Effect of Acacia Arabica Extracts on Bone Calcium Content

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ABSTRACT
Introduction: Osteoporosis is a disease characterized by low bone mass, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk. The available drug treatments have many side effects. So alternative indigenous medicine should be use, which have a lower side effect. Many herbal drugs are reported as anti-osteoporotic agents in ayurveda but they are not scientifically evaluated. Objective: To evaluate the effect of Acacia arabica on bone calcium using in-vitro bone culture experiment. Method: The aqueous and methanolic extracts of Acacia arabica prepared using hot maceration and soxhlet apparatus method respectively. All extracts evaluate for the effect on bone calcium using in-vitro bone culture experiment. Calcium content was measured and all the data were evaluated using graphpad prism software. Result: The aqueous and methanolic extracts of Acacia arabica caused a significant increase in the calcium content in the diaphyseal and metaphyseal tissues in in vitro bone-culture experiments. Conclusion: The effect could be due to the steroidal content present in drug extracts. So Acacia Arabica can be used as an anti-osteoporotic agent.

Keywords: Bone calcium, Acacia arabica, osteoporosis.

I. INTRODUCTION
Since ancient times herbal medicines have been used by traditional medical practitioners for the treatment of osteoporosis. Many of these herbal formulations are prepared using a collection of plant materials according to traditional formulas. Although these herbal medicines have been prescribed to patients for so many years, most of them may have not been subjected to scientific investigation to determine whether these herbal drugs truly have the potential to be benefit of the patients.

The therapeutic activity of herbal medicinal compounds towards osteoporosis is often attributed depend on there dose concentration. So the present study was carried out to investigate the effective concentration of Indian traditional herbal drugs like Acacia arabica and Terminalia arjuna using in-vitro bone culture.

The term half maximal effective concentration (EC₅₀) refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after some specified exposure time.¹ It is commonly used as a measure of drug’s potency.

The EC₅₀ of a graded dose response curve therefore represents the concentration of a compound where 50% of its maximal effect is observed. The EC₅₀ of a quantal dose response curve represents the concentration of a compound where 50% of the population exhibit a response, after a specified exposure duration. It is also related to IC₅₀ which is a measure of a compound's inhibition (50% inhibition). For competition binding assays and functional antagonist assays IC₅₀ is the most common summary measure of the dose-response curve. For agonist/stimulator assays the most common summary measure is the EC₅₀.²

Concentration measures typically follow a Sigmoidal curve, increasing rapidly over a relatively small change in concentration. The point at which the effectiveness slows with increasing concentration is the IC₅₀. This can be determined mathematically by derivation of the best-fit line. While relying on a graph for estimation is more convenient, it yields less precise and less accurate results.

It has some limitation like, The effects of a stressor or drug generally depend on the exposure time. Therefore, the EC₃₀ (and similar statistics) will be a function of exposure time. The exact shape of this time function will depend upon the stressor (e.g., the specific toxicant), its mechanism of action, the organism exposed, et cetera. This time dependency hampers the comparison of potency or toxicity between compounds and between different organisms.²

According to herbal medicine Acacia arabica is also of great use in dental problems. Vernacularly it is known as a Babool and it possesses anti diabetic properties and often recommended for proper union.
of fractured bones and also for strengthening otherwise healthy bones.  

2. MATERIAL AND METHODS

2.1 Plant Material

In the present study, the bark of Acacia Arabica collected from the ayurvedic store LVG, Ahmedabad, India. The barks are authenticated using organoleptic tests, macroscopic and microscopic observations. The voucher specimen was deposited in my institute. Soon after authentication, all barks were dried at room temperature, until they were free from the moisture. Finally the barks were subjected to get course powder and then passed through sieve no. 44 to get uniform powder. The sieve powder was stored in air tight, high-density polyethylene container before extraction.

2.2 Plant Preparation

The powdered barks (500 g) was subjected to hot continuous extraction (Soxhlet) with methanol. After the residue extraction, solvent was distilled off, and excess solvent was completely removed by using a rotary flash evaporator to get reddish-brown semi solid mass and dried by using Mini Lyotrap freeze dryer at 20 °C (yield: 28.17%). Water extract was prepared by maceration with distilled water for 24 hours. The water extract obtained was dried at 60 °C on water bath and its percentage yield was calculated (7.5% w/w). The obtained extracts were then evaluated for antosteoporotic activity using in vitro method.

2.3. Chemicals

Dulbecco’s modified Eagle’s medium (DMEM) (high glucose, 4.5 g dl⁻¹) and a penicillin–streptomycin solution (penicillin 5000 U mg⁻¹; streptomycin 5000 mg ml⁻¹) were purchased from Sigma Laboratories.

2.4. Male Rats Bone Tissue Cultures

Male Wistar rats weighing 90–100 g (4 weeks old) or male mice (ddY strain; 6 weeks old) were used. The animals were fed with a commercial laboratory chow containing 1.1% calcium and 1.1% phosphorus at a room temperature of 25°C, with free access to distilled water.

