Effect of soybean extract after tooth extraction on osteoblast numbers

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ABSTRACT

Background: Many researches were done to find natural materials that may increase and promote bone healing processes after trauma and surgery. One of natural material that had been studied was soybean extract which contains phytoestrogen, a non-steroidal compounds found in plants that may binds to estrogen receptors and have estrogen-like activity. Purpose: The aim of this study was to investigate the effect of soybean extract feeding on the number of osteoblast cells in alveolar bone socket after mandibular tooth extraction. Methods: This study was studied on male Rattus norvegicus strain Wistar. Seventeen rats divided into three groups were used in this study. Group 1 fed with carboxy methyl cellulose (CMC) solution 0,2% for seven days, and the left mandibular central incisives was extracted; group 2 fed with soybean extract for seven days and the left mandibular central incisives was extracted; group 3 received the left mandibular central incisives extraction followed by soybean extract feeding for seven days after the extraction. All groups were sacrificed on the seventh day post-extraction, and the alveolar bone sockets were taken for histopathological observation. The tissues were processed and stained using hematoxylin and eosin to identify the amount of osteoblast cells. The number of osteoblast cells was counted using an Image Tool program. The data was analyzed statistically using the One-Way ANOVA test. Results: Significant differences were found on the number of osteoblast cells in alveolar bone after tooth extraction between groups. Group 2 (fed with soybean extract) is higher than group 1 (fed with CMC) and group 3 (fed with soybean extract after extraction). Conclusion: Soybean extract feeding that given for seven days pre-tooth extraction can increase the number of osteoblast cells compared with the group that were not given soybean extract feeding and also with the group that were given soybean extract feeding for seven days post-tooth extraction.

Key words: Tooth extraction, soybean extract, phytoestrogen, osteoblast

ABSTRAK

Latar belakang: Pada masa sekarang, banyak dilakukan penelitian-penelitian untuk menemukan bahan-bahan alami yang dapat mendukung dan meningkatkan proses remodeling tulang untuk mengembangkan perawatan penyakit osteoporosis dan juga untuk mengembangkan penyembuhan tulang pasca trauma dan pasca pembedahan. Salah satu bahan alami yang banyak diteliti adalah ekstrak kedelai yang mengandung fitoestrogen, suatu senyawa non-steroid yang terdapat dalam tumbuhan, yang dapat berikatan dengan reseptor estrogen dan memiliki bioaktivitas yang sejenis dengan hormon estrogen. Tujuan: Tujuan dari penelitian ini adalah untuk meneliti pengaruh ekstrak kedelai yang mengandung fitoestrogen terhadap jumlah sel osteoblas pada tulang alveolar pasca pencabutan gigi. Metode: Penelitian ini dilakukan pada tikus wistar jantan. Tujuh belas ekor tikus dibagi menjadi tiga kelompok sampel dalam penelitian ini. Kelompok sampel tersebut mendapatkan perlakuan yang berbeda-beda. Kelompok 1 dilakukan feeding larutan CMC 0,2% selama tujuh hari, kemudian dilakukan pencabutan satu gigi insisives sentral kiri rahang bawah, kelompok 2 dilakukan feeding ekstrak kedelai selama tujuh hari, kemudian dilakukan pencabutan satu gigi insisives sentral kiri rahang bawah, kelompok 3 kelompok yang dilakukan pencabutan satu gigi insisives sentral kiri rahang bawah, kemudian diberikan feeding ekstrak kedelai selama tujuh hari pasca pencabutan gigi. Semua kelompok dikorbankan pada hari ketujuh pasca pencabutan gigi, dan soket bekas pencabutan gigi tersebut diambil untuk dibuat seduhan histopatologis. Jaringan tersebut diproses dan dilakukan pengecatan dengan hematoxylin and eosin untuk melihat sel osteoblas. Setiap preparat diperiksa di bawah mikroskop cahaya dan sel osteoblas dihitung...
Tooth extraction is a treatment that should be done for
severely damaged tooth. The process of wound healing after
tooth extraction generally consists of several phases, begins
with the formation of blood clots in the socket, continued to
inflammation process, infiltration of leukocytes, formation
of connective tissue, formation of granulation tissue,
epithelialization and regeneration of new bone to close
the remaining empty socket. When a tooth is removed, the
socket is filled with blood, which coagulates and form blood
clot. The inflammatory stage occurs during the first week of
post extraction. White blood cells enter the socket to remove
contaminating bacteria and any debris from the area, such
as bone fragments that are left in the socket. Fibroplasia
also begins during the first week, with the growth of
fibroblasts and capillaries. Finally, in the end of the first
week of healing, osteoclasts accumulate along the crystal
to start bone resorption, followed by osteoblasts that
responsible to the process of bone formation. The cortical
bone was resorbed from the socket and new trabecular bone
is laid down across the socket. As bones fill the socket,
the epithelium moves toward the crest and eventually
becomes level with adjacent gingival crest. This processes
continue and usually take sometime before a socket heals
and becomes the edentulous alveolar ridge. Factors that
can be related to the healing process is generally divided
into common factors and local factors. The common factors
includes: age, nutrition, vitamins, hormones, metabolic
status, and circulation status. Local factors includes: blood
supply, foreign bodies, tissue movement and damage, type
of tissue, infections, mechanical factors, size, location,
and type of wound. Those factors can also be related to the
healing process of tooth extraction.3

