Effects of *Jatropha curcas* L. Seed Extract to Protein Level of VEGF and Histological Image of Central Vein in Liver Tissue

**BACKGROUND**

Tumor caused by mechanism of rapid proliferation of normal cells and uncoordinated with tissue activity, by low cellular organ dysfunction. Hence, it required to inhibition proliferation of the tumor. The tumor cell activate various channels to supply oxygen, such by angiogenesis or by formation of blood vessels. Decreased activity of VEGF may cause suppression of tumor cell activity. *Jatropha curcas* L. reported has various activities as an anti-tumor agent, antimetastatic and antiangiogenic in melanoma.

**OBJECTIVE**

The study is purposed to investigate the effect of *Jatropha curcas* L. seed extract to protein level of VEGF and histological of central vein liver tissue.

**METHODS**

Mice was orally given by various doses of Jatropha seed extract with 0, 5, 50 and 250 mg/KgBW for 28 days. The VEGF levels in liver was measured by ELISA and observation of histological image of central vein in liver by Hematoxylin-Eosin staining. Data were analyzed statistically using one way ANOVA.

**RESULTS**

The VEGF level in Jatropha doses of 25 and 250 mg/KgBW has increased to 407 and 377 pg/mL; and has decreased by jatropha doses of 5 and 50 mg/KgBW to 270 and 280 pg/mL. Analysis by linear regression performed increasing trend in ANOVA, \( p < 0.05 \). The central vein abnormal histology demonstrated dosing jatropha seed extract 250 mg/KgBW. In this study showed that the extract of Jatropha seeds can damage the central vein. But there are efforts to repair damaged liver cells through angiogenesis through increased VEGF protein.

**CONCLUSION**

The *Jatropha curcas* L. has no evidence anti-tumor effect by anti-VEGF pathway. Jatropha seed extract high dose (250 mg / KgBW) causes damage in the central vein.

**Key words:** Jatropha, VEGF, central vein, liver.

**REFERENCES**

[Insert references here]
Herbal Research Center
YARSI Research Institution

Conference Program and Abstract Book
International Conference of Herbal Medicine

Integration of Science, Technology and Industry on Herbal Medicine for Clinical Application

October 6th – 7th, 2016
YARSI Tower, YARSI University, Jakarta

Supported by speakers from:
Conference Program and Abstract Book

INTERNATIONAL CONFERENCE OF HERBAL MEDICINE

Integration of Science, Technology and Industry on Herbal Medicine for Clinical Application


06-07 October, 2016
Herbal Research Centre
YARSJ Research Institution

YARSJ University, Jakarta - INDONESIA
Author:
ICHM Organizing Committee

Published by:
Universitas YARSI
Jl. Legian Sopomo Cempaka Putih Jakarta 10510
Tel. : +62 21 4206674
Fax  : +62 21 4206671

Year 2016
1st Print

Copyright © 2016 by Universitas YARSI

All rights reserved. No part of this publication and abstracts may be reproduced, distributed, or transmitted in any form or by any means, including photocopying, recording, or other electronic or mechanical methods, except in the case of quotations can only be as “personal communication” with prior written permission of the publisher or the authors. For permission requests, write to the publisher, addressed at the address above.

