

Assessment of Lectin Activity in a Toxic and a Non-toxic Variety of *Jatropha curcas* using Latex Agglutination and Haemagglutination Methods and Inactivation of Lectin by Heat Treatments

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Abstract: Lectin activity in a toxic (Cape Verde) and non-toxic (Mexico) variety of *Jatropha curcas* seed meal was investigated using latex agglutination and haemagglutination assays. Lectin activity expressed as reciprocal of the minimum quantity (in mg) of *Jatropha* meal per ml of the assay mixture which produced agglutination with the latex beads was 2.88 ± 0.57 and 1.71 ± 0.00 (mean \pm SD, $n = 3$) for the toxic and the non-toxic varieties, respectively, which did not differ significantly ($P > 0.05$), while with the haemagglutination assay these values were 102 and 51, respectively, and differed significantly ($P < 0.05$). The lectins were inactivated by heating and moist heat was more effective than dry heat. The results suggest that lectins may not be responsible for short term toxicity caused by consumption of raw *Jatropha* meal. © 1998 SCI.

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Key words: lectin activity; *Jatropha curcas*; latex agglutination; haemagglutination; Euphorbiaceae

INTRODUCTION

Jatropha curcas, a multipurpose tree of significant economic importance belongs to the Euphorbiaceae family. The plant is widely distributed in wild or semi-cultivated stands in Central and South America, Africa, India and South East Asia (Cano 1986; Cano *et al* 1989; Heller 1996). The seed weighs about 0.75 g and the kernel represents about 65% of the seed mass. Reports on the chemical composition of the kernel revealed protein and lipid contents of 27–32% and 58–60%, respectively (Liberalino *et al* 1988; Aderibigbe *et al* 1997). Besides being a source of oil, *Jatropha* also provides a meal which may serve as a highly nutritious protein supplement in animal feed if the toxins are removed (Makkar and Becker 1997). *Jatropha* meal

(fully defatted) has a crude protein content of between 53 and 63% and about 90% of this is present as true protein (Aderibigbe *et al* 1997).

Several studies with animals (Adam 1974; Ahmed and Adam 1979a,b; Liberalino *et al* 1988; El Badawi *et al* 1992, 1995) have shown that the seeds are toxic. The meal has also been found to be toxic to fish (Makkar and Becker 1997). This has therefore restricted its use as a food or feed source. The toxic and/or irritant compounds isolated so far from *Jatropha* seeds include curcin (Siegel 1893; Felke 1913; Mourgue *et al* 1961; Stirpe *et al* 1976) and the 12-deoxyl-16-hydroxyphorbol (Adolf *et al* 1984). β -D-Glucoside of β -sitosterol is also present in the kernel in large amount (Mitra *et al* 1970). The toxicity of *J curcas* is considered to be primarily caused by a lectin—curcin (Siegel 1893; Felke 1913; Cano Asselieh *et al* 1989).

Hitherto, studies carried out on lectins of *Jatropha* (Felke 1913; Cano Asselieh *et al* 1989; Aderibigbe *et al* 1997) have used one or another haemagglutination assay. This study compared the latex agglutination test

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(Kaul *et al* 1991) and haemagglutination test (Gordon and Marquardt 1974) to determine lectin activity of *Jatropha* meal. The objectives of this study were to quantify lectin activity in a toxic and non-toxic variety of *J. curcas* using the latex agglutination and haemagglutination methods and to establish heat treatment conditions for inactivation of lectins.

MATERIALS AND METHODS

Jatropha meal samples

The seeds were deshelled by hand, ground with a coffee grinder and defatted in petroleum ether (bp 40–60°C) using a Soxhlet apparatus. The defatted kernel is described as meal. *Jatropha* meal samples from the toxic (Cape Verde) and non-toxic (Mexico) varieties were used for the study. The chemical composition of these two varieties is available elsewhere (Makkar and Becker 1997).

Heat treatment

Moist heat (MH) treatment

Meal samples (4 g) of both varieties were weighed (in duplicate) into 50 ml beakers and 8 ml distilled water was added to bring the moisture level to 66%. The contents were made into a paste using a glass rod. The beakers were covered with aluminium foil and placed in a waterbath (100°C), or in an autoclave (121°C). The treatment times were 20, 40 and 60 min at 100°C and 10, 20, 30 and 40 min at 121°C. The samples were allowed to cool in a desiccator, placed in a freezer (6 h) and freeze-dried.

Dry heat (DH) treatment

Meal samples (4 g) of both varieties were weighed into beakers and subjected to heat treatment at 130°C and 160°C for 20, 40 and 60 min in an oven.

Latex beads preparation

The method of Kaul *et al* (1991) was used to adsorb ovalbumin onto latex beads.

Collection and trypsinisation of blood

The method of Gordon and Marquardt (1974) was used for preparation of trypsinised cattle erythrocytes. A 1% suspension of these erythrocytes was used for the assay.

Preparation of meal extract for the agglutination assay

The meal (0.5 g) was weighed in a 50 ml capacity polypropylene centrifuge tube (Greiner Labortechnik, Sol-

ingen, Germany) and then 10 ml of 0.9% (w/v) NaCl solution was added. The contents were homogenised using an Ultra-Turrax (20000 rpm) for 5 min (2 × 2.5 min) with intermittent cooling by keeping the tube in an iced water bath. The tubes were then centrifuged at 3500 × *g* for 10 min, the supernatants were collected into Eppendorf tubes, and centrifuged a second time at 9500 × *g* for 5 min. The supernatants were collected and serially diluted with two-fold increments using 0.9% (w/v) NaCl.

