

Analysis of Antinutritional Substances and Improvement in the Protein Digestibility of Sword Bean (*Canavalia gladiata* Jacq.) Seeds during Processing

A Doss, M Pugalenth, V Vadivel*, G Subhashini** and Anitha Subash**

Department of Botany, Government Arts College, Coimbatore - 18, Tamil Nadu, India

*Department of Biotechnology, Karpagam University, Coimbatore - 21, Tamil Nadu, India

**Department of Biotechnology, Biochemistry and Bioinformatics,

Avinashilingam Deemed University for Women, Coimbatore - 43, Tamil Nadu, India

e-mail: pugalmuthiah@rediffmail.com

ABSTRACT

Cost-effective processing methods were studied to remove the antinutritional compounds in red and white sword bean (SB) (*Canavalia gladiata* Jacq.) seeds. Soaked and soaked and autoclaving effectively reduced the total free phenolics (69-76%), tannins (53-71%), L-Dopa (73-78%), Phytic acid (55-80%), oligosaccharides such as raffinose (74-80%), Stachyose (73-84%) and verbascose (79-88%), trypsin inhibitor activity (57-77%) and also improvement in the *in vitro* protein digestibility (90-98%) in white and red SB seeds. Adoption of this processing method will enhance the utilization of SB and provide alternative/additional protein source for both human beings and animals.

Key words: *Canavalia gladiata*, Processing methods, Antinutritional compounds, *In vitro* protein digestibility

Sword beans (*Canavalia gladiata*) are one of many underutilized but exceptionally productive, large-seeded tropical legumes. It has many desirable agronomic features such as high biomass production, resistance to drought, pest and diseases, high fertility index and high seed productivity (800-1000 kg/ha) on fertile land, which enable them to grow well under tropical conditions. In India, the SB seeds are consumed by certain ethnic groups and poor village people. In Asia, the young pods and seeds of SB are used as a green vegetable. Immature pods are consumed as a green vegetable in Sri Lanka and boiled seeds are widely consumed by tribal and village people in Southern India (Siddhuraju and Becker 2001).

The nutritive value and protein quality of SB seeds seems to be similar to that of most of the edible legume grains and hence, they are advocated to be a good source for extending protein-sources. Despite the desirable nutritive features, the SB seeds are not extensively utilized as food/feed mainly due to the presence of certain antinutritional compounds. Presence of antinutritional compounds such as total free phenolics, tannins, Concanavalin A (Con A) lectin, L-Canavanine (a non-protein amino acid), phytic acid, oligosaccharides, protease inhibitors and [alpha]-amylase inhibitors were reported in the raw SB seeds (Pugalenth *et al.* 2010). Although, few reports are available on the nutritional value and antinutritional compounds of SB seeds, only limited information is available on the effect of certain common processing methods on the levels of nutritional and antinutritional profiles of SB seeds. Identification of suitable processing method will enhance the opportunities for the

versatile utilization of SB seeds as an alternative/additional and economic source of protein in the diets of human beings/animals. Hence, the present study was carried out to evaluate the nutritional value, antinutritional profiles and protein quality of raw and differentially processed SB seeds collected from South India with a view to identify a viable processing device, which will remove the maximum levels of antinutritional compounds without affecting the nutritive quality of SB seeds.

MATERIALS AND METHODS

Collection of the seed sample

White and red seeds of sword bean (SB) were collected from Kuppenatham (red coat), Madurai district and Arachalur (white coat), Erode District, Tamil Nadu, India, from the natural stands. Soon after collection, after removing immature and damaged seeds, the mature seeds dried in the open sun for 2 days and stored in plastic containers in refrigerator (5°C) until further use.

Processing methods

Soaking (T₁): The whole seeds of SB were soaked in distilled water for 4h in the bean water ration of 1:10 (W/V). After soaking, the water was drained off and the seeds were dried at 55°C for 6 hours in a hot air oven.

Cooking (T₂): Another set of seeds was cooked in distilled water (100°C) in the bean water ration of 1:10 (W/V) for 40 minutes. The cooked seeds were rinsed with distilled water and dried at 55°C for 6 hours in a hot air oven.

Autoclaving (T₃): Separate batches of seeds of SB were autoclaved at 15 lb pressure (121°C) in distilled water in the

bean water ratio of 1:10 (W/V) for 30 minutes. After treatment, the seeds were rinsed with distilled water and dried at 55°C for 6 hours in a hot air oven.

Germination (T₄): The seeds of SB were subjected to germination for 24 hours in a moist filter paper in a petri dish to reduce the antinutritional factors. The treated seeds were powdered and analyzed for various antinutritional factors.

Roasting (T₅): The seeds of SB were subjected to roasting for 20 minutes along with sand at 110°C to reduce the antinutritional factors.

Soaking in distilled water + Autoclaving (T₆): The seeds soaked in water for 2 hours were subjected to autoclaving for 30 min at 15 lb pressure (121°C) in the bean: water ration of 1:10 to reduce the antinutritional factors. The treated seeds were powdered and analyzed for various antinutritional factors.

Soaking in distilled water + Cooking (T₇): The seeds soaked in water for 2 hours were cooked for 30 minutes in distilled water in the bean: water ration of 1:10. The treated seeds

were powdered and analyzed for various antinutritional factors.

