Short Communication

Effect of Solid State Fermentation and Other Processes on the Lathyrus sativus (Kheshari Dal) Toxin by Chick Bioassay

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Introduction

The importance of legumes in human diet is well recognised. Legumes are the main sources of protein in the 3rd world countries. However, legumes intake is a problem due to the presence of antinutritive factors and toxicants[1]

In Bangldesh and India, Lathyrus sativus legume grows abundance even in adverse condition. Although these seeds contain toxic compounds, it is a principal, dietary source in these countries[2]. When people eat these legumes continiously over a period of two or three months, they develop a disorder involving nerves termd 'Lathyrism[3].

The actual neurotoxic compound of Lathyrus sativus was isolated by Rao [(4)] and identified it as βN-Oxalyl-L-α, β diamino-propionic acid (ODAP) which is responsible for human Lathyrism. According to Padmanaban[1], ODAP is probabl the main neurotoxin compound in the Lathyrus sativus seeds.

There have been reports that different porcessing and cooking procedures remove the neurotoxins in Lathyrus sativus seeds. Cooking the seeds for 30 minutes in excess water and draining or steeping the dehuskd seeds in hot water with prior soaking overnight, most of the toxic factors present are removed[5]. Soaking seeds is saturated lime water for overnight, followed by boiling for 25 minutes eliminate the seed toxin. Autoclaving seeds soaked in lime for 10 minutes compltely destroyed the Lathyrus toxins. Toxin analysis was done by paper chromatography in these studies[6].

The objective of this study was to process Lathyrus sativus seeds by various methods for neurotoxin removal or destruction using less concentrateed toxin (0.5 ml) instead the sensitive chidk of 1 ml and bioassay was done to evaluate the effectiveness of these processing treatments[7].

Materials and Methods

Fermentation and extraction of Toxins from the treated seeds.

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Sample of Lathyrus sativus seeds were obtained from the local market. After receiving the seeds, undesirable matters were removed manually. The seeds were than dehulled by burr mill in dry condition so that molds cannot grow on the dehulled seeds. After removing the seeds coat, the seeds were kept at room temperature. At the regular periods the seeds were examined for the presence of moldy or damaged seeds. Before fermentation, the seeds were soaked in water overnight (seeds and water ration being 1 : 3). After soaking water was drained off, washed and cleaned. Preparation of Tempeh (solid state fermented product) was followed by the modified method of william Shurtlef and Akiko Aoyagi (1985)[8]. The seeds were then steamed for 10 minutes to remove some of the Microflora. Then the seeds were dried at oven for removal of excess water for one hour. After removal of excess water the seeds were inoculated with 0.5% inoculum of Rhizopus oligosporus (Tempeh inoculum) obtained from the Nutrition Reesearch Development Centre at Bogor, Indonesia. The inoculated seeds were packed in petridishes and incubated at 30°C for 24 hours. After incubation, the fermented seeds were kept at 0°C.

The raw dehulled seeds (50 gram portions) were powdered and blended with 300 ml of distilled water for 10 minutes. Extractions of mixture were made by heating and stirring for 15 hours. After extraction, the mixture was centrifuged at 3,000 rpm for 20 minutes in a centrifugee and th supernatant was decanted. The residue was extracted again and combined with the first extraction after decantation. the decanted supernatant was then concentrated to 12.5 ml for an appropriate toxin concentration of 20 mg/ml (4) by rotatory evaporator (E Corning, England). The concerntrated raw seed extracts was then adjusted to p 6.8 with 40% NaOH.

A second batch of the seeds was soaked with 150 ml of water overnight, washed twice, steamd for 10 minutes and extracted as above. A third batch of the seeds was soaked and washed twice and steamed for 10 minutes and inoculated with 0.5% tempeh inoculum (Rhizopus oligosporus). A 4th batch of seeds was soaked overnight and washed five times, extracted as before. A fifth batch of seeds was soaked overnight and washed five times and inoculated with the incluum of Rhizopus oligosporus Packed with petridish and inoculated for 30 hours at 30°C[7]. the sixth batch of seeds was soaked in saturatd lime water and was extracted as before. The seventh batch of seeds was soaked in saturated lime water and autoclaved for 10 minutes, was extracted as

before. The lime was purchased from local market of Dhaka. All the seeds of different batchs were extracted and concentrated as before and kept for Bioassay of chicks.

Seeds of all th batches were extracted by the same procedure to same volume. For toxicological evaluation of the seven seeds extracts from different processing procedures, a one day old chick bioassay was accomplished[7]. Male white chick weighing 30—35 gms were obtained from the Dept. Of Poultry, Mirpur, Dhaka. The chicks were divided into eight groups and were individually ip injected with 0.5 ml of the seed extracts. Each chick in group one was injected with 0.5 ml of sterile water (Control) by (ip) injection. Group two received extracts of raw seeds. Group three received extracts of soaked overnight and washed twice. Group four received extracts of soaked overnight, washed twice and fermented with Rhizopus oligosporus for 30 hours at 30°C. Group five received extracts of soaked overnight and washed five times. Group six received extracts of soakd overnight and washed fiv times and fermented as before. Group seven received extracts of lime water soaked seeds for overnight. Group eight recieved extracts of lim water soaked an autoclaved seeds. All samples after soaking was steamed for 10 minutes. Chicks were maintained on chick starter (Dhaka, Bangldesh) Placed in an electrically heated room at 35°C.

