradiation on the antinutritional constituents of seeds of *Mucuna pruriens* was assessed on exposing to doses of 2.5, 5.0, 7.5, 10, 15 and 30 kGy. Except for 2.5 kGy, the rest showed significant dose-dependent increase in phenolics. Tannin concentration did not differ significantly up to 7.5 kGy, while it significantly increased at higher doses. Excluding 2.5 kGy, the rest of the treatments showed significant decreases in the phytic acid and complete degradation was attained at 15 and 30 kGy. The L-DOPA concentration showed a dose-dependent decline. A trace amount of hemagglutination activity was seen on human erythrocytes in raw seeds, which was completely absent on irradiation (>5 kGy). Concentration of Polonium-210, a natural radionuclide falls within the admissible limits for consumption. As *Mucuna* seeds serve as food, feed or as pharmaceuticals, it may be necessary to set the ionizing radiation to a specific dose to retain optimum levels or to eliminate phenolics, tannins, phytic acid and L-DOPA. As irradiation is a physical and cold process, it may be ideal and emerge as an important technique to improve the nutritional or pharmaceutical quality of *Mucuna* seeds and its products.


*Keywords:* *Mucuna pruriens*; Gamma irradiation; Antinutritional factors; Phenolics; Phytic acid; L-DOPA

## 1. Introduction

Legumes are an economically valuable source of protein in the developing countries, wherein animal proteins are scarce. Being rich in proteins, carbohydrates, calorific value, fibre and vitamins, legumes constitute staple food in many countries (Deshpande, 1992). Exploitation of underexplored wild legumes is an important approach to combat the protein-energy malnutrition in developing countries. The nutritional quality and overall acceptability of legumes has been impaired, largely by the occurrence of antinutritional factors (e.g. lectins, phenolics, phytic acid and trypsin inhibitors) (Liener, 1994; Thompson & Erdman, 1982).

Among the wild legumes, the genus *Mucuna* (common name, velvet beans) is widespread in tropical and sub-tropical regions of the world and is considered an alternative protein source. Besides their high nutritional value (Bressani, 2002), seeds of *Mucuna* have great demand in local markets, mainly for the presence of L-DOPA (3,4-dihydroxy-L-phenylalanine), a potential neurotransmitter used in the treatment of Parkinson's disease (Nagashayana, Sankarankutty, Nampoothiri, Mohan, & Mohankumar, 2000; Prakash & Tewari, 1999). The greatest impediment to promotion of *Mucuna* as food or feed is the presence of antinutrients, which are high in contrast to other unconventional legumes (e.g. *Canavalia*, *Sesbania*). The most important antinutritional compounds of *Mucuna* include: phenolics, tannins, lectins, phytic acid and trypsin inhibitors (Ravindran & Ravindran, 1988; Siddhuraju, Vijayakumari, & Janardhanan, 1996).

Hydrothermal treatments, fermentation and germination have been shown to be effective in reducing the antinutrients of *Mucuna* seeds (Siddhuraju & Becker, 2001; Wanjekeche, Wakasa, & Mureithi, 2003). Radiation-processing of food and agricultural commodities has been proved successful to ensure safety and quality and for satisfying consumers' requirements in recent years (Diehl,

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1995; FAO/IAEA/WHO, 1999; Gunes & Tekin, 2006; Mayer-Miebach, 1993). This technology has also been successfully commercialized by governments of about 40 countries (FAO/IAEA/WHO, 1999; Wilkinson & Gould, 1998). No information is available on the effects of radiation-processing to inactivate or to bring down the antinutrients in *Mucuna* seeds. In view of the need to provide baseline data for commercialization and exploitation of this valuable legume, the principle objective of the present study was to investigate the impact of gamma irradiation on some of the antinutritional constituents (phenolics, tannins, phytic acid, L-DOPA and lectins) of *Mucuna* seeds. In view of the accumulation of natural radionuclides in vegetation, cereals and grains and their impact on human health, polonium-210 concentration in *Mucuna* seeds was also assessed.

