Tingkat Kepuasan Pengguna Rawat Inap Rumah Sakit Pemerintah di Indonesia
Olah raga sebagai Salah Satu Bentuk Aktivitas Fisik


"Karena itu, pemerintah terus melibatkan dia dalam kegiatan olah raga, baik di sekolah-sekolah, kantor-kantor pemerintah dan swasta, maupun di tengah-tengah kehidupan masyarakat."


Tinggal bagaimana kita menyikapi hal ini, berpartisipasi aktif dalam olahraga demi kesehatan atau tetap mengikuti gaya hidup yang tak menyehatkan. Pilihan ada pada Anda. Redaksi.
Alteration of Ossification Rate on Fetal Humerus and Femur Swiss Webster Mice (*Mus musculus*) as the Teratogenic Effects of Gadung (*Dioscorea hispida* Dennst)

**Abstract**

Background: On every 100 g dry tuber of gadung (*Dioscorea hispida*) contains approximately 0.45% diogenin. Methodology: Pregnant Swiss Webster mice (*Mus musculus*) were given single dosage dried tuber of *D. hispida* which were equivalent with 20, 40, and 80 mg/kg body weight (bw) diogenin by gavage on gestation day (GD) 6 and 11, whereas the control group were administrated sterilized distilled water. The dams were killed by cervical dislocation on GD 18, and then were dissected for fetal observation. Humerus and femur bone developments of the obtained fetus were measured after staining with Alizarin Red S. Results: Ossification length rate of femur increased by 40 mg/kg bw treatment on GD 6 and 11, meanwhile only on GD 11 ossification length rate of humerus influenced to increase after 40 mg/kg bw treatment. Generally ossification width rates were not influenced by *D. hispida* treatment. Conclusion: Administration the single dosage of dried tuber of *D. hispida* which is equivalent with diogenin 40 mg/kg bw by gavage on GD 11 of mice influence significantly to increase ossification rate trough the acceleration of epiphyseal osteogenetic activity in the fetal humerus and femur.

Key words: *Dioscorea hispida*, diogenin, mice, humerus, femur, ossification

**Introduction**

Gadung (*Dioscorea hispida* Dennst) is a climbing plant which is well distributed and easy to find in Bengkulu, Sumatera, Indonesia. It was reported that tuber of the plant contains average 0.45% diogenin ([C$_2$H$_2$O$_2$]), a steroidal saponin, on every 100 g dry tuber,¹ (Sutarno and Astramidjio, 1998). Diosgenin were tested on preimplantation development of mouse embryos. It caused embryonic arrest at about the 16-cell stage, and the influence was not reversible by mevalonic acid. Meanwhile cholesterol was able to rescue 50% of the embryos but the effect of Diosgenin could be non-specific and probably caused by its entry...
into the plasma membrane (Surani et al., 1983). Estrogenic action of diosgenin on the mammary epithelium of ovariectomized (OVX) mouse has been reported. Diosgenin when administered at the dose levels of 20 and 40 mg/kg body weight (bw) for a period of 15 days stimulated the growth of mammary epithelium. This was indicated by the increase in DNA content, increase in number of ducts and appearance of terminal end buds. There was a significant increase in the mammary development scores in the presence of diosgenin. Concomitant treatment of estrogen and diosgenin showed augmentation of estrogenic effect of diosgenin especially at the higher dose level (40 mg/kg bw). Diosgenin showed a lack of progesterogenic action as was apparent from the absence of alveolar development even in the presence of exogenous estrogen (Aradhana, 1992).

Diosgenin present on *Trigonella foenum* and other plants has been shown to suppress inflammation, inhibit proliferation, and induce apoptosis in a variety of tumor cells, but through a mechanism that is poorly understood. Diosgenin suppresses proliferation, inhibits invasion, and suppresses osteoclastogenesis through inhibition of NF-kappaB-regulated gene expression and enhances apoptosis induced by cytokines and chemotherapeutic agents (Shishodia and Aggarwal, 2006). The antitumorporotic activity of the 90% EIOH fraction of the water extract of rhizomes of *Dioscorea spongiosa* and methyl-diosdiosin, its major constituent, were examined in the model of postmenopausal bone loss using OVX rats or mice. After 6 weeks treatment, the proximal tibia of rats or mice and the distal femora of mice were scanned by peripheral quantitative computed tomography (pQCT). The 90% EIOH fraction (100 mg/kg bw) significantly inhibited bone loss in bone mineral content (BMC) and bone mineral density (BMD) in total, cancellous and cortical bones, and the decrease in bone strength indices induced by OVX, without side effect on the uterus (Yin et al., 2004). Furthermore it was reported that Diosgenin which was extracted from the root of *Dioscorea villosa*, has been reported to demonstrate an opportunity for medical application. Vascular endothelial growth factor-A (VEGF-A) plays an important role in bone-related angiogenesis, a critical process occurring during bone formation and fracture healing. Diosgenin up-regulates VEGF-A and promotes angiogenesis in preosteoblast-like cells by a hypoxia-inducible factor-1 dependent mechanism involving the activation of src kinase, p38 MAPK, and Akt signaling pathways via estrogen receptor (Men et al., 2005). It was also reported that diosgenin inhibits melanogenesis by activating the Pi3K pathway, and also suggests that diosgenin may be an effective inhibitor of hyperpigmentation (Lee et al., 2007).