Analysis of antinutritional compounds

The total free phenolics and tannins of raw and processed seeds were extracted and estimated by the method of Sadasivam and Manickam (1992), Burns (1971), respectively. The L-Dopa content was quantified according to the method of Brain (1976), whereas, the phytic acid content was determined by following Wheeler and Ferrel (1971) method. The oligosaccharides were extracted by following the method of Somiari and Balogh (1993) and estimated according to the Tanaka *et al.* (1975). The haemagglutinating activity (HAA) was measured according to the Liener (1976) and the trypsin inhibitor activity (TIA) was determined by Sadasivam and Manickam (1992). The *in-vitro* protein digestibility (IVPD) of both the raw and processed samples was determined by the following the multienzyme technique (Hsu *et al.* 1977).

Table 1. Effect of various treatments of white and red seeds of Sword bean on total free Phenolics (TFP) and Tannins content

Treatments	White seed		Red seed	
	*TFP	**Tannins	*TFP	**Tannins
Raw seeds	4.08 ± 0.07	95.0 ± 4.3	4.10 ± 0.10	119.1 ± 12.8
T ₁	2.83 ± 0.01 (30.63%)	55.6 ± 0.28 (41.47%)	1.48 ± 0.01 (63.90%)	70.16 ± 0.28 (41.09%)
T ₂	1.81 ± 0.02 (55.63%)	56.16 ± 1.15 (40.88%)	1.78 ± 0.02 (56.58%)	75.83 ± 1.40 (36.33%)
T ₃	1.66 ± 0.05 (59.31%)	29.33 ± 0.28 (69.12%)	1.28 ± 0.02 (68.78%)	56.0 ± 0.5 (52.98%)
T ₄	1.26 ± 0.03 (69.11%)	56.5 ± 0.00 (40.52%)	1.05 ± 0.011 (74.39%)	71.5 ± 0.86 (39.96%)
T ₅	2.25 ± 0.01 (44.85%)	59.16 ± 0.57 (37.72%)	1.41 ± 0.028 (65.60%)	74.83 ± 0.28 (37.17%)
T ₆	0.95 ± 0.01 (76.71%)	27.3 ± 0.5 (71.26%)	0.95 ± 0.05 (76.82%)	53.2 ± 0.35 (55.33%)
T ₇	2.41 ± 0.01 (40.93%)	28.7 ± 1.0 (69.78%)	1.03 ± 0.063 (74.87%)	55.83 ± 0.28 (53.12%)

*All the values are mean of triplicate determination expressed on dry weight basis ± Standard Error

*g/100g of seed flour, **mg/100g of seed flour

RESULTS AND DISCUSSION

Total free phenolics (TFP) and tannins

The raw white and red seeds of SB contained 4.0-4.1% of TFP and 95-119 mg/100g tannins (Table 1). The TFP content (4.0-4.1%) of SB seeds was found to be lower when compared to the TFP content of certain common legume seeds such as *Sesbania sesban* (5.95%) (Hossain and Becker 2001); *Mucuna pruriens* var. *utilis* (9.7%) (Vadivel and Pugalenthi 2007), *M. pruriens* (7.75%), *M. cochichinensis* (6.53%) (Adebowale *et al.* 2005) and *Entada scandens* (6.23%) (Vadivel *et al.* 2008). The tannin content is comparable with red gram (97 mg/100g), Bengal gram (102mg/100g) and lentil (134mg/100g) (Salunke *et al.* 2006).

The reduction in TFP (76%) and tannins (55-71%) of white and black SB seeds in T₆ might be due to either the increased leaching out of phenolic substances or degradation by heat (Pugalenthi *et al.* 2005). The loss of phenolics and tannins during autoclaving might be due to leaching into the soaking medium and degradation by heat under pressure. In nutritional point of view, the content of both total free phenolics and tannins are not desirable for human

consumption. Phenolic compounds were reported to decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals (Liener 1994). They also have decreased the activity of digestive enzymes such as α -amylase, trypsin, chymotrypsin and lipase and may cause damage to the mucosa of digestive tract and also reduced the absorption of nutrients such as vitamin B₁₂.

L-Dopa

The raw red and white seeds of SB contained 3.45-4.1% (Table 2). These values are higher when compared to certain under-utilized legume seeds such as *Cassia floribunda* (1.6%); *Cassia obtusifolia* (1.3%) and *Canavalia ensiformis* (2.6%) (Vadivel and Janardhanan 2005). The maximum removal of L-Dopa content in both red and white seeds (77-78%) of SB was observed in T₆, which might due to increased leaching out and degradation by heat. This is in agreement with the earlier study by Siddhuraju and Becker (2003).

Phytic acid

The raw red seeds of SB contained higher level of phytic acid (949 mg/100g) than the white seeds (411

Improvement in the Protein Digestibility of Sword Bean

mg/100g) (Table 2). T₃, T₅ and T₇ exhibited 34-68% of loss of phytic acid and the maximum reduction (60-80%) was observed in both white and red SB seeds in T₆. The apparent decrease in the content of phytic acid during autoclaving

might be partly due to the leaching into the soaking medium or degradation of inositol hexaphosphate into penta and tetra phosphates by heat under pressure (Vijayakumari *et al.* 2007).