Results

As seen in Table 1, sterile water injected (ip) into each chick produced no symptoms of neurotoxicity. The percentage of symptom observeed was 0.00% from 0 to 3 hours. Injection of raw extracts from the raw seeds showed symptom within 30 minutes. Percentagee of symptoms observed 100. Injection of extracts from soaked and washed twice showed symptom 75% within 30 minutes and 100% within 3 hours. Injection of extracts from soaked, washed twice and fermented seeds showed symptom 50% withing 30 minutes and 75% within 3 hours. Treating chicks with the extracts of seeds soaked overnight and washed 5 tims showed symptom 75% within 30 minutes and 3 hours. Treating chicks with the extracts of seeds soaked overnight washed five times and fermented showed 0.00% symptom of toxicity within 30 minutes and 3 hours. Treating chicks with extracts from soaked overnight with saturated lime water showed symptom of neurotoxicity 75% within 30 minutes and 100% within 3 hours. Chicks treated with the extracts of seeds from lime water soaked and autoclaved showed 100% symptom of neurotoxicity in both in 30 minutes and 3 hours.

Tabl 1. Lathyrus sativus seeds toxin bioassay on chicks.

Treatments.	Number of Chicks assayed	(Number of chicks) Toxicity symptoms observed with in 30 minutes	Percentage (%)	Number of chicks) Toxicity symptoms observed within 3 hours.	Percentage (%)
1. Sterile water (Control)	4	0	0.00	0	0.00
2. Raw seed extracts	4	4	100	4	100
3. Extracts of seeds soaked & washed twice	4	3	75	4	100
4. Extracts of seeds soaked and washed twice & solid state fermented	4	2	50	3	75
5. Extracts of seeds soaked washed 5 tims and solid state fermented	4	3	75	3	75
7. Extracts of seeds soaked in saturated lime water	4	3	75	4	100
8. Extracts of seeds soaked in saturated lime water and autoclaved.	4	4	100	4	100

Discussion

As seen in Table 1, processing the extract of seeds by soaking twice and five tims showed removal of some toxins which was also observed by Moslehuddin et al. (1987) (7). Solid state formentation for 30 hours at 30°C showed some protection from the toxins. The percentage of neurotoxic symptom observed after the treatment of the chicks with the extracts of soaked, washed twice and solid state fermented seeds was 50% withing 30 minutes. Soaked twice showed only symptom 75%. It means that fermentation has some effect on the toxicity of the seeds. Moreover, the percentage of toxicity symptoms observed after the treatment of chicks with the extracts of soaked, washed five tims and solid state fermentation of the seeds is 0.00% in both 30 minutes and 3 hours. This means that solid state fermented seeds after five times washing showed complete reduction of the neurotoxins. On the other hand, washed five tims of the seeds also has some protection from the

neurotoxic symptoms observed in the experiments.

Soaking in saturated lime and autoclaving the seed extracts exhibited no protection. From thee observation of lime treatment, it is clear that chicks showed toxicity symptom 100% both in 30 minutes and 3 hours after injection to chicks.

In contrast to the previous reports (5,6) where by soaking in lime and autoclaving removed or destroyed the neurotoxins in the Lathyrus sativus seeds, our results using the chick bioassay with the same processing methods did not indicate a protective effect. On the otherhand, washing showed some effect on removal of some neurotoxins which was found by Mohan et al (1966) (5) and solid state fermentation also showed promising results in removal or destroying the neurotoxins present in the seeds. Further studies on this method ar essential to make the seeds free from toxicity and measuring the toxicity by developing instrumental analysis to see specific changes during processing.

Summary

Lathyrus sativus seeds were processed by different methods for removal or destruction of their natural toxins. Toxins were extracted from the seeds, partilly purified and injected intraperitonally into one day old male chicks as a sensitive bioassay for th effectiveness of th processed seeds. The chicks showed typical neurological symptoms such as head retraction, neck bending after injection of the concentrated extracts. Similar symptoms were observed in chicks injected with the extracts of seeds soaked overnight in water or in saturated lime followed by steaming, autoclaving and fermenting at 30°C for 30 hours. Toxicity symptoms were observed to all groups of the experiment except 1 and 6. Extracts from soaked overnight, washed five time showed 75% and two tims showed symptom 75% and 100%. Soaked 5 times and fermented for 30 hours showed symptom 0.00% and control containing sterile distilled water showed 0.00% symptoms. Washing twice and five tims have some effeect on reducing toxicity in the seeds and solid state fermentation also showed some reducation of toxicity from seeds. Lime water treatment and autoclaving appeared to have little effect on toxin removing or destroying.

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