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Jatropha Curcas Linn can Reduce the Expression of Hsp70 that will Result in Reduced Errors in Protein Folding and Promotion of Normal Protein Function in Proliferation and Apoptosis

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Abstract

Background: *Jatropha curcas* (*J. curcas*) as a local plant have phytochemical contents like phenolic that effect as anti-inflammatory and cytotoxicity properties. Research in the last decade about cancers showed that over expression of heat shock protein 70 (Hsp70) does to mis-folding and continues to triggering proliferation and suppressing apoptosis. Increased expression of Hsp70 is responsible for mis-folding of proteins and implicated in functions that lead and play a role in the pathogenesis of pain including cancer incidence. In this study, we examined the expression of Hsp70, pRb and caspase3 in Raji cells after treatment with *J. curcas*.

Methods: We first to determinethe plant. The plant is shrubby, woody and many are found in the tropics, names *J.curcas sp. (*Euphorbiaceae family). Second, to extraction of fresh leaves taken from *J. curcas*, washed clean, dried by the wind, sliced into small pieces and end dried by an oven at a temperature 65°C for 48 hours. And third, To developed a Raji cell culture (a cancer model) system and treated cells with fractionated extracts of *J. curcas* leaf. Fourth are evaluating the expression of Hsp70, pRb and caspase-3 that do by immuno histochemical analysis. A semi quantitative (devided into catagories: low, medium and strong) data was collected under light microscope view.

Results: Our results showed that the expression of Hsp70 in Raji cells after treatment with fractionation *J. curcas* leaf was strong as much as 9(30.00%) lower than control (14:46.60%). The expression of pRb after treatment with fractionation *J. curcas* leaf was strong as much as 15(50.00%) higher than control (9:30.00%). And the expression of caspase-3 in Raji cells after treatment with fractionation *J. curcas* leaf was strong as much as 13(43.3%) higher than control (9:30.00%).

Conclusion: Our results show that J. curcas leaf can reduce the expression of Hsp70, suggesting it can also reduce errors in protein folding and promotion of normal protein function in proliferation (by inhibit pRb protein expression) and apoptosis (by enhance caspase-3 protein expression).

Key words:

Jatropha curcas L; Hsp70; pRb; Caspase-3; Cancer

Introduction

Jatropha curcas Linn as a local plant have phytochemical contents, like anti-inflammatory and cytotoxicity properties of phenolic extract. Leaf of *J. curcas* contents higher phenolic compound, flavonoid and saponin. Application of different varieties of *J.curcas* in traditional medicine had been reported. However, information regarding the bioactive compounds and the therapeutic activities is still lacking [1]. These studies suggest that components from *J. curcas* may have anti-cancer function and potentially be useful for prevention and treatment strategies.

All organisms respond to heat (heat shock response) by up-regulating specific heat shock or stress proteins [2]. Research over the last decade has shown that increased expression of heat shock protein 70 (Hsp70) plays a role in protein folding errors from denaturised proteins and new protein translation, causing proteins to function abnormally [3-7]. Multiple hits – multiple steps – multiple stage stressors may experience distress and express Hsp70, among other cellular factors, to inhibit cell proliferation and enhance apoptosis [8-13].However, these efforts are not always successful and turn trigger cell proliferation and inhibits apoptosis and continue could lead to the development of cancer [14]. This concept gives the opportunity to be able to explain the increased incidence of cancer, then with granting the *J. curcas* the cancer can't occurs.

Cancer is initiated from the un-regulated of genes and pathways regulating critical cellular processes, such as cell proliferation and apoptosis [2]. Cell proliferation and apoptosis are a common phenomenon that usually happens that helps the reconstruction on a multi cellular organism [15]. Increasing cell proliferation of which many played by pRb are also a critical characteristic contributing to carcinogenesis [16]. Failure setting apoptosis genes of which many played by caspase3 are key of success carcinogenesis [8,10]. Note that

the above opinion expressed support, that molecules called Hsp70 molecular chaperone and also plays a role in homeostasis, alleged to be facilitating the flow of cell proliferation and apoptosis and acts on the staple groove changes towards cancer at the molecular level [9]. Heat shock protein is continuously and widely expressed in most normal tissues and cancer [11] and facilitates protein folding and translocation and prevents incorrect aggregation and degradation. Lots of protein folding error implies a function that leads and plays a role in disease pathogenesis (including cancer) of events like misfolding in pRb protein for proliferation and caspase3 for apoptosis [17,18]. When a high occurrence of cancer in developing countries including Indonesia is not addressed, it could impact the increased rate in morbidity and mortality, and lower the quantity and quality of human resources.

