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Research Article

Isolation of *Jatropha Curcas* Seeds Isolectins with Variable Affinity for Human and Animal Blood Types

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Abstract

Background: Lectins are carbohydrate-binding protein which agglutinate glycoconjugates in a reversible way, they are with wide applications in biological and medical sciences. *Jatropha curcas* belongs to the family *euphorbiaceae* and is distributed in many tropical and subtropical countries. The toxicity of this plant is known for long ago and has been attributed to several components among which is a protein called curcin.

Methods: *Jatropha curcas* seeds were pulverized and protein was extracted with suitable buffer. Protein extract thus obtained had undergone successive protein precipitations by salting-out using $(NH_4)_2SO_4$ (AS) at 40, 60, and 80% saturations. Lectin activity was detected by hemagglutination method using human- and animal blood types. AS-precipitated protein fractions that possess lectin activity were tested for their antimicrobial activity against the pathogenic *Staphylococcus aureus*, *Escherichia coli*, *Bacillus aueras*, and *Candida albicans*.

Results: At least three isolectins (Lec40, Lec60, and Lec80) were detected by hemagglutination (HA) and isolated by AS fractionation from the crude *Jatropha curcas* seed extract (CExt). The isolectins exhibited different tendency toward human and animal blood types. None of the isolectins could inhibit any of the used bacterial strains and *Candida albicans*.

Conclusions: In this study, though the detected lectins resemble their counterpart legume lectins, they, however, showed apparently unique and variable behavior toward human and animal blood types. Which might emphasize on the need for further structural analysis on the affinity sites of these proteins.

Keywords: *Jatropha curcas*; euphorbiaceae; lectin; hemagglutination; antimicrobial activity

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1. Introduction

Lectins are carbohydrate-binding proteins that recognize and bind reversibly to specific mono and oligosaccharides on cell surfaces, the extra cellular matrix, and secreted glycoprotein [1]. Due to lectin selective glycoconjugates binding property they have gained much of scientists' attention [2]. Plant lectins have been routinely used in research as a tool for glycoconjugates purification, and as bioactive agents for exploring some particular processes in cells or organisms. The current progress in plant lectin research, especially with respect to the study of the structure/ specificity/ function relationships of the different lectin categories will certainly refine and extend these molecules applications with emphasis to biomedical uses [3]. Much like other members of the family Euphorbiaceae, members of the genus *Jatropha* contain several toxic compounds [4]. In particular, the seeds contain the highly poisonous curcin, a dimeric lectin [5]. However, though of *Jatropha* apparent toxicity, its different parts have been used as medicine in certain geographic region [6] and is shown to exhibit antimicrobial activities [7]. Lectins have long been proven to be quite useful for clinical blood typing and structural studies of blood group substances [8]. The use of lectins as a tool for blood typing is known for years ago. In our laboratory, we have detected more than a single lectin in the seeds of *Jatropha curcas* and since no work has been done to characterize the affinity of these lectins towards human and animal blood types, this work was undertaken. Furthermore, since the previous work on the antimicrobial activity of *J. curcas* was focused on secondary metabolites we intended to fractionate seeds protein and study their antimicrobial effects on pathogenic bacterial and yeast strains.

2. Methods

Good and mature *Jatropha curcas* seeds were brought from North Kordofan state, Khartoum, Sudan.

2.1. Erythrocytes

Human blood samples (A, B, AB and O) were obtained from Blood Bank of Aldwali Hospital, Khartoum, Sudan. While animals blood (cow, goat, donkey and sheep) were obtained from Veterinary Hospital Bahri University, Khartoum, Sudan.

2.2. Protein estimation

Protein content was determined according to ref [9] using bovine serum albumin (BSA) as the standard.

2.3. Preparation of defatted acetone dried powder (ADP)

Jatropha curcas seeds were ground by coffee blender to obtain fine powder. Seeds powder was defatted with 500 mL petroleum ether and dehydrated with cold acetone and left at room temperature for drying. The obtained acetone dried powder (ADP) was quantified and used for protein extraction.