<table>
<thead>
<tr>
<th>No</th>
<th>Code</th>
<th>Presenter &amp; Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OP 01</td>
<td>Rahmat Santoso (Padjadjaran University) Formulation Gel with Cinnamon Bark Extract (Cinnamomum burmanii Nees ex. Bl) as Antioxidant</td>
</tr>
<tr>
<td>2</td>
<td>OP 02</td>
<td>Oentaraui Tjandra (Tarumanegara University) Comparative Study between the Effectiveness of Moisturizing Cream Containing Green Tea and Moisturizing Cream Containing Vitamin E in Geriatric Patient with Dry Skin</td>
</tr>
<tr>
<td>3</td>
<td>OP 03</td>
<td>Samsul Mustafa (YARSI University) The effect of soy extract on the expression of TERT pancreatic b-cells diabetes mellitus rat induced by alloxan</td>
</tr>
<tr>
<td>4</td>
<td>OP 04</td>
<td>Ida Susanti (Universitas Indonesia) Chemopreventive effect of Beta Glucan From Oyster Mushroom (Pleurotus ostreatus, Jacq P. Kurn) on carcino genesis of breast cancer induced by 7, 12 Dimethylbenz[a]anthracene (DMBA)</td>
</tr>
<tr>
<td>5</td>
<td>OP 05</td>
<td>Himni Marsiati (YARSI University) Viability of cultured HepG2 cells exposed to mangiferin and quercetin</td>
</tr>
<tr>
<td>6</td>
<td>OP 06</td>
<td>Nabila Alia Hanafiee (Syarif Hasyatussiah University) The Effects of Jatropha curcus Seed Extract to Protein Level of VEGF and Histological Feature of Central Vein in Liver Tissue</td>
</tr>
<tr>
<td>7</td>
<td>OP 07</td>
<td>Tira Bayuina (Syarif Hasyatussiah University) Effect of Jatropha curcus L. Seed Extract in LDH Activity and Histological Feature in Liver</td>
</tr>
<tr>
<td>8</td>
<td>OP 08</td>
<td>Susi Kusumantoro (Agency for Assessment and Application of Technology (BPPT)) Standardization of Labu Siam Fruit (Sectium edule Jacq Swartz) as Component of Cholesterol-Lowering Herbal Medicine</td>
</tr>
<tr>
<td>9</td>
<td>OP 09</td>
<td>Linda Wenti (YARSI University) Effect of Thymoquinone on Insulin Secretion in BRIN BD 11 Treated with Alloxan</td>
</tr>
<tr>
<td>10</td>
<td>OP 10</td>
<td>Eva Mariama (Maulana University) The Anticancer activities of Macaranga hirsuta leaves Against HeLa Cell</td>
</tr>
</tbody>
</table>
The Effects of *Jatropha curcas* Seed Extract to Protein Level of VEGF and Histological Feature of Central Vein in Liver Tissue

Nabilah Aulia Hasanuddin¹, Rz Ayu Fitri Hapsari², Endah Wulandari¹

¹A student of Medical Program, ²Department of Histology and ³Biochemistry, Faculty of Medicine and Health Sciences of State Islamic University Syarif Hidayatullah, Jakarta

**Background:** Tumor caused by mechanism of rapid proliferation of normal cells and uncoordinated with tissue activity, by with cause organ disfunction. Hence, it required to inhibition proliferation of the tumor. The tumor cell activate various channels to supply oxygen, such by angiogenesis or by formation of blood vessels. Decreased activity of VEGF may cause suppression of tumor cell activity. *Jatropha curcas* L. reported has various activities as anti-tumor agent, antimetastatic and antiproliferative in melanoma cases. The study is purposed to investigate the effect of *Jatropha curcas* L. seed extract to protein level of VEGF and histological of central vein liver tissue.

**Methods:** Mice was orally given by various doses of *Jatropha curcas* seed extract by 0, 5, 25, 50 and 250 mg/KgBW for 28 days. The VEGF levels in liver was measured by ELISA and observation of histological feature of central vein in liver by hematoxylin-Eosin staining. Data were analyzed statistically using one way-ANOVA.

**Results:** The VEGF level in Jatropha doses of 25 and 250 mg/KgBW has increased to 407 and 377 pg/mL; and has decreased by jatropha doses of 5 and 50 mg/KgBW to 270 and 260 pg/mL. Analysis by linear regression performed increasing trend in (ANOVA, p > 0.05). The central vein abnormal histology demonstrated dosing Jatropha seed extract 250 mg/KgBW. In this study showed that the extract of Jatropha seeds can damage the central vein. But there efforts to repair damaged liver cells through angiogenesis through increased VEGF protein.

