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Olah raga sebagai Salah Satu Bentuk Aktivitas Fisik

Ramadhan telah meninggalkan kita. Semarak Hari Raya Idul Fitri pun perlahan mulai sirna. Pemudik kembali ke kota. Pegawai kembali bekerja. Pedagang kembali menata barang dagangannya. Jalan-jalan raya di kota besar kembali padat. Aktivitas telah kembali seperti sedia kala.

Begitu pula dengan gaya hidup warga metropolitan, kembali seperti semula. Pola makan kembali pada konsumsi bahan pangan tinggi lemak dan rendah serat. Kebiasaan merokok masih berlanjut. Stres karena pekerjaan masih sering menghantui. Mobilitas warga lebih didominasi dengan sarana kendaraan bermotor dibandingkan dengan aktivitas fisik seperti berjalan kaki.

Ada baiknya kita meninjau ulang gaya hidup ala metropolitan tersebut. Gaya hidup ini merupakan keseharian sebagian besar warga kota besar seperti Jakarta. Padahal, sudah diketahui bahwa gaya hidup yang demikian ini akan berakibat pada semakin meningkatnya prevalensi penyakit kardiovaskular. Bukan hanya prevalensinya, kasus-kasus penyakit kardiovaskular juga terjadi pada usia yang lebih muda dibandingkan tahun-tahun sebelumnya.

Himbauan untuk selalu menerapkan gaya hidup sehat sudah digembar-gemborkan oleh banyak kalangan, termasuk pemerintah dan lembaga swadaya masyarakat. Anjuran mengatur pola makan, menghentikan kebiasaan merokok, melakukan aktivitas fisik, dan menghindari stres sudah kerap didengar. Bahkan, pemerintah sudah sejak 1948 menetapkan 9 September sebagai Hari Olah raga Nasional (HAORNAS). Olah raga di sini mencakup dua jenis, yakni olahraga kompetisi dan olah raga sebagai aktivitas fisik. Dalam pidato HAORNAS beberapa tahun lalu, Presiden Susilo Bambang Yudhoyono mengatakan bahwa kecintaan masyarakat akan olah raga masih perlu ditingkatkan. "Karena itulah, pemerintah terus mendorong dilakukannya kegiatan olah raga, baik di sekolah-sekolah, kantor-kantor pemerintah dan swasta, maupun di tengah-tengah kehidupan masyarakat."

Peran pemerintah dan swasta dalam memajukan olah raga, khususnya olah raga sebagai bentuk aktivitas fisik, semakin nyata saat ini. Hal ini terbukti dengan semakin banyak klub senam seperti senam jantung sehat, senam diabetes, senam osteoporosis, dan sebagainya. Peran ini semakin terasa dengan pemberlakuan Hari Bebas Kendaraan Bermotor setiap minggu ke-2 dan ke-4. Pada hari-hari tersebut, kendaraan bermotor dilarang melintas di Jalan Thamrin dan Sudirman. Sebaliknya, masyarakat bebas menggunakan jalan tersebut untuk berolah raga. Peran pemerintah juga dapat dilihat dari peresmian jalur sepeda di Jakarta Selatan. Meski baru beberapa kilometer, jalur ini membuktikan bahwa pemerintah memberikan respons positif atas kebutuhan warga akan sarana dan prasarana olah raga.

www.harianhi.com

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.**

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Alteration of Ossification Rate on Fetal Humerus and Femur Swiss Webster Mice (*Mus muculus*) as the Teratogenic Effects of Gadung (*Dioscorea hispida* Dennst)

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Abstract

Background: On every 100 g dry tuber of gadung (*Dioscorea hispida*) contains approximately 0.45 % diosgenin. Methods: Pregnant Swiss Webster mice (*Mus musculus*) were given single dosage dried tuber of *D. hispida* which were equivalent with 20, and 40 mg/kg body weight (bw) diosgenin 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 diosgenin 40 mg/kg bw by gavage on GD 11 of mice influence significantly to increase ossification rate trough the acceleration of epiphyseal osteogenic activity in the fetal humerus and femur.