2.4. Preparation of crude extract (CExt)

To thirty five gram of the ADP, 170 mL of child physiological saline (0.145M) (PhS) was added and extracted for four hours under cold conditions. The extract was filtrated through cheesecloth. The clear protein supernatant obtained after removal of small cell debris by centrifugation was named as crude extract (CExt). Hemagglutination unit and specific activity were calculated for CExt and further fractions as described earlier [10].

2.5. Ammonium sulphate (AS) protein fractions preparation

CExt protein was subjected to varying successive fractionations by addition of solid AS at 40%, 60%, and 80% saturations [11]. Precipitated proteins by salting-out were dissolved in minimal amount of PhS. The resultant protein fractions obtained after the suspension of precipitates in PhS were denoted here forth as Fr40, 60, and 80, respectively. And the lectin activities detected in these AS fractions were named as Lec40, 60, and 80 respectively.

2.6. Characterization of Fr40, 60, and 80

2.6.1. Erythrocytes suspension

Collected EDTA-treated blood samples (Human and animal) were taken, washed with several folds of PhS, 2% (v/v) erythrocytes suspension were prepared in physiological saline (0.145M). Trypsinized erythrocytes were prepared by addition of 0.05% trypsin to RBCs suspension and the mixer was incubated at 37°C for an hour. Trypsinized RBCs

were washed with four folds of PhS. Final RBCs suspension at 2% (v/v) was prepared as previously shown and used for all assays.

2.6.2. Hemagglutination Activity Assay (HA)

Hemagglutination test was performed in ELISA 96-well microtiter (U-shaped) plates, in a final volume of 100 µL. Agglutination was assessed after one hour incubation at room temperature. Hemagglutinating activity was expressed as titer, namely, the reciprocal of the highest dilution that gave a clear agglutination. The specific hemagglutinating activity was defined as titer (unit) per mg protein [12, 13].

2.6.3. Anti-microbial activity of protein fractions

For the disc diffusion antimicrobial tests, the test pathogens (100 µL/plate) (Table 1) were spread on Muller Hinton agar plates. Sterile paper discs of (6 mm diameter, 0.09 mm thickness, 5 discs/plate) were aseptically transferred on agar plates and were then soaked with equal volume 5 µL of 0.2 g/mL (w/v) fixed concentration of lec40, 60, and 80. The zone of inhibition was examined after the plates were incubated at 37°C for 24–48 h. The pathogens were also tested with both a standard antibiotic tetracycline (30 µg as positive control) and negative controls (solvents) [14].

3. Results and Discussion

50 gram of *Jatropha curcas* seeds were pulverized and extensively defatted with petroleum ether and extracted with suitable amount of PhS. The obtained clear protein solution was fractionated by the classical salting out technique using AS salt at 40, 60 and 80% saturations. The resultant precipitants were dissolved in minimal amount of PhS. Almost equal quantities of protein was precipitated in each AS fraction. Upon testing these fractions for lectin activity by HA, each faction exhibited detectable hemagglutinating activity, indicating the presence of lectin in multiform. These findings are similar to the one obtained by Osman *et al* [12] from seed extract of *Tamarindus indica* [12]. Whereas in contrary to De Oliveira *et al* in which only one lectin was present in seeds [15]. Maximum lectin activity was detected in Fra80 followed by Fra60 and finally Fra40. By this step the isolectin collectively were purified by almost 10 times (Table 2). Precipitation of *euphorbiaceae* family lectins with salting out using AS is reported in several publications [16-19]. However, none of these groups detected the presence of

isolectins separated by their order of AS solubility. These results could be attributed to the fact that in these reports CExt was brought in a single addition of AS to 60 [20] and 100% saturations [21] and no systematic protein fractionation, as in the current investigation, was done. Moreover, since lec40 is none reactive towards human blood types, these authors might have missed its detection. The affinity purified toxic lectin, curcin, from *J. curcas* seeds, was initially precipitated from its crude extract by AS at 60% saturation. Single lectin, the curcin, was obtained with a *p*I 8.54 [20]. Whereas a *p*I of 4.4 for a single lectin from the latex of *Synadenium carinatum* was reported [22].