**Conclusion:** The *Jatropha curcas* has no evidence anti-tumor effect by anti-VEGF pathway.

**Keywords:** Jatropha, VEGF, central vein, liver
Effects of *Jatropha curcas* L Seed Extract to Protein Level of VEGF and Histological Image of Central Vein in Liver Tissue

Nabilah Aulia Hasanuddin, Rr Ayu Fitri Hapsari, Endah Wulandari

1 A student of medical Program, 2 Department of Histology and 3 Biochemistry, Faculty of Medicine and Health Sciences of State Islamic University Syarif Hidayatullah, Jakarta.

Email: nblhaulia7@gmail.com

ABSTRACT

**Background:** Tumor caused by mechanism of rapid proliferation of normal cells and uncoordinated with tissue activity, by with couse organ disfunction. Hence, it required to inhibition proliferation of the tumor. The tumor cell activate various channels to supply oxygen, such by angiogenesis or by formation of blood vessels. Decreased activity of VEGF may cause suppression of tumor cell activity. Jatropha curcas L reported has various activities as anti-tumor agent, antimetastatic and antiproliferative in melanoma cases. The study is purposed to investigate the effect of Jatropha curcas L seed extract to protein level of VEGF and histological of central vein liver tissue. **Methods:** Mice was orally given by various doses of jathropha seed extract with 0, 5, 25, 50 and 250 mg/KgBW for 28 days. The VEGF levels in liver was measured by ELISA and observation of histological image of central vein liver tissue by Hematoxylin-Eosin staining. Data were analyzed statistically using one way-ANOVA. **Results:** The VEGF level in Jatropha doses of 25 and 250 mg/KgBW has increased to 407 and 377 pg/mL; and has decreased by jatropha doses of 5 and 50 mg/KgBW to 270 and 260 pg/mL. Analysis by linear regression performed increasing trend in (ANOVA, p > 0.05). The central vein abnormal histology demonstrated dosing jatropha seed extract 250 mg/KgBW. In this study showed that the extract of Jatropha seeds can damage the central vein. But there are efforts to repair damaged liver cells through angiogenesis through increased VEGF protein **Conclusion:** The Jatropha curcas has no evidence anti-tumor effect by anti-VEGF pathway. **Key words:** Jatropha, VEGF, central vein, liver

INTRODUCTION

Tumor (neoplasm) is an abnormal mass that derived from normal cells. The proliferation cell process is very fast and it is not coordinated with the needs of tissue, thereby that is disrupting function of organ. Neoplastic cells have the ability to continue grow progressively even though the stimulant that caused already lost or stopped. Tumors is a major cause of morbidity and mortality in the world, with an estimated 14 million new cases and 8.2 million deaths in 2012 and it is expected to increase reach out 70% in two decades which well come. Tumors was differentiated into benign and malignant tumors. Benign tumors do not invasion around tissue and that is growth is localized. Malignant tumors have ability to very high cell proliferation that is destructive. It can surrounding tissue and enter circulation to metastasize of other places. The Benign tumors have potential to develop into malignant. Both benign and malignant tumors need inhibition against for the proliferation (Price and Wilson. 2006).
Tumors are caused changes in the composition of genes that regulate growth, apoptosis, and DNA repair. Each tumor genes caused changes in physiology cell, for example the ability to generate their own growth signals, insensitivity to growth inhibitory signals, ability to avoid apoptosis, limitless replication potential, sustained angiogenesis, and metastatic ability. The excessive growth of cells need higher of nutrients and oxygen to increasing energy needs. Because, the existing blood vessels insufficient for supply oxygen tissues that generally hypoxic in tumor (Kumar et al. 2004).