Agglutination assays

In round-bottomed wells of microtitre plates (Greiner Labortechnik, Solingen, Germany) 15 µl of the latex bead (10% suspension in glycine buffer saline containing 0.1% bovine serum albumin) were mixed with an equal volume of the meal extract. The plates were gently shaken at room temperature for 3–4 h. The sedimentation pattern which indicated agglutination of the beads was a uniform circular clump at the bottom of the well, while a negative pattern (indicating no agglutination) was a suspended form similar to that in the blank. For the haemagglutination assay, plates were left at room temperature for 1–2 h and read. A positive pattern which indicated agglutination was a uniform coating of the bottom of the well by erythrocytes while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric, clear zone of equal size to the blank.

Both agglutination tests were also carried out in the presence of Ca²⁺, Mn²⁺ and Mg²⁺ ions at a concentration of 0.286 mM. The latex agglutination assay finally used for studies reported in this communication was comprised of: 10 µl of Mn²⁺ ions (1.15 mM to give a final concentration of 0.286 mM in the assay) added first to the microtitre plate, followed by 15 µl of the latex beads and finally 15 µl of the meal extract. The plates were treated in a manner mentioned above and read after 2–4 h. The haemagglutination assay mixture comprised of 20 µl of Mn²⁺ ions (1.43 mM to give a final concentration of 0.286 mM in the assay) added first to the microtitre plate followed 40 µl of trypsinised erythrocytes and 40 µl of the meal extract.

For both the assays lectin activity was expressed as reciprocal of the minimum quantity (in mg) of *J. curcas* meal per ml of the assay which produced agglutination. All data obtained were analysed statistically using the Student's *t*-test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

In this study the glycoprotein ovalbumin was used for coating the latex beads by physical adsorption. This protein was chosen because of its low cost and easy availability. Feeding studies conducted in our labor-

atory using rats and fish have established that the meal from the seeds obtained from Mexico is non-toxic and that from Cape Verde is toxic (Makkar and Becker 1997). In the absence of added Ca^{2+} or Mn^{2+} or Mg^{2+} ions, neither the toxic (Cape Verde) nor non-toxic (Mexico) meal caused agglutination in either assay of the undiluted meal extracts, ie at a concentrations of 19 mg ml^{-1} and 20 mg ml^{-1} in the latex agglutination and the haemagglutination methods, respectively. This explains the absence of haemagglutinating activity reported by Liberalino *et al* (1988) for *J curcas*. However, in presence of 0.286 mM of Ca^{2+} or Mn^{2+} or Mg^{2+} ions, agglutination was observed for both the toxic and non-toxic meals by both the assays. Amongst the three ions tested, Mn^{2+} was found to have the highest affinity in both the assays and therefore was used in further assays. The lectin activity (Table 1) was 2.88 ± 0.57 and 1.71 ± 0.00 (means \pm SD) for the toxic and non-toxic varieties, respectively, in the latex beads assay and this did not differ significantly ($P > 0.05$); however, in the haemagglutination assay lectin activity was 102 ± 0 and 51 ± 0 for the toxic and non-toxic variety and this differed significantly ($P < 0.05$). Both assays can be used to detect lectin activity in *Jatropha* meal, but the haemagglutination assay is better due to its higher sensitivity and shorter time duration for agglutination to appear.

We also determined lectin activity by preparing meal extracts in 0.5 M NaCl adjusted to pH 5.0 as suggested by Cano Asseleih *et al* (1987), results were similar to those observed above wherein meal extracts were prepared in 0.9% NaCl.

In the heat treated samples, results showed that MH treatment at 100°C for 20, 40 and 60 min; and DH treatment at 130°C and 160°C for 20, 40 and 60 min did not decrease the activity of lectin in either the toxic or non-toxic varieties (results not shown). However, for

samples obtained after MH treatment at 121°C for 10, 20, 30 and 40 min, decreased activity in agglutination was observed at 10 and 20 min by both the assays. Lectin was inactivated with the MH treatment at 121°C for 30 min for both the varieties using the latex beads assay (Table 1) and at 20 and 30 min for the toxic and non-toxic varieties, respectively, using the haemagglutination assay.

Lectins are known to be heat labile and their activity can be decreased by heat treatment (Pusztai 1991; Liener 1994). In an earlier study Aderibigbe *et al* (1997) found an increase in lectin activity following heat treatment which was attributed to some artifacts. It may be noted that in that study metal ions were not used. In the presence of metal ions (Mn^{2+}) the artifacts seem to disappear.

Both agglutination assays are based on agglutination of serially diluted meal extracts and therefore the values obtained (2.88 and 1.71 by the latex agglutination assay and 102 and 51 by the haemagglutination assay for the toxic and non-toxic varieties) differ by a maximum of only one dilution. Therefore, lectin activity for the non-toxic variety was not much different from that of the toxic variety. These observations suggest that the toxicity of *J curcas* meal cannot be attributed to lectin although the presence of lectin might aggravate the toxicity. The MH treatments are more effective than DH treatments in inactivating lectins of *J curcas* meals. Lectins of both toxic and non-toxic varieties can be completely inactivated by MH treatment (66% moisture, 121°C for 30 min).

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TABLE 1
Lectin activity in the moist heat (MH) treated *Jatropha* meal samples (66% moisture, 121°C) using the latex beads assay and haemagglutination method

Time (min)	Lectin activity ^a			
	Latex assay		Haemagglutination assay	
	Toxic (Cape Verde)	Non-toxic (Mexico)	Toxic (Cape Verde)	Non-toxic (Mexico)
0	2.88	1.71	102	51
10	0.43	0.43	1.17	1.17
20	0.43	0.43	ND ^b	0.21
30	ND	ND	ND	ND
40	ND	ND	ND	ND

^a Expressed as reciprocal of the minimum quantity (in mg) of *J curcas* meal ml^{-1} of the assay which produced agglutination.

^b ND not detected.

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