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Original research

Comparative effectiveness of two different forms of phytoestrogens as a graft material in bony defects

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ABSTRACT

Purpose: To compare the effectiveness of two forms of phytoestrogens in Soybeans as a graft material in bony defects.
Material and methods: Eighteen bone defects were created in the parietal bones of nine New Zealand white rabbits. Three groups were divided (Six defects each) and filled with either material A in group I, or material B in group II, or left empty as control group. One gm of the purified active fraction obtained from seeds of *Glycine max* L. (Soybean) Family Leguminosae, rich in isoflavones (Material A) was tested as well as a prepared semi solid pharmaceutical paste (Material B) of the crude bioactive fraction. Defects and surrounding tissue were decalcified and routinely processed for Haematoxylin and Eosin staining. The area percent of newly formed bone was estimated using an image analyzer computer system. Comparison of the results of all groups were carried out.co ANOVA test was used for statistical analysis.
Results: Regularly arranged, osteoid newly formed bone almost filled the surgical defect in group1, while group 2 revealed partial obliteration of the bony defect with dispersed irregularly arranged bone trabeculae, separated by fibrovascular marrow. Scarce bone trabeculae and wide fibrovascular marrow spaces were observed in the control group. This difference was statistically significant ($P < 0.0001$).
Conclusion: The results of this study suggest that phytoestrogens obtained from Soybeans seeds in form of semi solid pharmaceutical paste is more effective than the crude bioactive fraction of the same plant. Further comparative studies are needed on extended time intervals.

Introduction

Bone grafting and reconstruction is one of the main concerns in oral and maxillofacial surgery. However, its importance is so much applicable following trauma and in bony defects. Autogenous bone grafts are considered the golden standard in bone grafting. It could be harvested from extraoral sites such as iliac crest or intraoral sites (e.g. mandibular symphyseal region, maxillary tuberosity, ramus. . .etc.). In addition to its osteoinductive properties, it provides an osteoconductive feature as a scaffold for bone regeneration [1,2]. The major limitations of using autogenous grafting includesdonor site morbidity, pain and infection [3]. Moreover, graft resorption posed a severe problem. This encouraged the introduction of a variety of bone substitutes to aid in bone grafting. This included allografts, alloplasts and xenografts [4].

Bone remodeling is the function of the activity of 2 different cell lines. Osteoblasts, responsible for bone formation, respond to changes in the activity of osteoclasts, the bone resorbing cells. Many hormones, growth factors, and cytokines play a regulatory role in maintaining bone homeostasis by their effects on these 2 cell lines, and estrogen in particular is responsible for suppressing osteoclast activity and thereby preventing bone resorption [5,6].

The decrease in serum estrogen after menopause,showed a direct relationship with bone loss and osteoporosis, and estrogen replacement therapy is considered to be effective in preventing bone loss [7]. Treatment by using estrogen enhances osteoblast differentiation and bone formation [8]. This direct positive effect was detected on the osteoblastic activity. However, an inverse relation-