In the hypoxic conditions, tumor cells activate various channels to supply the needs of O₂. One of them through it is the angiogenesis process, or the formation of new blood vessels. The new vessels will facilitate the growth and survival of tumor cells by optimizing supply of nutrients and oxygen that accompany cell proliferation. The process of angiogenesis is stimulated by vascular endothelial growth factor (VEGF), which works to increasing the endothelial cell migration and mitosis, forming a vascular lumen, and vascular permeability increasing through fenestration vessel formation. The activity of VEGF decreased thought thres is suppress tumor cell activity. The anti-VEGF compounds can inhibit activity of proliferation endothelial cell in capillary formation, and it can suppress growth tissue. The anti-VEGF compounds were shown to inhibit the growth of tumors is bevacizumab and ranibizumab (Sukhramani and Suthar, 2010).

Until now, It is various efforts to suppress tumor growth continued. Jatropha (Jatropha curcas L) informed as one of role as anti-tumor agent. Lin (2003) showed that curcín compounds contained in Jatropha seeds have anti-tumor activity through the binding mechanism of N-glycosidase that inactivates ribosomes into it can not synthesize proteins. In this case allegedly curcín compound can suppress the synthesis of VEGF as a molecular protein (Lin et al., 2003). Moreover, Balaji (2009) showed that the compound methanolic of Jatropha curcas has antimetastatic and antiproliferative activity on melanoma cases in the lungs mouse (Balaji et al., 2009).

This study uses the liver as a model because the liver has a capillary tissue with high permeability. In addition, the liver plays an important role in the metabolism and the transport of oxygen and nutrients through the vaskular (Tortora and Derrickson., 2012). Based on the above problems this study was conducted to determine the effect of extract Jatropha curcas L seed in VEGF levels and histology central vein of liver tissue.

**METHODE**

**Materials.** Materials utilized in the study were as followed: rat of Sprague-Dawley, extract Jatropha curcas L seed from BALITRO institution (Bogor), the materials for ELISA technique were Mouse VEGF ELISA kit (Cusabio Biotech, Newark, New Jersey), phosphate buffered saline (PBS) pH 7.4, terraced alcohol, toluene, paraffin, Hematoxylin-Eosin (HE), canada balsam.

**Methods.** The study performed analytical experimental study. Liver tissues were obtained from 25 rats with 5 group given doses of extract jathropha seed (0, 5, 25, 50 and 250 mg/KgBW) for 28 days. The study had been approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, University of Indonesia. The study was conducted in Faculty of Medicine and Health Sciences of State Islamic University Syarif Hidayatullah, Jakarta. The evaluation of VEGF protein level using ELISA was performed at the Laboratory of Biochemistry and evaluation histology of central vein at laboratory of histology.
**ELISA technique for detecting the level of VEGF protein.** The level of VEGF protein was measured using ELISA Kit Cusabio. The specimens used were 30 mg homogenates of liver tissues in 100 µL phosphate buffered Saline (PBS) pH 7.4. The steps were as follows: the method was optimized by performing antigen titration through dilution of standard protein. The standard concentrations were 0; 0.0625; 0.125; 0.25; 0.5 (ng/mL) for quantifying protein VEGF level. After the standard had been made, a microplate was prepared, which had been coated with primary antibody. About 100 µL of each specimen and standard were transferred to microplate well and subsequently were incubated for 2 hours at 37°C. Following the incubation, the supernatant was discarded and the well was rinsed 3 times with Wash Buffer. About 100 µL HRP-avidin was added and then it was incubated for 1 hour at 37°C. The supernatant was discarded and the well was rinsed 5 times with Wash Buffer. About 90 µL TMB-substrate was transferred into the well and it was incubated in a dark room for 15-30 minutes at 37°C. Furthermore, 50 µL stop solution was added and a color yielded, in which the absorbance could be read using ELISA reader at 450 nm wavelength.