Methods

Determination of plant

The *J. curcas* fresh whole plant (Euphorbiaceae) of shrubby plants woody and many are found in the tropics was collected from the farm Faculty of Mathematic and Natural Science, University of Sebelas Maret, Solo, Central Java, Indonesia and determining the plant by him. A voucher specimen was on No.043/UN27.9.6.4/Lab/2015. Determination based on C.A. Backer and R.C. Bakhuizen van den Brink. 1963. Flora of Java.Volume.1.Noordhoff N.V, Groningensave in Faculty of Mathematic and Natural Science, University of Sebelas Maret, Solo, Central Java, Indonesia.

J. curcas leaf extracts preparation

The extraction of leaves taken from *J. curcas* washed clean, dried by the wind, sliced into small pieces, and dried by an oven at a temperature 65°C for 48 hrs. From as many as 1000g leaves *J. curcas* dried until produce 203,76 g the dried leaf. Dry substances then it is put in a solvent ethanol 900 ml during 3 × 24 hours. A solution of strained so as to separate residue from filtrate with volatilized. Coarse extracts the results of the extraction of dissolved with a solvent aquadest until the concentration 90%. Crude extracts of centrifuged. Supernatant earned poured into sterile conical tube, and then added the acetone little by little with a 1:1 comparison to precipitate the proteins at a temperature of 40°C. Solution is centrifuged again. Supernatant removed and the sediment that still contains a bit of acetone-dried with paper towels. Conical tubes covered with parafilm hollow and done drying technique with freeze dry for 1 hour.

The result is stored in the cooling temperature cupboards 40C. If will be used then dissolved in a little phosphate of sodium 5 mM, pH 6.5 without NaCl. A solution of vortexed until late at, centrifuged, the sediment dumped and supernatant which is the fraction of protein included in conical tube [19,20]. Partition done with chloroform and the success of a partition monitored with the methods chromatography thin layer and a top part is taken fractionation of from the upper part of the results of the process of partition is by using liquid chromatography vacuum. All fractions produced monitored the profile of its chemical content using chromatography thin layer method until fractions have a profile almost the same merged into one faction. C-Faction chosen (combined F12 and F13) is who assigns grades the percentage of death cell line that was the greatest [19,20].

Treatment (Raji cells were treated with fractionated extracts of *J. curcas* leaf)

The first stage was to develop the Raji cells (as a cancer cells cause virus) culture using the Fresney method with many modifications [21]. Raji cells were plated at 2×10^4 cells in a 24-well micro-culture plate in 100 ml of RPMI. Create RPMI control and solvent plus DMSO 0.25%. Subsequently a precipitate during 24hr in culture medium than the Raji cell were grown in microplate with media plus extract with a nonlethal concentration of fractionation *J. curcas* leaf. of sampling was performed for 24 hrs [20].

Immunohistochemical staining

Immunohistochemical staining was performed with a TSA-indirect method (Nen Life Science Product, Renaissence) using monoclonal antibodies against Hsp70 (1:500), pRb (1:500) and caspase3 (1:500) produced by Stressgen. Images were collected using a X100 objective lens (Nikkon) [21].

Ethical clearance

This research has been ratified by the feasibility of conduct to be done by Research and Ethical Committee Distric Hospital of Muwardi and Faculty of Medicine (University of Sebelas Maret) led by Dr. Hari Wuyoso

Results

Raji cells were treated with fractionated extracts of *J. curcas* leaf and Hsp70 expression was evaluated by immunohistochemical analysis (Figure 1). The results showed that Hsp70 expression was lower (mean strong is 9:30.0%) after treatment compared with expression before treatment (mean strong is 14:46.6%). In Table 1 showed the expression patterns of Hsp70 in Raji cells before treated with the active compounds of fractionation *J. curcas* leaf was strong 15(50.0%) in ethyl acetate, 17(56.6%) in ethanol water, 11(36.6%) in chloroform and 13 (43.3%) n-Hexane solvent. In comparison (Table 2), expression of Hsp70 protein in Raji cells treated with the active compounds of fractionation *J. curcas* leaf extracts was strong 11(36.6%) in ethyl acetate, 10(33.3%) in ethanol water, 8(26.6%) in chloroform and 6(20.0%) n-Hexane solvent.