Table 2 Effect of various treatments of white and red seeds of Sword bean on L-Dopa and Phytic acid content

Treatments	White seed		Red seed	
	*L-Dopa	**Phytic acid	*L-Dopa	**Phytic acid
Raw seeds	4.16 ± 0.1	411.6 ± 18.5	3.45 ± 0.8	949 ± 10.3
T ₁	2.49 ± 0.03 (40.14%)	344.3 ± 8.0 (16.35%)	1.36 ± 0.10 (60.57%)	553.5 ± 9.83 (41.67%)
T ₂	2.76 ± 0.02 (33.65%)	252 ± 5.40 (38.77%)	2.48 ± 0.02 (28.11%)	433 ± 4.33 (54.37%)
T ₃	1.06 ± 0.02 (74.51%)	185 ± 8.78 (55.05%)	0.916 ± 0.087 (73.62%)	365.5 ± 8.66 (61.48%)
T ₄	1.28 ± 0.141 (69.23%)	356 ± 3.77 (13.50%)	1.07 ± 0.043 (68.98%)	568 ± 4.33 (40.14%)
T ₅	2.00 ± 0.00 (51.92%)	208 ± 4.33 (49.46%)	1.28 ± 0.141 (62.89%)	382.5 ± 7.5 (59.69%)
T ₆	0.875 ± 0.108 (78.96%)	164.3 ± 6.82 (60.08%)	0.78 ± 0.03 (77.39%)	188.5 ± 1.73 (80.13%)
T ₇	1.15 ± 0.141 (72.35%)	268 ± 4.33 (34.88%)	1.24 ± 0.173 (64.05%)	299.5 ± 3.77 (68.44%)

*All the values are mean of triplicate determination expressed on dry weight basis ± Standard Error

*g/100g of seed flour, **mg/100g of seed flour

Table 3 Effect of various treatments of white and red seeds of Sword bean on Oligosaccharides (g/100g of seed flour) content

Treatments	White seed			Red seed		
	Raffinose	Stachyose	Verbascose	Raffinose	Stachyose	Verbascose
Raw seeds	1.46 ± 0.01	1.82 ± 0.020	4.16 ± 0.011	1.39 ± 0.01	2.01 ± 0.015	4.57 ± 0.015
T ₁	0.37 ± 0.015 (74.6%)	0.67 ± 0.02 (63.18%)	0.84 ± 0.05 (79.80%)	0.31 ± 0.011 (77.69%)	0.58 ± 0.01 (71.14%)	0.89 ± 0.01 (80.52%)
T ₂	0.61 ± 0.01 (58.21%)	1.01 ± 0.01 (44.58%)	1.02 ± 0.01 (74.48%)	0.61 ± 0.01 (56.11%)	0.96 ± 0.015 (52.23%)	1.06 ± 0.15 (76.80%)
T ₃	0.43 ± 0.01 (70.54%)	0.78 ± 0.05 (57.14%)	1.02 ± 0.01 (75.48%)	0.35 ± 0.05 (74.82%)	0.71 ± 0.01 (64.67%)	1.01 ± 0.01 (77.89%)
T ₄	0.48 ± 0.01 (67.12%)	0.84 ± 0.011 (53.84%)	1.02 ± 0.15 (75.48%)	0.46 ± 0.015 (66.90%)	0.92 ± 0.15 (54.22%)	1.18 ± 0.01 (74.17%)
T ₅	0.5 ± 0.17 (65.75%)	0.88 ± 0.15 (51.64%)	1.10 ± 0.02 (73.55%)	0.40 ± 0.11 (71.22%)	1.01 ± 0.4 (49.79%)	1.03 ± 0.17 (77.46%)
T ₆	0.29 ± 0.05 (80.13%)	0.33 ± 0.015 (81.86%)	0.48 ± 0.01 (88.46%)	0.30 ± 0.015 (78.41%)	0.31 ± 0.05 (84.57%)	0.74 ± 0.05 (83.80%)
T ₇	0.4 ± 0.01 (72.60%)	0.48 ± 0.15 (73.62%)	0.98 ± 0.05 (76.44%) ¹	0.34 ± 0.05 (75.53%)	0.51 ± 0.01 (74.62%)	0.91 ± 0.01 (80.08%)

*All the values are mean of triplicate determination expressed on dry weight basis ± Standard Error

Oligosaccharides

The raw seeds of white and red SB contained raffinose (1.46 and 1.39%), stachyose (1.82 and 2.01%) and verbascose (4.16 and 4.57%) (Table 3). These values are higher when compared to *Canavalia ensiformis* (Pugalenthi *et al.* 2006). T₁ and T₇ treatments resulted in reduction of the oligosaccharides during soaking was influenced by the solubility of the individual oligosaccharides and the diffusion rate (Upadhyay and Garcia 1988). The loss of oligosaccharides in T₂ is in agreement with our earlier study in *Canavalia ensiformis* (Pugalenthi *et al.* 2006). Present study has shown that T₆ is more effective in reducing the oligosaccharides (78-80% of raffinose), (81-84% of stachyose) and (83-88% of verbascose) in SB seeds. This is in good agreement with the earlier study in *Bauhinia purpurea*, in which, higher loss of raffinose (81%), stachyose (70%) and verbascose (79%) was observed by Vijayakumari *et al.* (2007) during autoclaving. The

reduction in the levels of oligosaccharides during hydrothermal processing in probably results from their molecular decomposition to form simple di- and monosaccharides (Vijayakumari *et al.* 2007).