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

Abstrak

Latar belakang: Pada setiap 100 g umbi kering Gadung (*Dioscorea hispida*) mengandung sekitar 0,45% diosgenin. Metodologi: Mencit (*Mus musculus*) Swiss Webster diberi dosis tunggal umbi kering *D. hispida* yang setara dengan 20, and 40 mg/kg berat badan (bb) diosgenin secara *gavage* pada umur kebuntingan (UK) 6 dan 11 hari, sementara kelompok kontrol hanya menerima pelarut air destilata steril. Induk mencit dibunuh pada UK 18 hari dengan cara dislokasi leher, lalu dibedah untuk pengamatan fetus. Perkembangan humerus dan femur fetus diukur setelah sebelumnya diwarnai dengan Alizarin Red S. Hasil: Laju panjang osifikasi femur meningkat akibat berlakuan 40 mg/kg bb pada UK 6 dan 11 hari, sementara hanya pada UK 11 hari laju panjang osifikasi humerus meningkat setelah mendapat perlakuan 40 mg/kg bb. Secara umum, laju lebar osifikasi tidak terpengaruh oleh perlakuan *D. hispida*. Kesimpulan: Pemberian secara gavage dosis tunggal umbi kering *D. hispida* yang setara dengan diosgenin 40 mg/kg bb pada mencit UK 11 hari nyata meningkatkan laju osifikasi melalui percepatan aktivitas osteogenik epifisis pada humerus dan femur fetus.

Kata kunci: *Dioscorea hispida*, diosgenin, mencit, humerus, femur, osifikasi

(Aceng Ruyani dkk., Medika 2011, Tahun ke XXXVII, No. 9, p. 596–603)

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 % diosgenin [$C_{27}H_{42}O_3$], a steroidal saponin, on every 100 g dry tuber,[1] (Sutarno and Atmowidjijo, 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 antiosteoporotic activity of the 90% EtOH fraction of the water extract of rhizomes of *Dioscorea spongiosa* and methylprotodioscin, 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% EtOH 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 indexes 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 *at 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 clearly, cutting to be smaller pieces, and then dried at 68.3°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 ware done in a room at 23-27 °C and 83% 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

administrated 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 (a/b; 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 (C/A; 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 administered 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. hispida* 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).

Alterations of ossification rate on experimental animal embryos commonly are used in studying teratogenic effect of certain substance. Ossification is the process of bone formation, in which connective tissues, such as cartilage are turned to bone or bone-like tissue as endocondral ossification (Brighton *et al.*, 1973). Beside of the other process intra membranous ossification, cartilage is present during endochondral ossification. It is also an essential process during the rudimentary formation of long bones, the growth of the length of long bones (humerus and femur), and the natural healing of bone fractures (Brighton and Hunt, 1986). The ossified tissue is invaginated with blood vessels. A 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

Table 1: Malformation in mice after administration dry tuber of D. *hispida* 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

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

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 removing bone. This process, termed bone remodeling continues throughout life. Bone of the developing skeleton can be reliably differentiated in whole-mount preparations with Alizarin Red S staining after fixation (Jensh 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 increase the index of length ossified part in femur 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 (e) 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 are sinus marginalis 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 pentagonal pieces, and the AER seems irreducible. Stilopodium 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).

Patton and Kaufman (1995) explained pattern of ossification on mice fore- and hind- limb during a period GD 15-18. The humerus has a primary centre of ossification by GD 15, and then this center rapidly extend along the shaft of the bone to include the deltoid tuberosity by GD 16. Meanwhile a small primerly centre of ossification in first

Table 2: Length humerus bone development in mice after administration dried tuber of D. *hispida* 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.