Based on mentioned above indicated that diosgenin is a futuristic natural product with the opportunity for medical application. Meanwhile at the level of morphological and histological observation, Sugiyanto (1993) concluded that diosgenin is toxic substance and has a slight teratogenic effect on the developing mice fetuses. Furthermore this study was aimed to understand alteration ossification rate of fetus limb as the teratogenic effect of diosgenin in mice.

**Material and Methods**

**Preparation Dry Tuber of *D. hispida***

Fresh tubers of *D. hispida* were collected from the local official authority of agriculture in Rejang Lebong district, Bengkulu province, Indonesia. The fresh tubers were washed carefully, cutting to be smaller pieces, and then dried at 68.9°C for 72 hours (Desrosier, 1998). The dried tuber of *D. hispida* was crushed to be finely flour, filtered using the 180 mesh size, and stored at -4°C ready to use as tested material.

**Experimental Animals**

Swiss Webster mice (*Mus musculus*) were used as experimental animals. Rearing the animals were done in a room at 23-27°C and 83%-94% humidity. Food and water were given ad libitum. When female mice achieved their sexual maturity (10-12 weeks old) they were mated with a male (1:1). A vaginal plug detected on the following morning was defined as day 0 of gestation (Sudarwati et al., 1995).

**Dosage and Sample Collection**

It was reported that each of 100 g dry tuber of *D. hispida* contains diosgenin approximately 0.45%, the remaining substances are carbohydrate 88.34%, protein 5.28%, and lipid 0.23% (Sutarno and Atmowidjijo, 1998). Aradhana (1992) reported that effective dosage of diosgenin in mice is 20-40 mg/kg bw. Equivalent with single dosage of diosgenin 20 and 40 mg/kg bw, were given by gavage on gestation day (GD) 6 and 11, whereas the control group were
administered sterilized distilled water. The dams were killed by cervical dislocation on GD 18; their fetuses were isolated, weighed, observed for morphological malformation, and then fixed with alcohol 96% during one week for skeletal staining.

**Skeletal Staining**

The fixed fetuses were stained by 0.01% Alizarin Red S on 1% KOH to make color of ossified part of the bones were red. Calcium forms an Alizarin Red S-calcium complex in chelating process, and then it was determined as ossified part of the fetus skeleton (Conn et al., 1960; Ruyani et al., 1991).

**Humerus and Femur Measurement**

Humerus and femur bones were isolated from the stained bone fetuses, and then their both length and width of ossified parts were determined below:

1. Length ossified part: Length of bone (a) was measured between two tips of the bone by caliper; meanwhile ossified part (b) was measured on stained (red) area of the bone using the same caliper. Furthermore index of length ossified part was determined by dividing the length of bone with the ossified part (arb; Ruyani et al., 1991).

2. Width ossified part: Cross section was made at a middle of the bone, and then obtained ring was measured by micrometer under the microscope. Diameter of cross section (a) and lumen (b) of the bone were used for calculating width of cross section (A) and lumen (B) respectively. Width of ossified part (C) was obtained by diminishing width of cross section with width of lumen. Furthermore index of width ossified part was determined by dividing the width of ossified part with the width of cross section (CIA; Ruyani et al., 1991).

**Results and Discussion**

Qin et al. (2009) revealed that diosgenin did not show any sign of toxicity up to oral dose of 562.5 mg/kg bw in mice. The effect of diosgenin on the prenatal development of the mouse embryos has been studied. Diosgenin was administrated daily to pregnant mice on GD 6 up to GD 13 with the doses 15 mg, 20 mg, and 30 mg per day per mouse. It was concluded that diosgenin is toxic substance and has a slight teratogenic effect on the developing fetuses (Sugiyanto, 1993). Furthermore this research succeeded in collecting some 173 fetal mice from 24 pregnant dams as shown in Table 1. Treatment with the single dosage of D. hirsuta dry tuber equivalents diosgenin 20 and 40 mg/kg bw by gavage on GD 6 of mice, each appeared only one (3%) fetal malformation, contrary the similar treatment on GD 11 did not cause any morphological abnormality of the produced fetus. The facts revealed that treatment with the single dosage of 20 and 40 mg/kg bw in pregnant mice were not significantly affected to appear the morphological changes in the resulting fetus. Of course teratogenic effects of the compound it is not sufficient if only be studied through the morphological changes, the next stage needs to be studied in more detail, such as, anatomy and histology (Ruyani et al., 1991).