## 2. Materials and methods

### 2.1. Seeds and properties

About 10 kg of freshly dried seeds of *Mucuna pruriens* L. (DC.) were obtained from local markets of southern India. Seeds were sorted by discarding damaged and immature ones. They were stored in air-tight containers at room temperature (25 ± 1 °C) prior to further use. Weight of 1000 seeds, seed coat and cotyledon were determined gravimetrically. Seed dimensions (length, width, thickness and hilum length) of randomly selected seeds ($n = 20$) were measured using dial calipers. Moisture of the seeds was determined, based on oven-drying (105 ± 1 °C), until a constant weight was attained. Seed samples (control and gamma-irradiated) were powdered (Wiley mill, 30 mesh) for further analysis.

### 2.2. Irradiation

Seed samples (each ~50 g) packed in biaxially oriented polypropylene bags (BOPP, 25 μm) were irradiated at different doses of gamma irradiation (2.5, 5.0, 7.5, 10, 15 and 30 kGy) at room temperature (25 ± 1 °C) using a $^{60}$Co source (dose rate, 6.5 kGy/h) at ISOMED, Bhabha Atomic Research Centre (BARC), Mumbai, India. The absorbed dose was measured by employing Fricke dosimetry (Fricke & Hart, 1966). Packed seed samples without irradiation served as control.

### 2.3. Phenolics and tannins

Total phenolics of the seed flours were assayed after extracting twice with methanol (50%, 5 ml) in a water bath (95 °C, 10 min) (Rosset, Bärlocher, & Oertli, 1982). The pooled extract was made up to 10 ml; the extract (0.5 ml) was mixed with an equal quantity of distilled water and treated with 5 ml $Na_2CO_3$ (in 0.1 N NaOH). After 10 min, 0.5 ml Folin–Ciocalteu's reagent (diluted 1:1 with distilled water) was added and the colour developed was read at 725 nm. Tannic acid served as a standard.

To determine tannins, the vanillin-HCl method (Burns, 1971) was employed. The seed flour (1 g) was extracted with methanol (10 ml, 28 °C, 12 h), vortexed and decanted. This process was repeated and the supernatants were pooled and made up to 25 ml. The extract (1 ml) was treated with reagent mixture (5 ml) (4% vanillin in methanol and 8% conc. HCl in methanol, 1:1). After 20 min, the colour developed was read at 500 nm (Spectronic 21, Miltonroy, India), using catechin (Sigma®) (50–250 μg) as standard.

### 2.4. Phytic acid and L-DOPA

The extraction and estimation of phytic acid in the seed flour was done by adapting standard procedures (Deshpande, Sathe, Salunkhe, & Cornforth, 1982; Sathe, Deshpande, Reddy, Goll, & Salunkhe, 1983). In brief, a known amount of the sample (2 g) was extracted (2 h) with 1.2% HCl (10 ml) containing sodium sulphate (10%) at room temperature (25 ± 1 °C) and centrifuged. The volume was made up to 10 ml with the same extract. Phytic phosphorus was estimated before and after precipitation of phytic acid by $FeCl_3$. Five ml of the above extract were taken and 3 ml of $FeCl_3$ solution ($FeCl_3$, 2 g + concentrated HCl, 16.3 ml, diluted to 1 l) were added, stirred, boiled for 75 min in a boiling water bath, cooled and left at room temperature (1 h) prior to centrifuging (2000*g*, 10 min) and filtered (Whatman #1). The supernatant (made up to 10 ml with distilled water) was used for assay. Analysis of soluble phosphorus was done by the method described by Bartlett (1959), using ammonium molybdate reagent. The absorbance was read at 430 nm after 30 min with $KH_2PO_4$ as standard. Phytate phosphorus was determined by the following formula:

$$\text{Phytate phosphorus} = [A \times 28.18] \div 100$$

where, $A$ = phytic acid.