Gestation day (GD)	Equivalent with diosgenin (mg/kg bw)	Number of dam	Number of stained fetal bone	Length (X±SD)		
				Bond (mm) [a]	Ossified part (mm) [b]	Index [b/a]
6	0	3	27	3.00±0.42	1.73±0.37	0.57±0.07[a]
	20	3	24	3.28±0.31	2.08±0.38	0.63±0.11[a]
	40	3	29	3.65±0.62	2.14±0.52	0.60±0.15[a]
11	0	3	24	2.94±0.17	1.70±0.39	0.57±0.08[a]
	20	3	22	2.57±0.29	1.24±0.12	0.49±0.06[a]
	40	3	26	4.03±0.48	2.54±0.40	0.63±0.06[b]

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

Table 3: Width humerus bone development in mice after administration dried tuber of D. *hispida* 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.

Gestation day (GD)	Equivalent with diosgenin (mg/kg bw)	Number of dam	Number of stained fetal bone	Length (X±SD)		
				Bond (mm^2) [a]	Ossified part (mm^2) [b]	Index [b/a]
6	0	3	27	0.38±0.09	0.26±0.09	0.71±0.15[a]
	20	3	24	0.35±0.06	0.28±0.07	0.80±0.13[a]
	40	3	29	0.40±0.10	0.28±0.11	0.75±0.16[a]
11	0	3	24	0.38±0.10	0.26±0.08	0.70±0.15[a]
	20	3	22	0.32±0.06	0.19±0.06	0.60±0.15[a]
	40	3	26	0.52±0.10	0.37±0.10	0.71±0.17[a]

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

Gestation day (GD)	Equivalent with diosgenin (mg/kg bw)	Number of dam	Number of stained fetal bone	Length (X±SD)		
				Bond (mm) [a]	Ossified part (mm) [b]	Index [b/a]
6	0	3	27	2.52±0.42	1.21±0.23	0.40±0.06[a]
	20	3	24	2.80±0.32	1.39±0.25	0.59±0.08[b]
	40	3	29	2.88±0.54	1.75±0.59	0.62±0.06[c]
11	0	3	24	2.47±0.41	1.19±0.23	0.49±0.06[a]
	20	3	22	2.32±0.19	1.03±0.02	0.45±0.06[a]
	40	3	26	3.23±0.47	1.91±0.34	0.59±0.06[b]

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 dry tuber of D. *hispida* 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.

Gestation day (GD)	Equivalent with diosgenin (mg/kg bw)	Number of dam	Number of stained fetal bone	Length (X±SD)		
				Bond (mm^2) [a]	Ossified part (mm^2) [b]	Index [b/a]
6	0	3	27	0.28±0.09	0.20±0.08	0.75±0.11[a]
	20	3	24	0.31±0.05	0.24±0.06	0.76±0.12[a]
	40	3	29	0.32±0.09	0.24±0.05	0.78±0.08[a]
11	0	3	24	0.25±0.09	0.18±0.08	0.73±0.11[a]
	20	3	22	0.22±0.05	0.11±0.05	0.51±0.15[b]
	40	3	26	0.37±0.12	0.28±0.09	0.73±0.16[a]

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

Table 5: Width femur bone development in mice after administration dried tuber of D. *hispida* 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 scarified on GD 18.

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 prenatal 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 occurs both calcification of the matrix and apoptosis of the hypertrophic chondrocytes (Rajpurohit *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 the single dosage of 40 mg/kg bw by gavage on GD 10 of mice influence significantly to increase ossification rate trough the acceleration of epiphyseal osteogenic activity in the fetal femur. Meanwhile (Corbière *et al.* 2004) also suggested that diosgenin is a plant steroid which is known to induce apoptosis. The facts of this research revealed that the diosgenin administration cause to accelerate endocondral 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 indicated 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 AAH61468 respectively (NCBI). Although our currently study did not apply a protein analysis in order to understand mechanism of diosgenin in appearing a teratogenic effect, but some previous similar research could be use to explain the phenomenon. For example, (Men *et al.* 2005) proposed angiogenic signaling pathway activated by diosgenin in MC3T3-E1 cells. Diosgenin up-regulates VEGF-A and promotes angiogenesis in MC3T3-E1 cells by means of a HIF-1–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 diosgenin 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. Diosgenin suppresses proliferation,

inhibits invasion,and suppresses osteoclastogenesis through inhibition of NF-kappaB-regulated gene expression (Shishodia and Aggarwal, 2006).