Haemagglutinins (Lectins)

Lectins are toxic glycoproteins that have the ability to bind with carbohydrate moieties on the surface of the human red blood cells (RBC) and cause them to agglutinate. Lectins can combine with intestinal mucosal cells and cause interference with the absorption of available nutrients (Liener 1994). The globulin fraction of *C. gladiata* exhibits weak agglutinates activity without any specificity against A, B and O human blood groups (Table 4). Nonetheless, albumin protein specifically agglutinates the human B blood group. This is in agreement with an earlier report in the *Tamrindus indica* (Pugalenthi *et al.* 2004). Lectins are highly sensitive to heat treatment. Haemagglutinating

Table 4 Effect of various treatments of white and red seeds of Sword bean on Trypsin Inhibitor activity (TIU) and Haemagglutination activity (HAA)

Treatments	White seed				Red seed		
	*TIA	HAA		*TIA	HAA		
		Albumin (A,B & O)	Globulin (A,B & O)		Albumin (A,B & O)	Globulin (A,B & O)	
Raw seeds	241.6 ± 2.8	++++	++++	325.0 ± 5.0	++++	++++	
T ₁	120.33 ± 0.5 (51.19%)	++	++	171.66 ± 2.8 (47.18%)	++	++	
T ₂	145.66 ± 1.15 (39.72%)	+++	+++	250.0 ± 0.00 (23.07%)	++	++	
T ₃	125.8 ± 1.40 (47.93%)	++	++	223.3 ± 1.44 (31.29%)	++	++	
T ₄	101.6 ± 2.8 (58.09%)	++	++	145.3 ± 0.57 (52.29%)	+++	+++	
T ₅	164.1 ± 1.4 (32.07%)	+++	+++	215.0 ± 0.0 (33.84%)	+++	+++	
T ₆	102.04 ± 1.4 (57.90%)	+++	+++	73.3 ± 2.88 (77.44%)	+++	+++	
T ₇	171.6 ± 2.88 (28.97%)	++	++	116.5 ± 0.34 (64.15%)	++	++	

*All the values are mean of triplicate determination expressed on dry weight basis ± Standard Error

*TIU/100g sample, ++++ Blood cells clumped strongly, +++ Clumpy patches, ++ Grainy

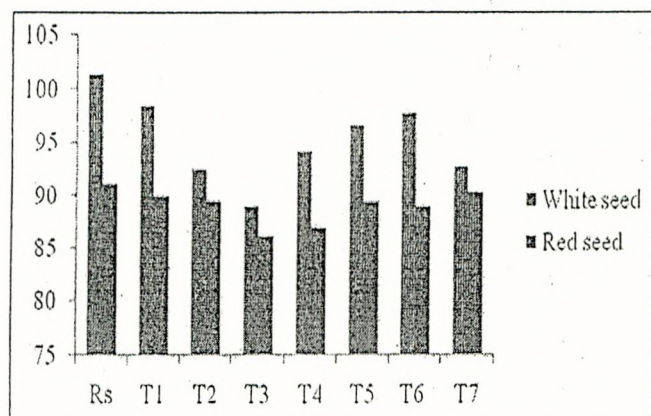


Fig 1 Effect of various treatments on *In-vitro* protein digestibility of white and red seeds of Sword bean

activity decreases during germination in *Glycine max*, *Phaseolus vulgaris*, *Vicia faba* and *Vigna radiata* (Valdebouze *et al.* 1980). A significant reduction in lectins activity has been noticed when the seeds of certain pulses were subjected to dry heat treatment and autoclaving (Siddharaju *et al.* 1996, Vijaya kumara *et al.* 1997) and cooking and autoclaving (Vijaya-Kumara *et al.* 1996).

Trypsin inhibitors

Highest trypsin inhibitor activity (TIA) was noticed in red seeds (77 TUI/g seed) than the white seeds (58 TUI/g

seed) (Table 4). These values are lower when compared with *Mucuna pruriens* (121 TUI/g seed) (Vadivel and Pugalenthil 2008). The maximum reduction of TIA of 57 and 77% was achieved for white and red SB, respectively in T₆.

In vitro protein digestibility (IVPD)

The raw white and red seeds of SB exhibited IVPD of 101.4 and 91.18%, respectively (Fig 1), which is higher with earlier reports in pigeon pea (60-72%), chick pea (65-79%) and soybean (62-71%) (Chitra *et al.* 1995). The IVPD of white and red SB seeds increased significantly by processing (Fig 1). T₁ resulted in significant improvement of protein digestibility in both the SB seeds. A similar improvement of IVPD under hydrothermal treatments have also been reported for *V. aconitifolia* (12.5%), *V. sinensis* (14.8%) and *Bauhinia purpurea* (16%) (Vijayakumari *et al.* 1998). The improvement in the protein digestibility of SB seeds after such hydrothermal treatment may partly due to reduction in the levels of various antinutrients and also due to increased accessibility of the proteins to enzymatic attack.

Among the various processing methods investigated, autoclaving the seeds soaked in water (T₆) appears to be more effective in reducing all the antinutritional substances and also significantly improved the protein digestibility in both white and red SB seeds. Adoption of such cost effective processing method could enhance the chances for increased and versatile utilization of this protein rich under-utilized legume as a food/feed.

LITERATURE CITED

- Adebowale Y A, Adeyemi A and Oshodi A A. 2005. Variability in the physicochemical and antinutritional attributes of six *Mucuna* species. *Food Chemistry* 89: 37-48.
- Burns R R. 1971. Methods for estimation of tannin in grain, Sorgham. *Agronomy Journal* 63: 511-512.