L-DOPA was analyzed by the protocol described in Fig. 1 (Fujii, Shibuya, & Yasuda, 1991). Powdered seed flour samples were extracted twice with ethanol (50%) including trifluoroacetic acid (TFA) (0.1%). After extraction, the filtrate was collected and concentrated in a rotary evaporator to dryness. The extract was dissolved in distilled water, filtered using an ultrafilter (TOYO ROSHI KAISHA Ltd., Japan) and kept overnight to remove compounds of higher molecular weight. The low molecular weight fraction was further purified on a ODS mini column (C18 Sep-Pak Cartridge, Waters) with 100% water. The extract was concentrated to dryness and L-DOPA was analyzed by HPLC and LC-ESI$^+$/MS. The conditions of the analysis are described in Fig. 1.

### 2.5. Lectins

Hemagglutination activity of lectins was determined by using a trypsin-treated human erythrocyte suspension (A, B, O) (Hankins, Kindinger, & Shannon, 1980) with a slight

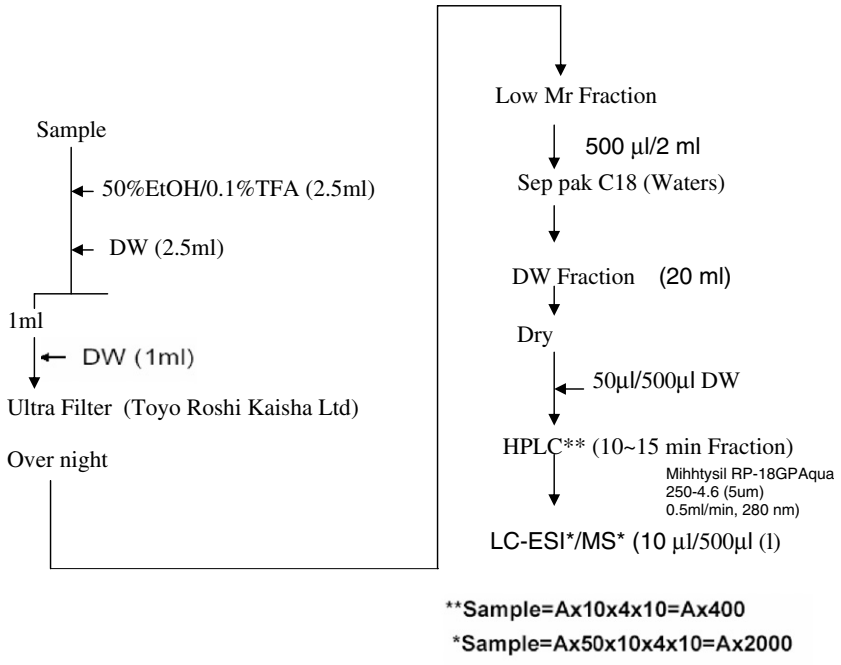


Fig. 1. Stepwise protocol for extraction of L-DOPA and conditions of HPLC and LC–MS.

modification. Blood samples (3 ml) collected from human subjects were directly transferred into a graduated tube containing autoclaved anticoagulant (1 ml) (saturated trisodium citrate), mixed and centrifuged (1000*g*, 5 min, 4 °C). The erythrocytes were washed thrice with phosphate buffered saline (PBS: 10 mM sodium phosphate buffer, pH 7.2 containing 150 mM NaCl) and re-centrifuged. The cells were treated with trypsin (50 µg/ml) [*N*-benzoyl-L-tyrosine ethyl ester (BTEE), 0.04 units/mg solid (Aldrich, 85658-4; purity, 98%)] for 1 h at room temperature (25 ± 1 °C), followed by centrifugation. The treated erythrocytes were washed thrice with excess PBS by centrifugation. The trypsin-treated erythrocytes were suspended in PBS (2%, v/v). For agglutination assay, trypsin-treated erythrocyte suspension in PBS was used according to Hankins et al. (1980). Twenty-five microlitres of the extract (extracted from 50 mg seed flour) were incubated with erythrocyte suspension (75 µl) in microtitre plates, incubated (30 min, 25 ± 1 °C) and examined for agglutination under a low power microscope.