No	In Solvent	Hsp70			Total
		tenuous	midts	strong	
1	Ethyl Acetate	13 (43.3%)	2 (06.6%)	15 (50.0%)	30 (100%)
2	Ethanol water	8(26.6%)	5 (16.6%)	17 (56.6%)	30 (100%)
3	Chloroform	12 (40.0%)	7 (23.3%)	11 (36.6%)	30 (100%)
4	n- Hexane	7 (23.3%)	10 (33.3%)	13 (43.3%)	30 (100%)
	Mean	10 (33.3%)	6 (20.0%)	14 (46.6%)	30 (100%)

Table 1: Immunohistochemistry staining using Hsp70 antibody anti Hsp70 protein in Raji cells before treated with *Jatropha curcas* Linn leaf. The expression of Hsp70 was strong 15(50.0%) in ethyl acetate, 17(56.6%) in ethanol water, 11(36.6%) in chloroform and 13 (43.3%) n-Hexane solvent.

No	In Solvent	Hsp70	Total

		tenuous	midts	strong	
1	Ethyl Acetate	17 (56.6%)	2 (06.6%)	11 (36.6%)	30 (100%)
2	Ethanol water	11 (36.6%)	9 (30.0%)	10 (33.3%)	30 (100%)
3	Chloroform	15 (50.0%)	7 (23.3%)	8 (26.6%)	30 (100%)
4	n- Hexane	11 (36.6%)	13 (43.3%)	6 (20.0%)	30 (100%)
	Mean	14 (40.0%)	7 (23.3%)	9 (30.0%)	30 (100%)

Table 2: Immunohistochemistry staining using Hsp70 antibody anti Hsp70 protein in Raji cells after treated with the active compounds of fractionation *Jatropha curcas* Linn leaf. The expression of Hsp70 was strong 11(36.6%) in ethyl acetate, 10(33.3%) in ethanol water, 8(26.6%) in chloroform and 6(20.0%) n-Hexane solvent.

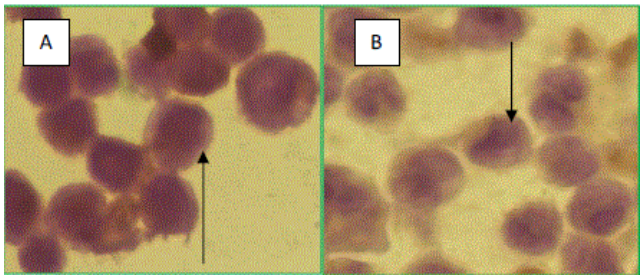

Figure 1: Raji cells are treated with fractionation of leaves *Jatropha curcas* Linn so the Hsp70 expression (brownish) before treatment was higher (A: X200) while the expression (bluish) after treatment was lower (B: X200).

We performed similar analyses to evaluate the expression of pRb in Raji cells after treatment with *J. curcas* leaf extracts. The results shown in Figure 2 demonstrate that pRb expression was lower (mean strong is 9:30.0%) before treatment compared to after treatment (mean strong is 15:50.0%). Table 3 showed the expression patterns of pRb in Raji cells before treated with the active compounds of fractionation *J. curcas* leaf was strong 13(43.3%) in ethyl acetate, 10(33.3%) in ethanol water, 6(20.0%) in chloroform and 9(30.1%) n-Hexane solvent. In comparison (Table 4), expression of pRb protein in Raji cells treated with the active compounds of fractionation *J. curcas* leaf extracts was strong 16(53.3%) in ethyl acetate, 15(50.0%) in ethanol water, 15(50.0%) in chloroform and 15(50.0%) n-Hexane solvent.

No	In Solvent	pRb			Total
		tenuous	midts	strong	
1	Ethyl Acetate	8 (26.6%)	9 (30.0%)	13 (43.3%)	30 (100%)
2	Ethanol water	15 (50.0%)	5 (16.6%)	10 (33.3%)	30 (100%)
3	Chloroform	17 (56.6%)	7 (23.3%)	6 (20.0%)	30 (100%)
4	n- Hexane	10 (33.3%)	11 (36.6%)	9 (30.1%)	30 (100%)
	Mean	13 (43.3%)	8 (26.6%)	9 (30.0%)	30 (100%)

Table 3: Immunohistochemistry staining using pRb antibody anti pRb protein in Raji cells before treated with the active compounds of fractionation *Jatropha curcas* Linn leaf. The expression of pRb was strong 13 (43.3%) in ethyl acetate, 10 (33.3%) in ethanol water, 6 (20.0%) in chloroform and 9 (30.1%) n-Hexane solvent.