**Preparing Histological Slides.** The liver tissue obtained from biopsy was immersed in cold 0.9% NaCl; then, it was cut in 3-5 mm thickness. Furthermore, a fixation was performed by transferring it into 10% formalin solution. Next, dehydration was performed by immersing the specimen in increasing concentration of 70% alcohol incubated for 24 hours, 80% alcohol incubated for 24 hours, 95% alcohol incubated for 24 hours, 100% alcohol for 2 x 24 hours. Afterward, clearing was performed by immersing the specimen in xylol 2 x 24 hours. Then, the embedding was performed, i.e. by infiltrating the specimen with liquid paraffin. After the tissue specimen was ready, it was cut into sections with microtome in 4-5 µm thickness. The sections were then taken using a brush and were transferred to a water bath so that they were allowed to widen. The sections were carefully transferred to a warm water bath at 40-46°C. At this point, the sections were trimmed and transferred onto a slide that had been smeared with eiwit (egg white and glycerin), which served as an adhesive. The slide and tissue specimen on it were set in a special shelf and transferred into incubator at 40-60°C for 24 hours or until the slide was ready for staining. Histologic staining is done with Hematoxylin-Eosin. Cell qualification was performed in 5 high power field (HPF) for each slide of liver tissue. High power field was determined as 40x magnification, which included: upper, lower, central, left, and right margins.

**Statistical Analysis.** The data are analyzed significant by one way-ANOVA was performed when the distribution in each group was normal; or Kruskal Wallis test was performed when the distribution in one of the groups was abnormal. Difference was considered significant when the $p < 0.05$.

**RESULT AND DISCUSSION**

**Result**

Data of VEGF protein level in liver tissue are presented in figure 1. The results are showed that VEGF protein level was given extract jatropha seed doses 25 and 250 mg/KgBW increased (407 and 377 pg/mL); the Jatropha doses of 5 and 50 mg/KgBW decreased (270 and 260 pg/mL). In a linear regression trend in Jatropha seed extract increased levels of VEGF not-significantly (ANOVA, $p > 0.05$).
Figure 1. Data of VEGF protein level in liver tissue with given extract jatropha seed doses 0, 5, 25, 50 and 250 mg/KgBW (ANOVA, p > 0.05).

Figure 2. Histology of central vein in liver tissue with it was given extract jatropha seed doses (A) 0, (B) 5, (C) 25, (D) 50 and (E) 250 mg/KgBW (a = normal of central vein, the hepatic lamina is demarcated; b = abnormal of central vein, the hepatic lamina is not demarcated).
Observation of histological image of central vein in liver by hematoxylin-Eosin staining in figure 2 and table 1. The central vein normal histology demonstrated dosing jatropha seed extract 250 mg/KgBW. The central vein abnormal histology demonstrated dosing jatropha seed extract 250 mg/KgBW. In this study showed that the high dose extract of Jatropha seeds can damage the central vein.

Table 1. Observation of central vein liver rat with given jatropha extract seed doses

<table>
<thead>
<tr>
<th>Doses of jatropha seed extract (mg/KgBW)</th>
<th>Lot of Central Vein in liver rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0</td>
<td>V</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
</tr>
<tr>
<td>25</td>
<td>V</td>
</tr>
<tr>
<td>50</td>
<td>V</td>
</tr>
<tr>
<td>250</td>
<td>V</td>
</tr>
</tbody>
</table>

Discussion

The increased VEGF stimulated by a variety of inflammatory cytokines and growth factors, such as epidermal growth factor (EGF), interleukin-1β (IL-1β), platelet derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), and transforming growth factor-β1 (TGF-β1). VEGF can also increased in the wound healing process especially granulation phase. VEGF can attract hematopoietic precursor and endothelial cells from bone marrow into the blood circulation (Speca et al., 2012). VEGF may also increased by genetic changes, it is include malignancy because loss of p53 tumor suppressor gene and the activity of oncogenes such as Kras, Vrsc, E6 and Her2 (Kerbel et al, 1998). This study was increased concentration of VEGF with the extract of Jatropha seeds, but the stimulation of mechanism has not known.