Our currently 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 trough the acceleration of epiphyseal osteogenic activity in the fetal femur. Mechanism of the facts should be verified through angiogenic 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 trough the acceleration of epiphyseal osteogenic activity in the fetal humerus and femur.

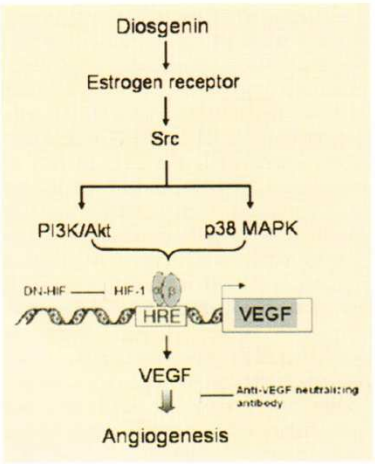

Figure 1: Diosgenin up-regulates VEGF-A and promotes angiogenesis in preosteoblast-like cells by a hypoxia-inducible factor-1 dependent mechanism involving the activation of src kinase, p38 MAPK, and Akt signaling pathways via estrogen receptor (reproduced with permission from Men *et al.*, 2005).

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References

1. Andersson, N., Lindberg, M.K., Ohlsson, C., Andersson K and Ryberg, B. 2001. Repeated *in vivo* determinations of bone mineral density during parathyroid hormone treatment in ovariectomized mice. *Journal of Endocrinology*,170, 529–537.
2. Aradhana. 1992. *Diosgenin-a grawth stimulator of mammary gland of ovariectomized mouse*. Indian J Exp Biol., 30 (5): 367-370.
3. Brighton, C.T., Yoichi S., and Hunt, R.M. 1973. Cytoplasmic structures of epiphyseal plate chondrocytes; quantitative evaluation using electron micrographs of rat costochondral junctions with specific reference to the fate of hypertrophic cells, *Journal of Bone and Joint Surgery*, 55-A: 771-784.
4. Brighton, C.T. and Hunt, R.M. 1986. Histochemical localization of calcium in the fracture callus with potassium pyroantimonate: possible role of chondrocyte mitochondrial calcium in callus calcification. *Journal of Bone and Joint Surgery*, 68-A (5): 703-715.
5. Conn, H.J., Darrow, M.A., and Emmel, V.M. 1960. *Staining procedures*. The Williams and Wilkins Co. Baltimore.
6. Corbière C, Battu, S., Liagre, B., Cardot, P.J., and Beneytout, J.L. 2004. SdFFF monitoring of cellular apoptosis induction by diosgenin and different inducers in the human 1547 osteosarcoma cell line. *J Chromatogr B Analyt Technol Biomed Life Sci.*,808(2):255-62.
7. Desrosier, W. 1998. *Teknologi Pengawetan Pangan*. UI Press. Jakarta.
8. Holmes, K., Roberts, O.L., Thomas, A.M., Cross, M.J. 2007. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. *Cell Signal*. 19 (10): 2003–2012.
9. Jensh?, R.P and Brent?, R.L. 1966. Rapid schedules for KOH clearing and Alizarin Red S staining of fetal rat bone. *Biotechnic & Histochemistry*, 41,179-183.
10. Lee, J., Jung, K., Kim, Y.S. and Park, D. 2007. Diosgenin inhibits melanogenesis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. *Life Sci.*, 81(3):249-54.
11. Men L.Y., Jen L. S., Chung L.C., Kuang W.T., Ching Y.Y., Wei F.C., Chiao C. C., and Min L. K. 2005. Diosgenin Induces Hypoxia-Inducible Factor-1 Activation and Angiogenesis through Estrogen Receptor-Related Phosphatidylinositol 3-kinase/Akt and p38 Mitogen-Activated Protein Kinase Pathways in Osteoblasts. *Mol Pharmacol* 68:1061-1073.
12. Patton, J.T. and Kaufman, M.H. 1995. The timing of ossification of the limb bones, and growth rates of various long bones of the fore and hind limbs of the prenatal and early postnatal labortory mouse. *J. Anat.*, 186: 176-185.
13. Qin Y, Wu X, Huang W, Gong G, Li D, He Y, and Zhao Y. 2009. Acute toxicity and sub-chronic toxicity of steroidal saponins from Dioscorea zingiberensis C.H.Wright in ro-

dents. *J Ethnopharmacol.*, 126(3):543-50.