Improvement in the Protein Digestibility of Sword Bean

- Chitra U, Vimala V, Singh U and Geervani P. 1995. Variability in phytic acid content and protein digestibility of grain legumes. *Pl Foods Human Nutrition* 47: 163-172.
- Ekanayake S, Skog K and Asp N G. 2007. Canavanine content in sword beans (*Canavalia gladiata*): Analysis and effect of processing. *Food Chemistry and Toxicology* 45: 797-803.
- Hossain M A and Becker K. 2001. Nutritive value and antinutritional factors in different varieties of sesbania seeds and their morphological fractions. *Food Chemistry* 73: 421-431.
- Hsu H W, Vavak D L, Satterlee L D and Miller G A. 1977. A multienzyme technique for estimating protein digestibility. *Journal of Food Science* 42: 1269-1271.
- Linner I E. 1976. Phytohaemagglutinins (Phytolectins). *Review in Plant Physiology* 27: 291-319.
- Linner I E. 1994. Implications of antinutritional components in soybean foods. *CRC Crit Review Food Science and Nutrition* 34: 31-67.
- Pugalenthi M, Siddhuraju P and Vadivel V. 2006). Effect of soaking followed by cooking and the addition of α -galactosidase on oligosaccharides levels in different *Canavalia* accessions. *Journal of Food Com Analysis* 19: 512-517.
- Pugalenthi M, Vadivel V and Doss A. 2010. Evaluation of nutritional value and protein quality of raw and differentially processed sword bean (*Canavalia gladiata* DC.) seeds. *African Journal of Food and Agriculture Nutrition Development* 10(7): 2850-2865.
- Pugalenthi M, Vadivel V and Siddhuraju P. 2005. Alternative food/feed perspectives of an under-utilized legume *Mucuna pruriens* var. utilis- A Review. *Pl Foods Human Nutrition* 60: 201-218.
- Sadasivam S and Manickam A. 1992. Phenolics. In: *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Ltd., New Delhi, India.
- Salunke B K, Patil K P, Wani M R and Maheswari V L. 2006. Antinutritional constituents of different grain legumes grown in North Maharashtra. *Journal of Food Science and Technology* 43: 519-521.
- Siddhuraju P and Becker K. 2001. Species/variety differences in biochemical composition and nutritional value of Indian tribal legumes of the genus *Canavalia*. *Nahrung Food* 45: 224-233.
- Siddhuraju P and Becker K. 2003. Comparative nutritional evaluation of differentially processed mucuna seeds (*Mucuna pruriens* (L.) DC. Var. utilis (Wall ex Wight) Baker ex Burck) on growth performance, feed utilization and body composition in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research* 34: 487-500.
- Siddhuraju P, Vijayakumari K and Janardhanan K. 1996. Chemical composition and nutritional evaluation of an under-exploited legume, *Acacia nilotica* L. *Food Chemistry* 57: 385-391.
- Somiari R T and Balogh E. 1993. Effect of soaking, cooking and α -galactosidase treatment on the oligosaccharides content of cowpea flours. *Journal of Science Food and Agriculture* 61: 339-343.
- Tanaka M, Thanakul D, Lee T C and Chichester C O. 1975. A simplified method for the quantitative determination of sucrose, raffinose and stacyose in legume seeds. *Journal of Food Science* 40: 1087-1088.
- Upadhyay J K and Garcia V V. 1988. Effect of soaking and cooking on reduction of oligosaccharides of cowpea (*Vigna unguiculata* L. Walp.). *Philipp Journal of Food Science and Technology* 12: 21-28.
- Vadivel V and Janardhanan K. 2005. Nutritional and antinutritional characteristics of seven South Indian wild legumes. *Pl Foods Human Nutrition* 60: 69-75.
- Vadivel V, Pugalenthi M and Megha M. 2008. Biological evaluation of protein quality of raw and processed seeds of gila bean (*Entada scandens* Benth.). *Trop Subtropical Agroecosystem* 8: 125-133.
- Vadivel V and Pugalenthi M. 2007. Biological value and protein quality of raw and processed seeds of *Mucuna pruriens* var. utilis. *Livestock Research for Rural Development* 19: Retrieved on July 9, 2007.
- Vadivel V and Pugalenthi M. 2008. Removal of antinutritional/ toxic substances and improvement in the protein digestibility of velvet bean (*Mucuna pruriens*) seeds during processing. *Journal of Food Science and Technology* 45(3): 242-246.
- Valdebouze P, Beegeron E, Gaborit T and Delort Laval J. 1980. Contents and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. *Journal of Plant Science* 60: 695-701.
- Vijayakumari K, Pugalenthi M and Vadivel V. 2007. Effect of soaking and hydrothermal processing methods on the levels of antinutrients and in vitro protein digestibility of *Bauhinia purpurea* L. seeds. *Food Chemistry* 103: 968-975.
- Vijayakumari K, Siddhuraju P and Janardhanan K. 1996. Effect of different post harvest treatment on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. *International Journal of Food Science Nutrition* 47: 263-272.
- Vijayakumari K, Siddhuraju P, Pugalenthi M and Janardhanan K. 1997. Chemical composition, amino acid contents and protein quality of the little known legume, *Bauhinia purpurea* L. *Journal of Science Food Agriculture* 73: 279-286.
- Vijayakumari K, Siddhuraju P, Pugalenthi M and Janardhanan K. 1998. Effect of soaking and heat processing on the levels of antinutrients and digestible proteins in seeds of *Vigna acônitifolia* and *Vigna sinensis*. *Food Chemistry* 63: 259-264.
- Wheeler E L and Ferrel R E. 1971. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry* 48: 312-320.



Antioxidant status of oxidant challenged rats treated with *Bacopa monnieri* leaf extract

Radha Palaniswamy*, Sumathi Sundaravadivelu and Padma Palghat Ragnathan

Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore – 641 043

Received on: 19-06-2011; Revised on: 08-07-2011; Accepted on: 01-10-2011

ABSTRACT

The present study was conducted to evaluate the enzymic antioxidant effect of *Bacopa monnieri* on oxidant challenged rats. The oxidative status of liver and kidney of male Wistar albino rats aged 6-8 weeks subjected to oxidative stress by CCl_4 and subsequent oral administration of *Bacopa monnieri* leaf extract (500 mg/kg) for 21 days was studied. The antioxidant status in the experimental animals was assessed by analyzing the enzymic antioxidants in the liver and kidney of the rats exposed to ethanol- CCl_4 treatment in the presence and the absence of the leaf extract. The results revealed that the extract could effectively counteract the oxidative insult, which manifested as a decrease in the activities of the antioxidants in both the organs under study.