Table 1: Malformation in mice after administration dry tuber of D. hirsuta which were equivalent with 0, 20, and 40 mg/kg bw diosgenin by gavage on gestation day (GD) 6 and 11, and then the dams were killed on GD 18

<table>
<thead>
<tr>
<th>Gestation day (GD)</th>
<th>Equivalent with diosgenin (mg/kg bw)</th>
<th>Number of dam</th>
<th>Number of fetus (%)</th>
<th>Fetus Morphology</th>
<th>Malformation (%)</th>
<th>Normal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0</td>
<td>3</td>
<td>33 (100)</td>
<td>0 (0)</td>
<td>33 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3</td>
<td>29 (100)</td>
<td>1 (3)</td>
<td>28 (97)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>29 (100)</td>
<td>1 (3)</td>
<td>28 (97)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>3</td>
<td>34 (100)</td>
<td>0 (0)</td>
<td>34 (100)</td>
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<td></td>
<td>20</td>
<td>3</td>
<td>22 (100)</td>
<td>0 (0)</td>
<td>22 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>26 (100)</td>
<td>0 (0)</td>
<td>26 (100)</td>
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</tr>
</tbody>
</table>

Physiological process involving the growth of new blood vessels from pre-existing vessels is determined as angiogenesis. Though there has been some debate over this, vasculogenesis is the term used for spontaneous blood-vessel formation, and intussusception is the term for new blood vessel formation by splitting off existing ones. Meanwhile angiogenesis is a normal process in growth and development, as well as in wound healing. However, this is also a fundamental step in the transition of tumors from a dormant state to a malignant state. These blood vessels...
bring minerals like calcium and deposit it in the ossifying tissue. Bone formation is a
dynamic process, with cells called osteoblasts
depositing minerals, and osteoclasts remov-
ing bone. This process, termed bone remo-
deling continues throughout life. Bone of the
developing skeleton can be reliably differen-
tiated in whole-mount preparations with
Alizarin Red S staining after fixation (Jens
and Brent, 1966; Ruyani et al., 1991).

The effects of treatment with 20 and 40
mg/kg bw by gavage on GD 6 and 11 of mice
could be viewed from index of length ossified part (Table 2 and 4). Obtained data indicated
that 40 mg/kg bw caused significantly to in-
crease the index of length ossified part in fe-
mur on GD 6 and 11 compared 40 mg/kg bw
and the control. Meanwhile the index of
length ossified part in humerus only on GD
11 which was increased significant by 40
mg/kg bw treatment. Furthermore index of
width ossified part in femur and humerus (Table 3 and 5) were not generally influenced
by 20 and 40 mg/kg bw treatment on GD 6
and 11, except 20 mg/kg bw treatment
caused significantly to decrease the index of
width ossified part in femur on GD 11. Based
on the realities it should be generalized that,
(a) GD 11 is more sensitive rather than GD 6,
(b) femur bone ossification is more sensitive
rather than humerus, (c) 40 mg/kg bw treatment is an effective single dosage of
diosgenin to increase ossification rate in mice
embryos, and (d) epiphyseal osteogenic
activity is more influenced by 40 mg/kg bw
treatment rather than periosteal osteogenic
activity. These sensitive period (a and b) in
accordance with the results of research
(Wanek et al. 1989) whom divides the normal
development of limb Swiss Webster (SW) mice
into fifteen stages starting from GD 10 until to 5 days after birth. At the end of GD 11
(stage 4), shoots a member of the distal-
chip flattened shape and form the proximal
part of stalks. Apical ectodermal ridge (AER)
formed visible, and there is sinus marginals
at a distance of approximately 1-3 cells below
the AER. Furthermore at the end of GD 12
(stage 7), limb bud began to form a penta-
gonal pieces, and the AER seems irreducible.
Stilpodium and zeugopodium would appear
elongated. Condensation of mesenchyme
cells to be finger ridge-4 and 3 was evident,
while the condensing mesenchyme cells will
be the second finger ridge began to form
(Wanek et al., 1989).