### 2.6. Polonium-210

An electrochemical deposition method, as described by Iyengar, Ganapathi, Kannan, Rajan, and Rajaram (1990) was employed for the measurement of $^{210}$Po in the samples. In brief, a known weight of the dry sample (20 g) was taken in a 500 ml conical flask and leached with 4 N nitric acid (50 ml) to evaporate to dryness. For rapid oxidation of the organic matter, hydrogen peroxide was frequently added, followed by the slow addition of concentrated nitric acid. The process was repeated until a white residue was obtained. The residue was then converted into chloride form by digesting it twice with 1:1 concentrated hydrochloric acid, followed by concentrated hydrochloric acid. After complete drying, the residue was dissolved in 0.5 N HCl and filtered through Whatman filter paper (#42) to remove trace residue present in the solution and taken for $^{210}$Po plating.

The solution was kept on a hot plate-cum-magnetic stirrer for electroplating and temperature was maintained at 90 °C. A brightly polished silver disc (background counted on both sides) was suspended in the solution. The solution was stirred to allow $^{210}$Po to deposit on a background counted brightly polished silver disc by electro-chemical exchange for 6 h. Ascorbic acid was added to the solution, for minimizing the interference of unwarranted compounds (ferric ions) and for better deposition of $^{210}$Po on silver discs. Subsequently, the disc was removed from the solution, washed with distilled water, rinsed with alcohol and dried under an infrared lamp. The dried silver disc was counted on both sides for $^{210}$Po activity in a ZnS (Ag) alpha counter (Electronics Corporation of India Ltd., Bombay, India). From the counts, the $^{210}$Po activity A was calculated using the following formula (Iyengar et al., 1990):

$$A = [S \pm \mathrm{SD}]\frac{100}{E} \times \frac{100}{E_\mathrm{p}} \times \frac{1000}{W} \times \frac{100 - M}{100}\,\mathrm{Bq\ kg^{-1}}$$

where $S$ is net counts per second; SD the standard deviation; $E$ the efficiency (%) of the alpha counter; $E_\mathrm{p}$ the plating efficiency (%) determined using $^{210}$Po standard; $W$ the weight of the dry sample taken for analysis in grammes and $M$ is the moisture (in %) of the sample.

The chemical recovery was 94 ± 2% and counting efficiency was 30%. The background count rate was 0.4 cpm. The efficiency of the detector (α-counter) was determined

using a $^{239}$Pu standard source. The results of $^{210}$Po activity were expressed on wet weight basis.

### 2.7. Statistical analysis

All the data presented are the means of three independent determinations with standard deviation. The statistical significance of data was tested by one-way analysis of variance (ANOVA) using ORIGIN® (version 6.0, Microcal Software Inc., Northampton, MA 01060, USA).

## 3. Results and discussion

### 3.1. Seed features

The seeds of *Mucuna* assessed were black in colour with flat to varied shapes. Mean weight of one thousand seeds, seed coat and cotyledon weights per seed were $441 \pm 7.74$, $0.13 \pm 0.1$ and $0.33 \pm 0.1$ g, respectively. The seed length, breadth and hilum length were $1.23 \pm 0.15$, $0.78 \pm 0.12$ and $0.439 \pm 0.02$ cm, respectively. Variation in physical features of seeds (e.g. size, shape) influences the cooking qualities within the accessions and not all the seeds cook at the same time (Ezeagu, Maziya-Dixon, & Tarawali, 2003; Wanjekeche et al., 2003). Moisture of the seeds was 9.16% (±0.26) and did not show significant loss ($P > 0.05$) after irradiation up to a dose of 10 kGy (Fig. 2). However, in contrast to control seeds, higher doses (15 and 30 kGy) showed significant decrease in seed moisture ($P < 0.05$) (control vs. 30 kGy: 9.16 vs. 8.16%).