14. Rajpurohit, R., K. Mansfield, K. Ohyama, D. Ewert and I. M. Shapiro. 1999. Chondrocyte death is linked to development of a mitochondrial membrane permeability transition in the growth plate. *J. Cell. Physiol.* 179: 287–296.
15. Ruyani, A. 2010. *Model teratoproteomik, penerapan teknis analisis protein dalam penelitian bidang toksikologi perkembangan.* UNIB PRESS, Bengkulu.
16. Ruyani, A., Sudarwati, S., Sutasurya, LA., Sumarsono, SH., Kim, D.J., and Chung, J.H. 2005. A teratoproteomics analysis: Heat shock protein 70 is up-regulated in mouse forelimb bud by methoxyacetic acid treatment. *Birth Defects Research A Clinical and Molecular Teratology*, 73 (7), 517-21.
17. Ruyani, A., Muktiningsih, dan Barlian, A. 2004. Penggunaan teknik proteomik dalam penelitian bidang ilmu dasar dan terapan. *Medika*, 30, 179-184.
18. Ruyani, A., Sudarwati, S., dan Sutasurya, L. A. 1991. Pengaruh seng sulfat terhadap perkembangan tulang femur embrio ayam (Gallus gallus) galur Tegel TM 70. *Proceedings ITB*, 24, 1-13.
19. Shishodia, S. and Aggarwal, B.B. 2006. Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. *Oncogene.*, 25(10):1463-73.
20. Sudarwati, S., Surjono, T. W., and Yusuf, A. T. 1995. Kelainan perkembangan awal anggota depan mencit A/J yang diinduksi asam metoksiasetat (MAA). *JMS* (Suppl. H), 60–70.
21. Sugiyanto, J. 1993. Effect of diosgenin on the development of mice embryos (*Mus musculus*). *Biologi*, 1(6): 273-281.
22. Surani MA, Kimber SJ, and Osborn JC. 1983. Mevalonate reverses the developmental arrest of preimplantation mouse embryos by Compactin, an inhibitor of HMG Co A reductase. *J Embryol Exp Morphol.*, 75:205-23.
23. Sutarno, H dan Atmowidjoyo, S. 1998. *Mengenal Lahan Kering dan Prospek Pengembangan Usaha Tani Tanaman Penghasil Karbohidrat di Nusa Tenggara Timur.* Prosea Indonesia. Bogor.
24. Wanek, N., Muneoka, K., Holler-Dinsmore, G., Burton, R. and Bryant, S.V. 1989. A staging system for mouse limb development. *J. Exper. Zool.*, 249, 41-49.
25. Wu, L. N., B. R. Genge, D. G. Dunkelberger, R. Z. LeGeros, B. Concannon and R. E. Wuthier. 1997. Physiochemical characterization of the nucleational core of matrix vesicles. *J. Biol. Chem.* 272: 4404–4411.
26. Yin, J., Tezuka, Y., Kouda, K., Le Tran, Q., Miyahara, T., Chen, Y., and Kadota, S. 2004. In vivo antiosteoporotic activity of a fraction of Dioscorea spongiosa and its constituent, 22-O-methylprotodioscin. *Planta Med.* 70(3):220-6.
27. Yin, M., Koyama, E., Zasloff, M and M. Pacifici, M.. 2002. Antiangiogenic treatment delays chondrocyte maturation and bone formation during limb skeletogenesis. *J. Bone Miner. Res.* 17: 56–65.
28. Zhou, Z., Apte, S.S., Soininen, R., Cao, R., Baaklini, G.Y., Rauser, R.W., Wang, J., Cao, Y., and Tryggvason K. 2000. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. *Proc Natl Acad Sci U S A.*97(8):4052-7.