Key words: CCl_4 , ethanol, oxidative stress, *Bacopa monnieri*, enzymic antioxidants

INTRODUCTION

Generation of free radicals is an integral feature of normal cellular functions in contrast to excessive generation or inadequate removal of free radical results in destructive and irreversible damage to the cells¹. The imbalance between cellular production of free radicals and ability of cells to defend against them is referred as oxidative stress². The generation of reactive oxygen radicals in mammalian cells profoundly affects the numerous cellular functions and causes diseases, which affects pulmonary system³ and neurological system⁴. Natural products play an important role in two aspects- it enhances activity of original natural antioxidant and neutralize ROS by enzymic and non-enzymic mechanisms. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases⁵. Sustained interest in the use of antioxidants for treatment of human disease and the role of dietary antioxidant in prevention of disease development offer better understanding for the development of newer and better therapeutic entities. The holistic therapeutic effects of dietary antioxidants display a first line antioxidant defense⁶. The plant selected for the present study *Bacopa monnieri* (L.), commonly known as "Brahmi" is a member of the family Scrophulariaceae. *B. monnieri* is a classic brain and nerve tonic used for the treatment of cognitive disorders of aging. It helps to improve protein activity and protein synthesis, especially in brain cells, which can impact cognition and memory, and decrease senility⁷.

MATERIALS AND METHODS

The experiments were carried out on healthy female, Swiss albino mice (6-8 weeks old) of 25-30g body weight procured from Small Animal Breeding House, Kerala Agricultural University, Thrissur, Kerala. The mice were maintained at room temperature with food and water *ad libitum*.

EXPERIMENTAL DESIGN

Healthy male Wistar albino rats aged 6-8 weeks were procured from Small Animal Breeding Station, Trissur. The animals were randomly divided into seven groups of six animals each after an acclimatization period of two weeks. They were fed with standard pellet diet and 10% alcohol in drinking water *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee (623/02/b/CPSEA).

TREATMENT GROUPS

The oxidant used to induce stress *in vivo* was CCl_4 , which is metabolized to the oxidative moiety by the cytochrome P450 2E1 (CYP2E1) isoenzyme. In the animals treated with oxidant, a 20 day pretreatment with alcohol (10%) in drinking water was given to induce CYP2E1 activity. CCl_4 was administered as a single subcutaneous injection (2.0ml/kg body weight) diluted 1:1 in paraffin

oil on the 21st day. The methanolic extract of *B. monnieri* leaves was prepared such that the final concentration was 500mg/ml. The methanol was evaporated and the residue was resuspended in water for gavage feeding. The plant extract was administered at a dose of 500mg/kg body weight. The experimental design was as follows:

- Group I: Control
- Group II : Alcohol
- Group III : Alcohol + CCl_4
- Group IV : Alcohol + methanolic extract of *B. monnieri* leaves
- Group V : Alcohol + methanolic extract of *B. monnieri* leaves + CCl_4
- Group VI : Alcohol + Silymarin
- Group VII : Alcohol + Silymarin + CCl_4

Silymarin (a standard hepatoprotective antioxidant) was given at a dose of 25mg/kg body weight/day. Both the plant extracts and silymarin were administered by gastric intubation (gavage) for 21 days. After the treatment period of 21 days, the animals were sacrificed on the 22nd day by cervical dislocation.

The animals were quickly dissected. The liver and kidney were immediately excised and stored in 0.1M ice cold tris-HCl buffer (pH 7.4) in cryovials at -85°C till the assay. The tissues were examined for the activities of enzymic antioxidants.

PARAMETERS ANALYSED

The parameters analysed were superoxide dismutase⁸, catalase⁹, peroxidase¹⁰, glutathione S-transferase (GST)¹¹, and glutathione reductase (GR)¹². The enzyme activities are expressed as specific activity (units / mg protein). The protein content of the enzyme extracts was estimated¹³. The results obtained were subjected to statistical analysis using one way ANOVA followed by post-hoc Fischer analysis.

RESULTS AND DISCUSSION

The activities of the enzymic antioxidants SOD, CAT, POD, GR and GST were analyzed in the liver and kidney of the experimental animals after ethanol and CCl_4 exposure and the protective effect of the extract in counteracting the damage were analyzed.

Alcohol treatment caused a slight, but significant ($P < 0.05$), decrease in the activities of all the enzymic antioxidants in the liver and kidney of rats subjected to oxidative stress assessed except glutathione reductase over untreated controls. CCl_4 caused a further decrease in their activities, indicating oxidative damage. *B. monnieri* leaf administration nullified the alcohol- CCl_4 effect by bringing the activities of the enzymic antioxidants analysed back to control levels in both the organs under study. The activities in the animals treated with *B. monnieri* leaf extract were higher than the levels in the untreated controls. The activities in the liver and kidney of rats not exposed to CCl_4 were much higher than the control values, showing that the leaves can improve the antioxidant status of animals under alcohol-induced oxidative stress. These results indicate that the leaf extract effectively counteracted the oxidative effects of both CCl_4 and alcohol. The effect of *B. monnieri* extract was comparable to that of silymarin, the standard antioxidant.

SOD, CAT and GPx constitute a mutually supportive team of enzymes, which provide defense against the intermediates of dioxygen. CAT and GPx protect

*Corresponding author.