Table 2: Length humerus bone development in mice after administration dried tuber of D. hipda which were equivalent with 0, 20, and 40 mg/kg bw dios-
genin by gavage on gestation day (GD) 6 and 11, and then the dams were killed on GD 18.

| Gestation day (GD) | Equivalent with diosgenin (mg/kg bw) | Number of dam | Number of stained fetal bone | Bond (mm) [a] | Ossified part (mm) [b] | Index [c]
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>6</td>
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<td>3</td>
<td>27</td>
<td>3.09±0.42</td>
<td>1.73±0.37</td>
<td>0.57±0.10a</td>
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<td>20</td>
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<td>24</td>
<td>3.28±0.31</td>
<td>2.08±0.38</td>
<td>0.63±0.11b</td>
</tr>
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<td></td>
<td>40</td>
<td>3</td>
<td>29</td>
<td>3.05±0.62</td>
<td>2.14±0.52</td>
<td>0.60±0.15b</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>3</td>
<td>24</td>
<td>2.94±0.17</td>
<td>1.70±0.39</td>
<td>0.57±0.08b</td>
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<td>22</td>
<td>2.57±0.29</td>
<td>1.24±0.12</td>
<td>0.49±0.06a</td>
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<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>26</td>
<td>4.03±0.48</td>
<td>2.54±0.40</td>
<td>0.63±0.06b</td>
</tr>
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</table>

a, b, c indicated the results of LSR (Least Significant Ranges; Steel and Torrie, 1981) test for lines on the same column.

Table 3: Length humerus bone development in mice after administration dried tuber of D. hipda which were equivalent with 0, 20, and 40 mg/kg bw dios-
genin by gavage on gestation day (GD) 6 and 11, and then the dams were killed on GD 18.

| Gestation day (GD) | Equivalent with diosgenin (mg/kg bw) | Number of dam | Number of stained fetal bone | Bond (mm) [a] | Ossified part (mm) [b] | Index [c]
<table>
<thead>
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<tbody>
<tr>
<td>6</td>
<td>0</td>
<td>3</td>
<td>27</td>
<td>0.28±0.09</td>
<td>0.26±0.09</td>
<td>0.71±0.15a</td>
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<td>0.28±0.07</td>
<td>0.80±0.13a</td>
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<td>0.75±0.16a</td>
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<td>0.52±0.10</td>
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a, b, c indicated the results of LSR (Least Significant Ranges; Steel and Torrie, 1981) test for lines on the same column.
Table 4: Length femur bone development in mice after administration of D. nipitaria which were equivalent with 0, 20, and 40 mg/kg bw of asiagenin by gavage on gestation day (GD) 6 and 11, and then the dams were killed on GD 18.

<table>
<thead>
<tr>
<th>Gestation day (GD)</th>
<th>Equivalent with asiagenin (mg/kg bw)</th>
<th>Number of dam</th>
<th>Number of stained fetal bone</th>
<th>Bond (mm)</th>
<th>Osified part (mm)</th>
<th>Index [b]</th>
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<tr>
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<td></td>
<td>26</td>
<td>3.23±0.29</td>
<td>1.97±0.34</td>
<td>0.56±0.06</td>
</tr>
</tbody>
</table>

a, b, c indicated the results of LSR (Least Significant Ranges), Steel and Tonie, 1981 test for lines on the same column.

Table 5: Width femur bone development in mice after administration of D. nipitaria which were equivalent with 0, 20, and 40 mg/kg bw of asiagenin by gavage on gestation day (GD) 6 and 11, and then the dams were sacrificed on GD 18.

<table>
<thead>
<tr>
<th>Gestation day (GD)</th>
<th>Equivalent with asiagenin (mg/kg bw)</th>
<th>Number of dam</th>
<th>Number of stained fetal bone</th>
<th>Bond (mm)</th>
<th>Osified part (mm)</th>
<th>Index [b]</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>0</td>
<td>3</td>
<td>27</td>
<td>0.29±0.03</td>
<td>0.19±0.08</td>
<td>0.75±0.11</td>
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<tr>
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<td></td>
<td>24</td>
<td>0.31±0.05</td>
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<td>29</td>
<td>0.32±0.09</td>
<td>0.24±0.05</td>
<td>0.78±0.08</td>
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<td>24</td>
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<td>0.18±0.08</td>
<td>0.73±0.11</td>
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<tr>
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<td>22</td>
<td>0.22±0.05</td>
<td>0.11±0.05</td>
<td>0.51±0.15</td>
</tr>
<tr>
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<td>3</td>
<td>26</td>
<td>0.37±0.12</td>
<td>0.28±0.09</td>
<td>0.73±0.16</td>
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</table>

a, b indicated the results of LSR (Least Significant Ranges), Steel and Tonie, 1981 test for lines on the same column.