Nuts and seeds such as soybean, peanut, winged bean
seeds, coconut and others are the sources of proteins and
fats which are important in human life. Soybean is mostly
found in Indonesia and has protein content of 35%. The
improved varieties of soybean may even contain protein
levels of 40–43%. Compared to rice, maize, cassava flour,
green bean, meat, fresh fish, and egg, soybean has higher
protein content, nearly as high as the protein content found
in dried skim milk. Soybean can be processed into tempeh,
tofu, soybean sauce, soybean milk, etc. The processing
process of soybean into many kind of food is simple, and the
equipments needed are commonly found in the household,
except for the stripper, grinder, and mould.4

Soybean contained some chemical substances, one of
them is known as phytoestrogen, a non-steroidal compounds
found in plants, which have in part some structural
similarities to estrogen hormone, that is 17β-estradiol.5
Currently, four different families of phenolic compounds
produced by plants are considered as phytoestrogen: the
isoflavonoid, stilbenes, lignans and coumestans.6 Soybean
is the main source of phytoestrogen isoflavones group,
namely as genistein and daidzein.7

Endogenous estrogen levels are associated with human
bone homeostasis. Estrogen is known to play an important
role in maintaining bone mass. Lack of estrogen levels
may causes excessive bone resorption, and the estrogen
deficiency results in bone loss.8 Bone volume is maintained
by two phases of bone remodelling: one is bone resorption
by osteoclasts, and the other one is bone formation by
osteoblasts. It is known that bone remodelling is controlled
by various cytokines and hormones, such as interleukin-
1 (IL-1), interleukin-6 (IL-6), parathyroid hormone,
glucocorticoids, and estrogen hormone.9

Many researches were done to find some natural
materials that may increase and promote bone remodeling
in order to improve treatment in osteoporosis, and
bone healing process after trauma and after surgery.
Phytoestrogen such as genistein and daidzein found in
soybean were investigated and showed its estrogen-like
activity because of its structural similarities to 17β-
estradiol. Natural phytoestrogen found in soybean extract
can increase osteoblast cells proliferation in cells MC3T3-
E1, the osteoblast-like cell line from rat.10 Phytoestrogen
may as influence in increasing alkaline phosphatase activity
in MC3T3-E1 cells in vitro and found that phytoestrogen
can also be used as one of the osteogenic local agents in
vivo.5 The aim of this study was to investigate the effect
of soybean extract feeding on the number of osteoblast in
the alveolar bone socket after mandibular tooth extraction
histopathologically.