Dr. P. Radha,
Department of Biochemistry,
Biotechnology and Bioinformatics,
Avinashilingam Institute for Home Science
and Higher Education for women,
Coimbatore – 641 043, India.
Tel.: + 91-9786000399
E-mail: radhu_74@yahoo.co.in

Table 1. Effects of *B. monnieri* leaf extract on the activities of enzymic antioxidants in the liver of oxidant challenged rats

Parameters (Units / mg protein)	Groups						
	Control	Without CCl ₄ Alcohol	Leaf extract	Silymarin	With CCl ₄ Alcohol	Leaf extract	Silymarin
Superoxide dismutase*	5.25 ± 0.22	4.24 ± 0.07*	7.74 ± 0.13*	10.26 ± 0.45*	3.74 ± 0.10*	5.77 ± 0.1 ^{bcde}	8.66 ± 0.64 ^{abcd}
Catalase**	153.53 ± 0.85	111.89 ± 1.17*	205.63 ± 1.24*	244.25 ± 2.14*	97.38 ± 0.91*	173.60 ± 2.3 ^{bcde}	185.62 ± 0.86 ^{abcd}
Peroxidase***	10.53 ± 0.55	7.61 ± 0.41*	13.53 ± 0.49*	15.82 ± 0.47*	5.52 ± 0.45*	11.41 ± 0.47 ^{bcde}	13.33 ± 0.52 ^{abcd}
Glutathione S-transferase##	5.04 ± 0.17	4.27 ± 0.07*	7.90 ± 0.14*	10.65 ± 0.33*	3.38 ± 0.36*	7.16 ± 0.17 ^{bcde}	9.11 ± 0.10 ^{abcd}
Glutathione reductase***	0.33 ± 0.02	0.32 ± 0.02	0.45 ± 0.04*	0.55 ± 0.04*	0.19 ± 0.02*	0.25 ± 0.04 ^{bcde}	0.30 ± 0.03*

Values are mean ± SD (n = 6)

Statistically significant (P < 0.05) compared to

* Unit = amount of enzyme required to decrease the absorbance of 240nm by 0.05 units.

Unit = change in absorbance at 430nm/min

** Unit = Amount of enzyme that causes 50% reduction in NBT oxidation

Unit = m Moles of CDNB conjugated / min.

*** unit = m Moles of NADPH oxidized / min.

a - Control

b - Alcohol treated group

c - Alcohol + CCl₄ treated group

d - Plant extract / silymarin treated group

e - Silymarin + CCl₄ treated groupTable 2. Effects of *B. monnieri* leaf extract on the activities of enzymic antioxidants in the kidney of oxidant challenged rats

Parameters (Units / mg protein)	Groups						
	Control	Without CCl ₄ Alcohol	Leaf extract	Silymarin	With CCl ₄ Alcohol	Leaf extract	Silymarin
Superoxide dismutase*	5.19 ± 0.46	4.54 ± 0.41	9.34 ± 0.71*	14.58 ± 1.23*	4.12 ± 0.43	6.45 ± 0.53 ^{bcde}	12.69 ± 1.14 ^{abcd}
Catalase**	247.72 ± 2.70	186.28 ± 0.93*	350.43 ± 1.07*	401.39 ± 1.0*	182.51 ± 0.66*	306.64 ± 0.95 ^{bcde}	315.74 ± 1.28 ^{abcd}
Peroxidase***	14.37 ± 0.54	9.83 ± 0.72*	15.58 ± 0.55*	19.49 ± 0.5*	5.53 ± 0.48*	13.34 ± 0.49 ^{bcde}	15.89 ± 0.69 ^{abcd}
Glutathione S-transferase##	5.50 ± 0.18	4.23 ± 0.33*	8.80 ± 0.40*	11.67 ± 0.7*	4.20 ± 0.18*	7.85 ± 0.47 ^{bcde}	9.48 ± 0.20 ^{abcd}
Glutathione reductase***	0.41 ± 0.04	0.28 ± 0.03*	0.51 ± 0.03*	0.66 ± 0.02*	0.18 ± 0.04*	0.29 ± 0.03 ^{bcde}	0.41 ± 0.04 ^{abcd}

Values are mean ± SD (n = 6)

Statistically significant (P < 0.05) compared to

* Unit = amount of enzyme required to decrease the absorbance of 240nm by 0.05 units.

Unit = change in absorbance at 430nm/min

** Unit = Amount of enzyme that causes 50% reduction in NBT oxidation

Unit = m Moles of CDNB conjugated / min.

*** unit = m Moles of NADPH oxidized / min.

a - Control

b - Alcohol treated group

c - Alcohol + CCl₄ treated group

d - Plant extract / silymarin treated group

SOD against inactivation by H₂O₂ and SOD protects CAT and GPx against superoxide anion¹⁴.

There was a marked decrease in the percentage inhibition of superoxide dismutase, catalase and the level of GSH in the liver of CCl₄ treated rats when compared with the control group. However, the percentage inhibition of SOD, CAT and the level of GSH were significantly increased on administration of the aqueous extract of *Strychnos henningsii* in a dose-dependent manner¹⁵. The administration of the aqueous extract of the bark of *Terminalia arjuna* significantly elevated the reduced SOD, CAT and GST activities in the liver and kidney of CCl₄ challenged mice¹⁶.