VEGF concentration increased by pressure of oxygen as a regulator. Hypoxia can induces VEGF quickly. Otherwise normal oxygen levels (normoxia), it is decreased VEGF expression or stable. When cell hypoxia, hypoxia inducible factor-1 (HIF-1) is stable, a transcription factor that menstimlasi release of VEGF (Ziemer et al., 2001; Ohno et al., 2012). VEGF will binds to VEGF receptors on endothelial cells, then it is triggering a tyrosine kinase pathway for implementation of angiogenesis function. It is usefull for cell survival. VEGF can be potential for cancer treatment by angiogenesis. Angiogenesis process is include 1) mitogenesis endothelial cells of blood vessels, 2) mediates secretion and activation of enzymes that play a role in matrix degradation ekstrseluler, 3) maintaining endothelial cells from apoptosis, 4) mobilized endothelial precursor cells in the bone marrow to begin the process of vascularization, 5) stimulates the migration of endothelial cells at the site of angiogenesis (Nishida et al., 2006). Central vein is damaged on jatropha seed extract dose of 250 mg/ KgBW. Until at jatropha seeds extract dose of 50 mg/KgBW was not damage central vein. It is suspected VEGV stimulate to maintain structure of central vein from damaged.

The increased of VEGF concentration was highest at a dose of 25 m/KgBW. The increased VEGF may occur due to the toxicity extracts of jatropha seed that activate
protection mechanism of cells with increased expression of VEGF which it is aim to improve the condition of the cells through increased vaskularization (Sozmen et al., 2014). In the treatment group a dose extract jatropha seed of 5 mg/KgBW, increased levels of VEGF protein has not happened because it still relatively safe dose. It is not cause tissue damage. Lowest levels of VEGF protein found also in the treatment group a dose of 50 mg/KgBW. The high level of cell damage that causes the cell is not able to do a protection mechanism by increasing the expression VEGF (Augustin, 2004). On treatment group a dose extract jatropha seed of 250 mg/KgBW, increased levels of VEGF protein as compared to a dose of 50 mg/KgBW. This is most likely due to the removal of the resin does jatropha seed extract dose of 250 mg/KgBW which it can reduce the toxicity extract of jatropha seed. Cells can still respond protection by increasing the expression of VEGF.

This study showed that the jatropha seed extract was stimulates increased VEGF level. The function of VEGF plays a role in the process angiogenesis. The increasing concentrations of VEGF is caused by the compound of extracts of Jatropha seeds. The results of phytochemical screening, jatropha seeds have alkaloids and terpenoids compound which both have pro-wound healing activity. The process of wound healing stimulates increased expression VEGF (Ferara and Gerber, 2003; Nwala et al, 2013)

Jatropha seed extract with a low dose is not affect the central vein structure within 28 days. Angiogenesis process have several phase by the initiation of the VEGF protein. The phase is vasodilatation and increased vascular permeability, separation perisit, degradation of basale membrane by matrix-metalloproteinase enzymes, migration and proliferation of endothelial cells, capillary lumen formation, and cell mobilization periendothelium (Dulak et al., 2013). Another possibility was the process of angiogenesis at the level of capillary endothelial proliferation so that an image is not visible. Jatropha seeds extract of high dose (250 mg / KgBW) showed central vein have hepatic lamina undemacrated. This is due to exposure of toxic compounds by endothelial cell proliferation mechanism that causes the connective tissue of vascular occlusion vein (Haschek and Rousseaux, 1998).

CONCLUSION

The Jatropha curcas has no evidence anti-tumor effect by anti-VEGF pathway. Jatropha seed extract high dose (250 mg / KgBW) causes damage in the central vein.

ACKNOWLEDGMENT

The authors acknowledge the financial support of this study by the State Islamic University Syarif Hidayatullah by “Development of Science research grants 2016”.

REFERENCES