MATERIALS AND METHODS

This study was done in healthy male Wistar strain
rats, aged 2–3 months and weighed between 150–200
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Table 1. Number of osteoblast in control group (K)

<table>
<thead>
<tr>
<th>No.</th>
<th>Slides</th>
<th>Number of osteoblast (each slide)</th>
<th>Mean of osteoblast cell number each slide</th>
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</thead>
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<tr>
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<td>Bottom region</td>
<td>Middle region</td>
</tr>
<tr>
<td>1</td>
<td>K.4</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
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<td>40</td>
</tr>
<tr>
<td>5</td>
<td>K.9</td>
<td>41</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 2. Number of osteoblast in treatment group 1 (P1)

<table>
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<th>Mean of osteoblast number each slide</th>
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<tr>
<td>2</td>
<td>P1.2</td>
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<td>P1.5</td>
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<td>6</td>
<td>P1.6</td>
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Table 3. Number of osteoblast in treatment group 2 (P2)

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<th>No.</th>
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<th>Mean of osteoblast number each slide</th>
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<td>Bottom region</td>
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grams. Samples were divided into three groups: control group who received feeding of carboxy methyl cellulose (CMC) solution concentration of 0.2% equivalent to 9 cc/200 gram of rat’s body weight per day for seven days before-tooth extraction; the treatment group 1 (P1) which fed with soybean extract feeding using a dose of 9 cc/200 gram of rat’s body weight/day for seven days before-tooth extraction; treatment group 2 (P2) which fed with soybean extract feeding 9 cc/200 gram of rat’s body weight per day for seven days after-tooth extraction.

“Surya” white Soybean available in local market in Madiun, East Java was used in this study. The soybean extract was made using Akebono brand soybean extractor machine and sixty grams of dried Soybeans was soaked for 12 hours and inserted into the Soybean extractor machine. 1500 cc of warm water was added and 1500 cc of soybean extract liquid obtained.

The control group was fed with 0.2% CMC solution in a dose of 9 cc/200 gram of rat’s body weight per day for seven days. A feeding tube was connected into a syringe was the feeding instrument used in this purpose. Seven days after fed with CMC 0.2% solution, the experimental animals in the control group were anesthetized for tooth extraction. General anesthesia with ether solution was used and the process was done by putting the experimental animal into a glass jar contained of cotton wool soaked with ether solution. The experimental animals were inserted and the jar was closed for several minutes until the animal was unconscious. The left mandibular central incisives was removed using a modified forceps which was made for rat’s tooth rat extraction. Tooth extraction bleeding was stopped by pressing the socket wound using sterile cotton. Soybean and CMC feeding in the control groups were not given for seven days but only received a drinking water. The same procedure as in the control group and P1 group was used. Soybean extract feeding of 9 cc/200 gram of rat’s body weight per day was given for seven days post-tooth extraction in this group.
After all experiment procedures were completed, all animal samples were sacrificed using an overdosed ether solution on the seventh day post-extraction to examine the osteoblast cell that has begun to accumulate along the alveolar bone lining the socket. The mandible bone of the experimental animals were taken from the skull and inserted into the fixative solution of 10% formalin solution. The alveolar bones were taken for further histopathological slide preparation procedure. The tissues were cutting in serial in rotary microtome with 6μ of thickness followed by tissue staining using haematoxylin and eosin (HE). All slides fields were observed for the present of osteoblast cells in 400x of magnification. Three different areas were observed as those are the top, bottom and the middle regions of the tooth extracted socket which are adjacent to the post extraction alveolar bone. The number of osteoblast cells were counted using the computer program “Image Tool”, a program used to analyze and process free images. Analysis function of images in this program can be done in variety including the dimensional measurement, i.e. measurement of distance, angle, area, and also a certain scale of measurement in the form of points, lines, or areas and it is completed with a counting of its standard deviation. The statistical difference in the number of osteoblast cells during bone remodeling processes after tooth extraction among those three sample groups were statistical analyzed using the One-Way ANOVA test.

RESULTS

The results on osteoblast cells observation on those three areas of tooth extraction socket on the seventh day after-extraction is shown in table 1, 2, 3. Based on data, it is shown that the difference number of osteoblast cells are found higher in the sample group who received soybean extract feeding (P1, P2 groups), compared to the control group (K) in which soybean extract feeding was not given (Figure 1).