No	In Solvent	pRb			Total
		tenuous	midts	strong	
1	Ethyl Acetate	7 (23.3%)	7 (23.3%)	16 (53.3%)	30 (100%)
2	Ethanol water	10 (33.3%)	5 (16.6%)	15 (50.0%)	30 (100%)
3	Chloroform	9 (30.0%)	6 (20.0%)	15 (50.0%)	30 (100%)
4	n- Hexane	5 (16.6%)	10 (33.3%)	15 (50.0%)	30 (100%)
	Mean	8 (26.6%)	7 (23.3%)	15 (50.0%)	30 (100%)

Table 4: Immunohistochemistry staining using pRb antibody anti 208 pRb protein in Raji cells 209 after treated with Jatropha curcas Linn leaf. The expression of pRb was strong 210 16(53.3%) in ethyl acetate, 15(50.0%) in ethanol water, 15(50.0%) in chloroform and 211 15(50.0%) n-Hexane solvent. And the mean was 15(50%).

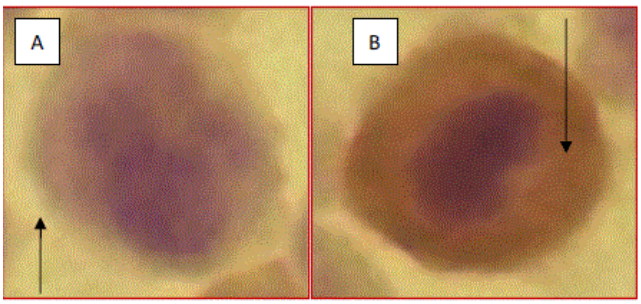

Figure 2: Raji cells are treated with fractionation of leaves *Jatropha curcas* Linn so the pRb expression (brownish) before treatment was lower (A: X400) while the expression (bluish) after treatment was higher (B: X400).

In (Figure 3), expression of caspase3 was higher (mean strong is 13:43.3%) after treatment compared with before treatment (mean strong is 9:30.0%). Table 5 showed the expression patterns of caspase3 in Raji cells before treated with the active compounds of fractionation *J. curcas* leaf was strong 6(20.0%) in ethyl acetate, 10(33.3%) in ethanol water, 12(40.0%) in chloroform and 7(23.3%) n-Hexane solvent. In comparison (Table 6), expression of caspase3 protein in Raji cells treated with the active compounds of fractionation *J. curcas* leaf extracts was strong 10(33.3%) in ethyl acetate, 12(40.0%) in ethanol water, 19(63.3%) in chloroform and 11(36.6%) n-Hexane solvent.

No	In Solvent	pRb			Total
		tenuous	midts	strong	
1	Ethyl Acetate	17 (56.6%)	7 (23.3%)	16 (53.3%)	30 (100%)
2	Ethanol water	15 (50.0%)	5 (16.6%)	15 (50.0%)	30 (100%)
3	Chloroform	15 (50.0%)	6 (20.0%)	15 (50.0%)	30 (100%)
4	n- Hexane	14 (40.0%)	10 (33.3%)	15 (50.0%)	30 (100%)

	Mean	15 (50.0%)	7 (23.3%)	15 (50.0%)	30 (100%)

Table 5: Immunohistochemistry staining using caspase3 antibody anti caspase3 protein 230 in Raji cells before treated with Jatropha curcas Linn leaf. The expression of caspase3 231 was strong 6(20.0%) in ethyl acetate, 10(33.3%) in ethanol water, 12(40.0%) in 232 chloroform and 7(23.3%) n-Hexane solvent. And the mean was 9(30.0%).

No	In Solvent	pRb			Total
		tenuous	midts	strong	
1	Ethyl Acetate	12 (40.0%)	8 (26.6%)	10 (33.3%)	30 (100%)
2	Ethanol water	10 (33.3%)	8 (26.6%)	12 (40.0%)	30 (100%)
3	Chloroform	9 (30.0%)	2 (06.6%)	19 (63.3%)	30 (100%)
4	n- Hexane	9 (30.0%)	10 (33.3%)	11 (36.6%)	30 (100%)
	Mean	10 (33.3%)	7 (23.3%)	13 (43.3%)	30 (100%)

Table 6: Immunohistochemistry staining using caspase3 antibody anti caspase3 protein in Raji cells after treated with the active compounds of fractionation Jatropha curcas Linn leaf. The expression of caspase3 was strong 10(33.3%) in ethyl acetate, 12(40.0%) in ethanol water, 19(63.3%) in chloroform and 11(36.6%) n-Hexane solvent. And the mean was 13(43.3%)

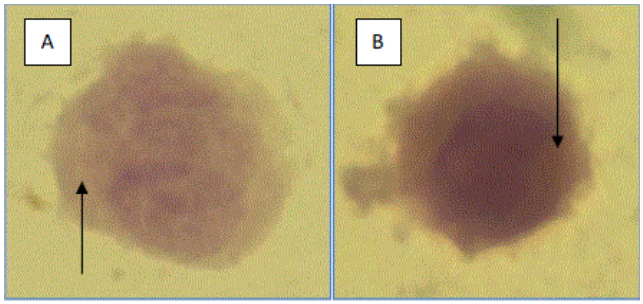

Figure 3: Raji cells are treated with fractionation of leaves *Jatropha curcas* Linn so the caspase3 expression (brownish) before treatment was lower (A: X400) while the expression (bluish) after treatment was higher (B: X400).