seen in the femur by GD 15 and is more extensive by GD 16. As stated previously on GD 18 we determined ossified part of fetal humerus and femur, so we studied only pre-natal long bone development. It is well accepted that chondrocytes in the primary center of ossification begin to grow (hypertrophy). They stop secreting collagen and other proteoglycans and begin secreting alkaline phosphatase, an enzyme essential for hypertrophic chondrocytes (Wu et al., 1997). Furthermore they occur both calcification of the matrix and apoptosis of the hypertrophic chondrocytes (Raupuroith et al., 1999). Defective vascular invasion of cartilage leads to enlargement of hypertrophic zones of growth plates and delayed formation of secondary ossification centers in long bones (Zhou et al., 1999). From the results of our current study, we suggest that administration of a single dosage of 40 mg/kg bw by gavage on GD 10 of mice influence significantly to increase ossification rate through the activation of epiphysial osteogenic activity in the fetal femur. Meanwhile (Corbiere et al. 2004) also suggested that asiagenin is a plant steroid which is known to induce apoptosis. The effects of this research revealed that the asiagenin administration cause to accelerate endochondral ossification and it is predicted that shortening of bones is a consequence of decreased chondrocyte proliferation in the proliferative zone of the growth plates.

During embryonic development it was described that vascular endothelial growth factor-A (VEGF-A) plays an important role in bone-related angiogenesis and a critical process occurring during bone formation (Holmes et al., 2007). Further information concerning two kind of VEGF-A, 137 amino acid (aa) and 190 aa, in mice can be accessed by number AAM55477 and AAH64168 respectively (NCBI). Although our currently study did not apply a protein analysis in order to understand mechanism of asiagenin in appearing a teratogenic effect, but some previous similar research could be used to explain the phenomenon. For example, (Men et al. 2005) proposed angiogenic signaling pathway activated by asiagenin in MC3T3-E1 cells. Asiagenin up-regulates VEGF-A and promotes angiogenesis in MC3T3-E1 cells by means of a HIF-1α-dependent mechanism involving the activation of the Akt and p38 MAPK signaling pathways via an estrogen receptor-mediated src kinase as illustrated in Fig 1. Other research revealed that asiagenin which was collected from fenugreek (Trigonella foenum graecum) and other plants, has been shown to suppress inflammation, inhibit proliferation, and induce apoptosis in a variety of tumor cells, but through a mechanism that is poorly understood. Asiagenin suppresses proliferation,
inhibits invasion and suppresses osteoclastogenesis through inhibition of NF-kappaB-regulated gene expression (Shishodia and Aggarwal, 2006).

Our current research revealed that treatment with the single dosage of dried tuber of *D. hispida* which is equivalent with 40 mg/kg bw by gavage on GD 10 of mice influence significantly to increase ossification rate through the acceleration of epiphyseal osteogenic activity in the fetal femur. Mechanism of the facts should be verified through angio- nogenic signaling pathway which was proposed by Men et al. (2005; Fig. 1). Meanwhile physiological and biochemical mechanisms of the signaling pathway can be explained by comparing the presence of certain proteins qualitatively and quantitatively which were obtained from cells or tissues under different conditions. This protein approach in the field of teratology, then called as teratoproteomics (Ruyani et al., 2004; Ruyani et al., 2005; Ruyani, 2010). Therefore teratoproteomics analysis may still be required to understand the phenomenon, and furthermore obtained data will be used as a consideration in implementing the natural diosgenin for some medical applications.

**Conclusion**

Administration the single dosage of dried tuber of *D. hispida* which is equivalent with diosgenin 40 mg/kg bw by gavage on GD 11 of mice influence significantly to increase ossification rate through the acceleration of epiphyseal osteogenic activity in the fetal humerus and femur.

![Figure 1: Diosgenin up-regulates VEGF-A and promotes angiogenesis in proosteoblast-like cells by a hypoxia-inducible factor-1 dependent mechanism involving the activation of src kinase, p38 MAPK, and Akt signaling pathways via estrogen receptor (reproduced with permission from Men et al., 2005).](image)

**Acknowledgment**

This research was supported by the local official authority of agriculture in Rejang Lebong district, Bengkulu province, Indonesia. We gratefully acknowledge Endang Dwi Hardani and Marini for their materials and valuable suggestions.

**References**

Jika hasil karya ilmiah Anda ingin dibaca oleh mereka - pemerhati bidang kesehatan dari pelosok negeri hingga manca negara, MEDICA adalah sarananya.

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