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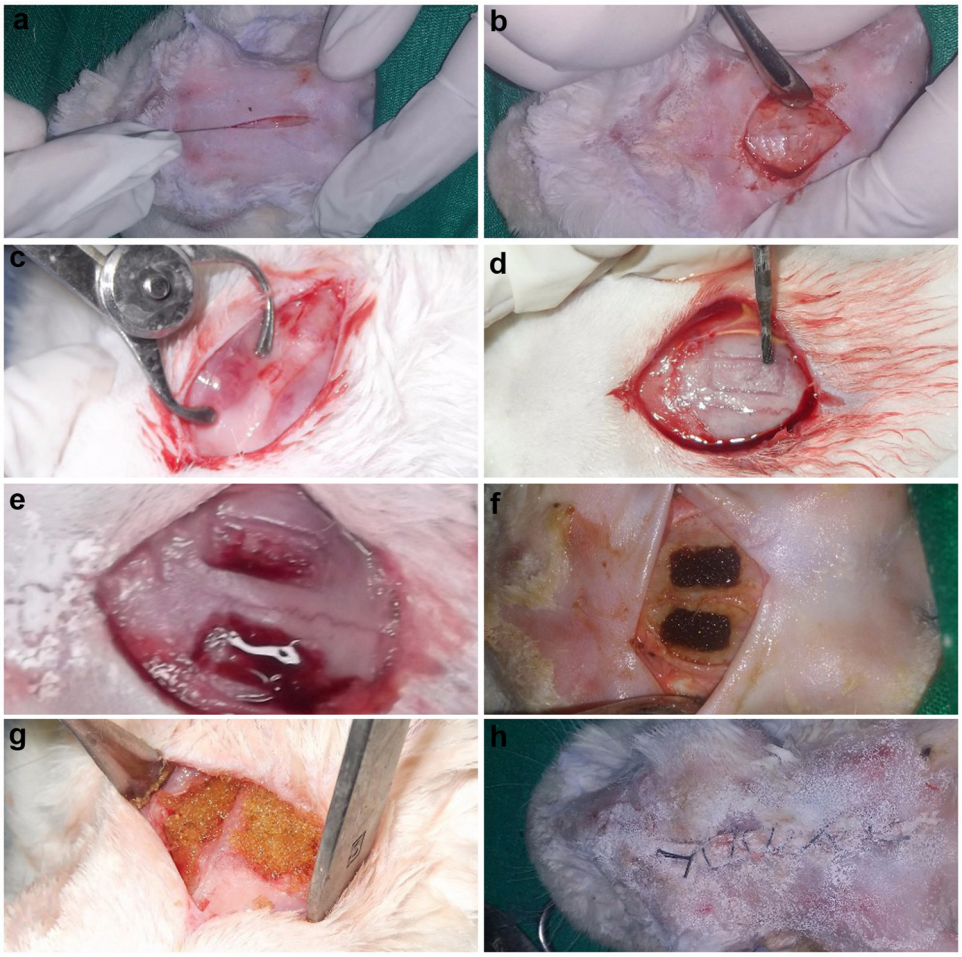

Fig. 1. Surgical procedure include the followings: a) cranial incision after wrapping and anesthesia, b) reflection and retraction of full thickness flap, c) measuring planned bone defect, d) creation of bone defect using surgical bur with copious sterile saline irrigation, e) 2 bone defect beds, f) filling defects with material A, g) filling defects with material B, h) after suturing of surgical wound and use topical antimicrobial.

ship was made upon osteoclast. This was detected in the form of a decrease in the action and differentiation of osteoclasts [9].

Therefore, estrogen is considered as an important treatment for bone osteoporosis and in maintenance of bone metabolism [9].

Phytoestrogens are plant-derived non-steroidal compounds that bind to estrogen receptors (ERs) and have estrogen-like activity. There are three classes ofphytoestrogens:isoflavones, coumestans and lignans [9]. They have played an important role in various medical conditionssuch as prevention and treatment of cardiovascular diseases, osteoporosis, diabetes and obesity, menopausal symptoms, renal diseases and various cancers [10,11].

A number of observational and dietary intervention studies confirm the general findings from the in vitro effects of phytoestrogens on bone cells in culture. Observational or epidemiologic studies [12,13] and dietary intervention studies [14–17] have shown significant relationships between phytoestrogens and surrogate markers for bone turnover that are indirectly consistent with reduced bone turnover markers indicative of osteoblast and osteoclast activity that have been measured include urinary calcium, magnesium and phosphorous, hydroxyproline, and collagen cross-links, while serum measures have included bone-specific alkaline phosphatase, tartarate-resistant acid phosphatase, osteocalcin, insulin-like growth factor I (IGF-I), and interleukin 6 [18]
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Kanno et al. [19,20] studied the effects of phytoestrogens and environmental estrogens on osteoblast differentiation.This was applied on MC3T3-E1 cells, a mouse calvaria osteoblast-like cell line. They increased alkaline phosphatase activity and enhanced bone mineralization in these cells.

Soybean (*Glycine max* L.) was first grown as a crop in China about 5000 years ago [12] and have been widely consumed as folk medicines in China, India, Japan and Korea forhundreds of years. Today is a major source of plant protein (70%) and oil (30%) and becomea globally important crop. Its nutrients become basic for humans consumption,beyond its by-products, that offer great diversities of products to the food industry. Soybeanoil is highly consumed world-wide and soy milk is often used as a milk substitute to peoplewho have lactose intolerance. In addition soybean has phytoestrogens which can be usedin replacement to women hormone [13]. One important group of minor compounds present in soybean that has received considerableattention is a class of phytoestrogen called the isoflavones [14].

However, they play significant roles in the prevention of several diseases, so they maybe considered health-promoting substances.

Materials and methods

I-a-Plant material

Seeds of *Glycine max* L. (Soybean) Family Leguminosae were purchased from Local Market and authenticated in the Herbarium of Flora Department, Agricultural Museum, Giza, Egypt. The seeds

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