DISCUSSION

The soybean extracts and its proteins, carbohydrates, fats, calcium, phosphorus, iron, provitamin A, vitamin B complex (except B12) and water contained showed statistically able to enhance the regeneration process of bone healing after tooth extraction. The calcium and phosphorus contain found in soybean extract will increase the intake of calcium and phosphorus, which are needed for the bone regenration process. Soybeans also have low sulfur amino acid content, which known as acids can inhibit calcium resorption through kidneys, and therefore may cause considerable loss of calcium in the urine. These facts supported the result of this study that overall there are a significant difference in bone regeneration process, marked by the different number of osteoblast cells that found higher in the treatment group who received a soybean extract feeding, compared to the control group who did not fed with the soybean extract. Statistical result of tests using the One-Way ANOVA showed a significant difference in the number of osteoblast cells between the control group and group P1 (p < 0.05). These results showed that soybean extract feeding on experimental animals seven days pre-extraction can affect the total amount of alveolar bone osteoblast cells compared to those the control group.

Phytoestrogens has a capability to bind to estrogen receptors in the body competitively with estrogen hormone, so the number of osteoblast cells production can be found higher. Thus occurred because the binding process between estrogen receptor and phytoestrogens may influence the osteoblast cells production by improving the process of synthesis, activation, receptor binding, and local growth factor binding protein in osteoblast, either directly or indirectly. Transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1) which may support the process of cell proliferation from osteoblast precursor cells that is preosteoblasts into osteoblasts also activated during the presence of phytoestrogen. The synthesis of bone matrix by osteoblast cells thereby affect in increasing of osteoid deposition during bone regeneration process.

Soybean extracts containing phytoestrogens will increase the anabolic function of osteoblast cells.
study on soybean extracts which contain phytoestrogens was reported can stimulate the protein synthesis in osteoblast cells in vitro. Synthesis of these proteins will support the process of new osteoblast cell proliferation by accelerating the process of cell differentiation from preosteoblast into osteoblasts.

Binding of phytoestrogens with estrogen receptors on osteoblast cells will improve the activity of alkaline phosphatase and osteocalcin synthesis by osteoblast cells. Osteocalcin is a non-collagen protein bone matrix, which synthesis stimulated by 1.25-dihydroxicolecalsiferol, which will stimulate 1.25-dihydrooxicolecalsiferol, which will increase the synthesis of 1.25-dihydroxikolcalciferol, which receptors are also present in the cell membrane of osteoblast. Soybean extracts containing phytoestrogens can increase the synthesis of 1.25-dihydroxikolcalciferol, which will provide a direct influence on the synthesis and stabilization of osteocalcin at the transcriptional and post-transcriptional phase, so that the process of osteocalcin synthesis by osteoblast cells will increase. Osteocalcin is an important component in the process of bone regeneration because osteocalcin is a non-collagen bone protein that functions in the process of calcium ions binding in bone regeneration, assisted by vitamin K1. Those reported facts can be used to explain our study in the present of osteoblast cells that produced in group P1 is found more mature, so that the new bone trabeculae also more in the experiment group of P1 than shown in the control group. In addition, alkaline phosphatase and osteocalcin produced by osteoblast cells indicates that osteoblast cells are already mature and can produce an optimal bone matrix.

The One-Way ANOVA test results also indicates a significant difference in osteoblasts cells contained shown in groups P1 and P2 (p < 0.05), where the mean of osteoblast cells number in groups P1 is higher than in group P2. This event can be explained that the soybean extract were given in different sequences in all experiment animals in groups P1 and groups P2.

The removal of tooth initiates some sequences of inflammatory process, fibroplasia, formation of granulation tissue, epithelialization. This pathophysiological sequence can be found during the formation of new bone to close the remaining empty post-extraction socket. Although the process of new bone regeneration in tooth extracted socket is present, but the observation showed that the increased number of osteoblast cells in groups P2 was not significantly found higher than in control group which were not given soybean extract. Control group did not receive soybean extract as in group P1 and group P2, so the process of bone regeneration occurred normally with normal amount of osteoblast cells proliferation. The 0.2% CMC solution feeding was given to the control group with purpose to achieve the same physiological conditions in their intestinal tract both the animal control group and treatment group. Although the process of new bone regeneration in tooth extracted socket is present, but the observation showed that the increased number of osteoblast cells in groups P2 was not significantlydifferent than the control group.

It is concluded that soybean extract feeding for seven days before-tooth extraction can increase the number of osteoblast.
REFERENCES