### 3.2. 3.2. Phenolics and tannins

In the present study, except for 2.5 kGy, rest of the doses showed a significant dose-dependent increase in total phenolics ($P < 0.05$) (control, 73.4 vs. 30 kGy, 116 g/kg) (Fig. 2). Siddhuraju, Osoniyi, Makkar, and Becker (2002) found increased phenolics in *Sesbania* and green gram (*Vigna radiata*) seeds on soaking, followed by irradiation. They attributed such increase in phenolics to higher extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation. However, irradiation is known to increase the activity of phenylalanine ammonia-lyase, which is responsible for the synthesis of phenolic compounds. For instance, gamma-irradiated (mean dose, 0.3 kGy) Moroccan citrus fruits (*Citrus clementina*) on storage (49 days, 3 °C) enhanced the synthesis of total phenolic compounds, which was directly correlated to increased activity of phenylalanine ammonia-lyase (Oufedjikh, Mahrouz, Amiot, & Lacroix, 2000). In our study, *Mucuna* seeds showed a definite dose-dependent increase in phenolic compounds, indicating enhancement of phenylalanine ammonia-lyase activity rather than mere extractability. Although phenolics are considered as one of the major antinutrients, considerable interest has been recently shown in their possible antioxidant activities and potential health benefits. Epidemiological studies have correlated the consumption of plant produce with high phenolics to reduce cardio-cerebrovascular diseases and cancer mortality (Hertog, Sweetnam, Fehily, Elwood, & Kromhout, 1997).

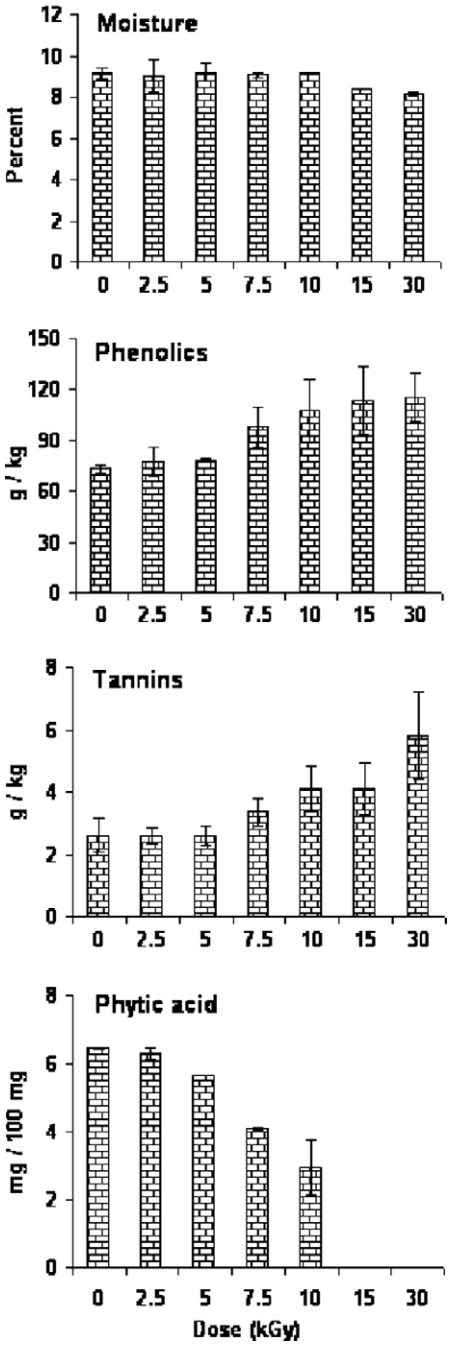


Fig. 2. Radiation dose-dependent changes in moisture and antinutritional features (phenolics, tannins and phytic acid) in seeds of *Mucuna pruriens*.