Discussion

Molecular chaperones known as heat shock proteins act as the central integrators of protein homeostasis. This molecules showed bind and stabilize proteins, facilitate protein folding and assembly, promote protein translocation across membranes and target proteins for degradation. These chaperone proteins make important settings on many of proteolysis. These chaperone facilitate transformation towards cancer on a molecular level, and supports the concept that in carcinogenesis, several events change the function of proteins and thus require serious attention in the development of cancer [9].

Heat shock proteins are classified based on molecular weight; for example, Hsp70 is a 70 kDa heat shock protein [22]. The main chaperone machinery includes Hsp40; Hsp70; nucleotide-exchange factor; the chaperonin complex-containing tailless complex polypeptide 1 (TCP-1), comprised of eight subunits (also called CCT); the chaperone pre-folding, composed of five subunits; small Hsp chaperones that form multimers of various sizes; and the Cpn60-Cpn10 complex (in which Cpn is a chaperonin also known as Hsp60-Hsp10) [23-28].

The protein folding process is complicated. Although it has long been known that amino acid chains in several ways to guide the adjustment makes the protein is active,there are still some other mechanisms the folding process. The tutorial will lead starting from the amino acid chain of birth to achieve a form/structure with infinite possibilities. It is clear that the system of quality control is instrumental to protect from consequences if the folding process fails and mis-folded proteins accumulate. This accumulation of misfolded proteins can result in cell death. It turns out the end of the last decade mechanism is triggered to be understood. Studies have shown a connection between faulty protein folding with a variety of ailments, such as disease prione, diabetes mellitus and cancer, from the presence of mis-folded and accumulated proteins. Trigger condition to be used as the principle target treatment [29]. As is known heat shock protein is continuously and thoroughly expressed in most normal tissues and cancer [6] and the implications for the interaction of many proteins such as folding, translocation and prevent improper aggregation and degradation. Increased expression of Hsp40 and Hsp70 is responsible for mis-folding of proteins implicated in functions that lead and play a role in the pathogenesis of pain including cancer incidence [17,18].

The biological function of proteins depends on the correct three-dimensional structures, which is done by the protein constituent of amino acids during the process of folding. Recent studies showed that several diseases result from consequences of protein folding errors and these are classified as protein conformational disorders (PCDs), such as Alzheimer's disease, transmissible encephalopathies, serpin-deficiency disorders, haemolytic anemia, Huntington's disease, cystic fibrosis, type II diabetes,amyotrophic lateral sclerosis, Parkinson's disease, dialysis-related amyloidosis and more than 15 other less studied diseases. In the majority of PCDs, incorrect protein folding results in amyloid-like aggregates in different organs and stimulates tissue damage and organ dysfunction [30].

Jatropha curcas halted in return in order to cure the disease. *Jatropha curcas* leaf extracts, contained appreciable amounts of phenolic compounds. These extracts also showed good antioxidant activity [1]. Some research suggests occuring decline in the expression of protein (emphasis expression gene) after the provision of phenolic on various plant [31-33]. Yan research revealed that phenolic can reduce a gene expression of T3SS (The type III secretion system) is a major virulence factor in many gram-negative bacterial pathogens. Plants defend themselves against bacterial pathogens by recognizing either the type 3 effectors or their actions and initiating a cascade of defense responses that often results in programmed cell death of the plant cell being attacked. He showed that a plant phenolic compound, suggesting that plants can also defend against bacterial pathogens by manipulating the expression of the T3SS [34].

Conclusion

Our results show that *J. curcas* leaf can reduce the expression of Hsp70, suggesting it can also reduce errors in protein folding and promotion of normal protein function in proliferation and apoptosis.

Acknowledgements

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Author Contribution

AP performed the study, made a protocol of the laboratory, prepared data analysis, collected data, contributed to the drafting, wrote and revising of the paper. MSF provide laboratory work, generated extracts, performed cell culture experiments. AYE provide laboratory work, generated extracts, performed cell culture experiments, OPA is a designer.

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