The depletion in the activities of the antioxidant enzymes SOD, CAT and GPx when treated with ethanol, was prevented by the methanolic extract of the root of *Opuntia ficus indica* f. inermis in rat stomach tissues¹⁷. The co-administration of the ethanol extract of *Aquilegia vulgaris* or silymarin resulted in a significant increase in the hepatic antioxidant enzyme activities, which was significantly reduced after CCl₄ administration in male Wistar rats⁸. An ethanolic extract of the whole plant of *Amaranthus spinosus* was found to increase the activities of SOD and CAT in CCl₄ induced hepatotoxicity in rats¹⁹.

When the extract of *Pleurotus ostreatus* was used to treat rats with CCl₄-induced toxicity, it enhanced the mean activities of CAT, SOD, GPx and GST in kidneys, heart and brain of rats²⁰. Pretreatment of rats with *Cystisus scoparius* plant extract caused a significant increase in the SOD, CAT, GPx, GST and GR activities in the liver against CCl₄ exposure²¹. These results are in agreement with our findings.

CONCLUSION

Finally it may be concluded that induction of hepatic cell damage in CCl₄ induced mice caused by generation of reactive oxygen species may be decreased by *Bacopa monnieri* leaf extract due to its effective scavenging property. Therefore from the present study it is clear that *Bacopa monnieri* can be used as a potential antioxidant and hepatoprotective agent. It can be recommended for herbal medication also. The present study, thus confirms the antioxidant potential evoked by *Bacopa monnieri* leaf extract administration *in vivo*.

REFERENCES:

- Srikumar R, Parthasarathy NT, Manikandan S, Narayanan GS Sheeladevi R, Effect of *Triphala* on oxidative stress and on all mediated immune response against noise stress in rats. Mol. Cell. Biochem, 283, 2006, 67-74.
- Schaller B, Prospects for the future role of free radicals in treatment of stroke, Free Rad.

Biol. Med. 38, 2005, 411-425.

- Solomidou M, Muzykantsov VR, Antioxidant strategies in respiratory medicine. Treat. Resp. Med, 5, 2006, 47-78.
- Leutner S, Schindowski K, Frolich L, Maurer K, Kratzsch T, Eckert, Muller WE, Enhanced ROS generation in lymphocytes from Alzheimer's patients. Pharmacopsychiatry, 38, 2005, 312-315.
- Heo SJ, Park EJ, Kim SJ, Jeon YJ, Antioxidant activity of enzymatic extracts from a brown sea weed *Ecklonia Cava* by electron spin resonance spectrometry and comet assay. Eur. Food Res. Technol, 221, 2005, 41-47.
- Tiwari AK, Imbalance in antioxidant defence and human disease: Multiple approach of natural antioxidant therapy. Curr. Sci, 81, 2001, 1179-1187.
- Kapoor YK, Dureja J, Chadha R, Herbs in the control of ageing. Drug Discovery Today, 14, 2009, 992-998.
- Misra S, Fridovich L, The role of superoxide anion in the antioxidant of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem, 247, 1972, 3170-3185.
- Luck H, In: Methods in enzymatic analysis - 2 (Ed: Bergmeyer, J.) Academic press, New York, 1974, 885.
- Reddy KP, Subhani SN, Khan PA, Kumar KV, Effect of light and benzyl adenine on dark-treated graying rice (*Oryza sativa*) leaves II, Change in peroxidase activity. Plant Cell Physiol, 26, 1995, 987-994.
- Habig WH, Pabst MJ, Jakoby W, The first enzymatic step in mercapturic acid IV formation. J. Biol. Chem, 249, 1974, 7130-7139.
- David M, Richard JS, In methods of enzymatic analysis - 3 (Ed. Bergmeyer J and Marianna Gra B), Verlag Chemic Wein Hein Dein Field, Beach Florida, Basel 1983, 358.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent. J. Biol. Chem, 193, 1951, 265-275.
- Parthasarathy NJ, Kumar AS, Manikandan S, Devi RS, Methanol-induced oxidative stress in rats lymphoid organs. J. Occupational Health, 48, 2006, 20-27.
- Oyedemi SO, Bradley G, Afolayan AJ, *In-vitro* and *-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr. J. Pharm. Pharmacol, 4, 2010, 70-78.
- Manna P, Sinha M, Sil PC, Aqueous extract of *Terminalia arjuna* prevents CCl₄ induced hepatic and renal disorders. BMC Complement. Altern. Med, 6, 2006, 1-10.
- Alimi H, Hfaiedh N, Bouoni Z, Hfaiedh M, Sakly, M, Zourgui, L, Rhouma KB, Antioxidant and anticarcinogenic activities of *Opuntia ficus indica* f. inermis root extract in rats. Phytomedicine, 17, 2010, 1120-1126.
- Jodynis-Liebert J, Adamska T, Ewertowska M, Bylka W, Matlaska I, *Aquilegia vulgaris* extract attenuates carbon tetrachloride-induced liver fibrosis in rats. Exp. Toxicol. Pathol, 61, 2009, 443-451.
- Zeashan H, Amresh G, Singh S, Rao CV, Hepatoprotective activity of *Amaranthus spinosus* in experimental animals. Food Chem. Toxicol, 46, 2008, 3417-3421.
- Jayakumar T, Sakthivel M, Thomas PA, Geraldine P, *Pleurotus ostreatus*, an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain. Chem. Biol. Interact, 176, 2008, 108-120.
- Raje S, Ahamed KFHN, Kumar V, Mukherjee K, Bandyopadhyay A, Mukherjee, P.K. Antioxidant effect of *Cystisus scoparius* against carbon tetrachloride treated liver injury in rats. J. Ethnopharmacol, 109, 2007, 41-47.

Source of support: Nil, Conflict of interest: None Declared
Journal of Pharmacy Research Vol.4, Issue 10, October 2011