Our study revealed high tannins in *Mucuna* seeds (2.62 g/kg). Their concentration did not differ significantly ($P > 0.05$) upon irradiation up to a dose of 7.5 kGy, while higher doses (10, 15 and 30 kGy) resulted in significant increase ($P < 0.05$) (control vs. 30 kGy: 2.62 vs. 5.81 g/kg). The variation in tannin concentration in legumes has been reported to range between 3.0 and 15.6 g/kg (Baramp-

ama & Simard, 1994). Elevation of tannins in *Mucuna* seeds by gamma irradiation may be attributed to their higher extractability. Some reports indicate that irradiation decreases tannins in seeds (Abu-Tarboush, 1998; Villavicencio, Mancini-Filho, Delincée, & Greiner, 2000). Such differences may be attributed to the differential response, variability in the genetic constituents (strains and varieties), geographical origin and other biological factors of legumes. Tannins belong to the family of high molecular weight phenolics and are 15–30 times more effective in free radical quenching activity than are trolox and other simple phenolics (Hagerman et al., 1998), indicating their health-benefiting properties.

The total phenolics and tannin concentration of raw *Mucuna* seeds in our study are comparable with some of the earlier observations (Siddhuraju & Becker, 2005; Siddhuraju et al., 1996). Most of the polyphenolics are present in the seed coat, which is dependent on the seed colour. The high phenol and tannin concentrations in our study may be directly related to the black seed coat colour. This view has been supported by earlier observations, wherein black seed *Mucuna* accessions have higher phenolic and tannin contents than have white seed accessions (Gurumoorthi, Pugalenthi, & Janardhanan, 2003). Dehulling of seeds is one way to bring down phenolics and tannins in *Mucuna* seeds. Depending on the nutritional or health benefits, a decision should be made to eliminate or to retain phenolics in adequate quantities in *Mucuna* products in food or pharmaceuticals. If the consumers prefer food products or preparations made out of *Mucuna* seeds to be less astringent, the seeds should be dehulled before further processing.

### 3.3. Phytic acid and L-DOPA

Except for the seeds irradiated at 2.5 kGy, the rest of the samples showed a significant decrease ($P < 0.05$) in the phytic acid between control (6.47) and irradiated seeds (10 kGy, 2.95) (Fig. 2). Phytic acid of seeds was completely eliminated on exposure to doses of 15 and 30 kGy. Decrease or elimination of phytic acid has been attributed to the low inositol and inositol phosphates by the action of free radicals generated during irradiation (De Boland, Garner, & O'Bell, 1975). Duodu, Minnaar, and Taylor (1999) indicated that phytic acid degradation by radiation is due to cleavage in the structure of phytic acid itself, which may also be true in the present study. The dry heat treatment has also been shown to reduce phytic acid in *Mucuna*-seeds from 47% to 36% (Siddhuraju et al., 1996).

Phytic acid in legumes has been reported to lower the nutritional value by limiting the bioavailability of minerals and essential trace elements (Gustafsson & Sandberg, 1995; Ryden & Selvendran, 1993). Recent reports indicate that, although phytic acid is a major antinutrient, it has rich antioxidant, anticarcinogenic and hypoglycemic activities (Graf & Eaton, 1990; Rickard & Thompson, 1997; Shamsuddin, Vucenik, & Cole, 1997) and can benefit health if present in low concentrations. Thus, the utilization of *Mucuna* seeds or its products as a source of phytic acid (antinutrient or health-promoting phytochemical) solely depends on the consumer's preferences.

The L-DOPA of *Mucuna* seeds in the current study showed a dose-dependent decline. The decrease was significant ($P < 0.05$) and was in the order of $5.15 < 4.38 < 4.13 < 3.76 < 3.40 < 2.69 < 2.62\%$ in control, 2.5, 5, 7.5, 10, 15 and 30 kGy samples, respectively (value of 3 independent determinations). *Mucuna* seeds, with a high amount of L-DOPA, may suffer in their utilization as food and feed (Carsky et al., 1998; Flores, Esnada, & Myhrman, 2002). In around 36 accessions of *Mucuna* seeds, L-DOPA varied between 2.2% and 7.2% (Lorenzetti, MacIsaac, Arnason, Awang, & Buckles, 1998). Variations of L-DOPA in *Mucuna* seeds have been attributed to the environmental conditions (e.g. latitude, intensity of light, nitrogen source) and genetic control (Lorenzetti et al., 1998; Wichers, Wijnsma, Visser, Malingre, & Huizing, 1985). Various processing methods have been employed by researchers to reduce the L-DOPA of *Mucuna* seeds, wherein the methods employed were based on the use of water, chemical and thermal treatments (Diallo et al., 2002; Wanjekeche et al., 2003).