3.1. Behavior of lectin toward human and animals blood types

Of the several distinct properties of lectins is their erythrocytes Hemagglutinating. Therefore, it's routine laboratory work to detect lectin activity by the hemagglutinating assay. Most researchers have focused their research on human erythrocytes ABO system antigens [23] which could be due to their ease to obtain and higher stability in comparison to animal erythrocytes. All human (A, AB, B and O) or animal (cow, goat, horse and sheep) blood types were either used raw or trypsin-treated. The lectin isoforms (Lec40, Lec60, and Lec80) exhibited variable interaction patterns with the blood types used. Like most of other *euphorbiaceae* lectins, *J. curcas* seed lectins agglutinated human and animal bloods differently. Lec60 and 80 agglutinated poorly human A, B and O blood types, whereasLec40 didn't clump any of the used human blood type. Interestingly, none of these isolectins could agglutinate AB blood type. On the other hand, agglutination was induced or enhanced when human blood types were treated with trypsin (Table 3). Treatment of RBCs with proteases such as trypsin, neuraminidase, pronase, and papin is known to increase sensitivity of RBCs agglutination by several folds. Treatment of RBCs with protease leads to the removal of polypeptides on the surface of erythrocytes and exposing number of lectins surface receptors [24]. These preferential agglutinations of human blood types with this lectin confirm the presence of this lectin in multiform. Our results of agglutination of human blood types with *Jatropha curcas* isolectins, are in accordance with some published papers on euphorbiaceae family lectins [18, 19] whereas in contrary to some other reports [21]. These differences might highlight interesting variability in the affinity sites of these isolectins and through some light on the apparent distinct, thought yet to be disclosed, physiological role of these proteins.

When blood of animals like cow, goat, horse and sheep were used, none were agglutinated by *jatropha curcas* isolectins. However, interestingly, upon treatment of

RBCs with trypsin, lec40 could only agglutinate, to good extend, cow and sheep bloods, whereas no effect was shown by lec60 and 80 (Table 3). Jawada *et al* in their work *E. tithymaloides* leaves lectin had shown that among the different animal blood types they used, only cow erythrocytes were agglutinated [21].

3.2. Antimicrobial activity of lectin

The evaluation of the antimicrobial activity of Lec40,60 and 80 was conducted using 3 strains of bacteria, in which two were gram negative, one was gram positive and fungi cultivated in Mueller – Hinton medium, with different concentrations of protein 100, 200 and 300ug, the antibiotic gentamycin was used as control. None of the isolectins lec30, 60 or 80 showed any inhibitory effect against the used bacterial and fungal strains (data not shown). A lectin from *E. helioscopia* was shown to express strong inhibitory effect on *pseudomonas aeruginosa*, *klebsiella pnuemoniae* and *Escherichia coli* [25]. Previous work with *Jatropha curcas* seed extract had shown inhibitory effect of J. curcas extract against *Fusarium oxysporum* however, since they didn't use *Candida albicana,* which we used in the present study, we couldn't correlate our results with theirs [14]

4. Conclusion

Though *Jatropha curcas* seed lectin is a typical legume family lectin in term of its presence in multiform, these lectins behaves differently from other lectins of the same family. With regards to RBCs hemagglutination, unlike majority of legume lectin, *Jatropha curcas* seed lectins agglutinated poorly tyrosinated or untyrosinated human and animal RBCs. These results conclusively indicate the peculiar active site of these proteins. Therefore, further studies on the affinity site of these lectins as well as their secondary and tertiary structure may pave explaining these interesting features of these proteins.

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