Earlier studies have indicated the toxicity of L-DOPA of *Mucuna* seeds without substantial scientific evidence. Nechama and Edward (1967) have attributed the toxicity of L-DOPA consumption only to those individuals having deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) in their erythrocytes. In fact, L-DOPA has been consumed for a long time by patients suffering from Parkinson's disease and its adverse effects are not available to unequivocally prove its toxicity. To eliminate L-DOPA from the nutritional point of view, the irradiation process is desirable and comparable with earlier reports. It may be possible to set the ionizing radiation to a specific dose to retain optimum levels of L-DOPA in *Mucuna* seeds for desired nutritional or pharmaceutical purposes.

### 3.4. Hemagglutination activity

*Mucuna* seeds exhibit trace amounts of hemagglutination activity in human blood groups. Irradiated seed samples, except for the 2.5 kGy, did not show hemagglutination activity. Seeds exposed to 2.5 kGy showed a slight hemagglutination activity, wherein a grainy (++) nature of the erythrocytes was seen. Relatively, blood group O in our study exhibited a much lower activity than did other blood groups and this observation is in agreement with earlier studies (Siddhuraju et al., 1996; Vijayakumari, Siddhuraju, & Janardhanan, 1996). Liener (1994) has reported the elimination of lectins in leguminous seeds by dry and wet thermal treatments. *Mucuna* seeds subjected to heat-treatment and autoclaving also showed decreased hemagglutination activity of human erythrocytes (A, B, O) (Siddhuraju et al., 1996). Lectins are known to inhibit digestive enzymes and reduce protein digestibility (Thompson, Tenebaum, & Hui, 1986).

### 3.5. Polonium-210 activity

The $^{210}$Po activity was not great in *Mucuna* seeds in our study ($0.74 \pm 0.6$ Bq/kg wet mass) and fell within admissible limits (UNSCEAR, 1993) This indicates that it is safe for consumption. It is notable that irradiation of *Mucuna* seeds up to 30 kGy did not alter the $^{210}$Po concentration. The alpha emitter, $^{210}$Po, is one the naturally occurring radionuclides with a $^{238}$U daughter product having 138 days of radiological halflife. Among the different types of natural radionuclides in the environment, $^{210}$Po is as hazardous as plutonium and five times more toxic than $^{226}$Ra (McDonald, Fowler, Heyraud, & Boxter, 1986). Presence of some of these radionuclides in vegetation or edible plant produce (e.g. beans, rice and vegetables) has been proved to be harmful as they are biomagnified through food chains (Bhat, Sridhar, Rajashekar, & Narayana, 2005).

## 4. Conclusions

*Mucuna pruriens* seeds showed significant dose-dependent increase in phenolics on gamma irradiation, except for 2.5 kGy. Tannins in seeds did not differ significantly up to 7.5 kGy, but a significant increase was seen at higher doses. In view of possible antioxidant activities and potential health benefits, phenolics and tannins in *Mucuna* seeds have to be treated with caution. Significant decrease in the phytic acid, between control and irradiated seeds, was seen, except for seeds exposed to 2.5 kGy. Seeds exposed to 15 and 30 kGy were devoid of phytic acid, while L-DOPA in seeds showed a dose-dependent decline. Hemagglutinating activity of seeds on human erythrocytes was present in traces and the Polonium-210 activity falls within the admissible limits of consumption. In view of the importance of *Mucuna* seeds as food, feed or pharmaceuticals, it may be necessary to set the ionizing radiation to a specific dose to achieve optimum benefits or to eliminate phenolics, tannins, phytic acid and L-DOPA. As irradiation is a physical and cold process, it may emerge as one of the important techniques for preserving or improving the nutritional or pharmaceutical quality of *Mucuna* seed or its products.

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