2.5. Bone Culture Experiments

Under ether anesthesia with cervical dislocation, the femurs of male rats were removed aseptically after bleeding and were then soaked in ice-cold 0.25M sucrose solution. The soft tissue and marrow were cleaned-off from the femur, and the diaphysis and metaphysis (not containing epiphyseal tissue) were separated by a morphological tool. The femoral-diaphyseal and femoral-metaphyseal tissues were cut into small pieces (the size of about 2 · 2 mm) by a pair of scissors. Diaphyseal or metaphyseal fragments (pieces of 3 or 4) were cultured for 48 h in a 35 mm dish in 2.0 ml of medium consisting of Minimum essential medium (MEM) (high glucose, 4.5 g dl⁻¹ supplemented with antibiotics (penicillin 100 units and streptomycin 100 µg ml⁻¹). In order to determine the effects of aqueous and methanolic extracts of acacia arabica on bone calcium content, bone tissues were cultured in a medium containing drugs extracts in different concentration. Both extracts were soluble in the culture medium. Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO2 and 95% air. After culture, the diaphyseal or metaphyseal tissues were removed, washed with ice-cold 0.25 M sucrose solution and dried for 16 h at 110°C. The calcium content was determined by atomic absorption spectrophotometry. The concentration of calcium in the medium containing drugs were in the range of 0.05–1.0 mg ml⁻¹ of medium. The calcium content in the bone tissue was expressed as mg per g of dry bone. All experimental data are mean ± SEM for six animals.

2.6. Statistics

Data were obtained from three measurements and expressed as means ± standard deviations. Data of tissue cultures were expressed as means ± standard errors. Statistical evaluation is done by using Graphpad Prism 5 using nonlinear regression analysis and one way ANOVA.

3 RESULT

3.1 Effect of Acacia Arabica extracts on the calcium contents in the femoral-diaphyseal and femoral-metaphyseal tissues using in-vitro bone culture method

As shown in table 1, the calcium content in the diaphyseal or metaphyseal tissues significantly increased when the bone tissues were cultured in the presence of 10–100 µg/ ml Aqueous and Methanolic extract of Acacia Arabica. In 500 - 1000 µg/ ml concentration the calcium content was decrease in the diaphyseal or metaphyseal tissues.
Table 1: Effect of Acacia Arabica extracts on the calcium contents in the femoral-diaphyseal and femoral-metaphyseal tissues

<table>
<thead>
<tr>
<th>Bone culture</th>
<th>Calcium content mg/gm of bone</th>
<th>0 µg/ml</th>
<th>10 µg/ml</th>
<th>100 µg/ml</th>
<th>500 µg/ml</th>
<th>1000 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia arabica (Aqueous extract)</td>
<td>Metaphyseal</td>
<td>181.07 ± 0.28</td>
<td>190.32 ± 0.04 *</td>
<td>212.14 ± 1.21 *</td>
<td>204.71 ± 0.81 *</td>
<td>203.35 ± 2.22 *</td>
</tr>
<tr>
<td></td>
<td>Diaphyseal</td>
<td>242.89 ± 0.23</td>
<td>251.83 ± 2.31 *</td>
<td>291.78 ± 1.16 *</td>
<td>288.53 ± 1.29 *</td>
<td>287.50 ± 1.47 *</td>
</tr>
<tr>
<td>Acacia arabica (Methanolic extract)</td>
<td>Metaphyseal</td>
<td>181.07 ± 0.28</td>
<td>185.40 ± 0.82 *</td>
<td>226.28 ± 0.84 *</td>
<td>224.99 ± 0.44 *</td>
<td>224.55 ± 0.32 *</td>
</tr>
<tr>
<td></td>
<td>Diaphyseal</td>
<td>242.89 ± 0.23</td>
<td>251.13 ± 1.16 *</td>
<td>296.82 ± 0.33 *</td>
<td>295.56 ± 0.28 *</td>
<td>295.52 ± 1.87 *</td>
</tr>
</tbody>
</table>

3.2 EC 50 determination of Acacia Arabica

Evaluation of Table 1 data by using nonlinear regression analysis by using statistical test log agonist vs. response-variable slope (four parameter). From this statistical test I found the EC 50 value of Acacia Arabica as describe in Table 2. EC 50 determination graph for aqueous and methanolic extract of Acacia arabica on femoral metaphyseal and femoral daiphyseal tissues are showing in figure 1.

![Fig. 1: EC 50 determination of Aqueous and Methanolic extract of Acacia Arabica (on Metaphyseal and Diaphyseal bone tissue)](image-url)
4. DISCUSSION
Although hormone replacement therapy has been shown to be effective in prevention and treatment of post-menopausal bone loss, alternatives are continuously being searched because of actual or possible side effects, or contraindications limiting their use, and poor compliance of patients. In traditional herbal medicine, there are many exceptional herbal drugs that have potential competence of preventing and curing osteoporosis; however, not much research has been done on its mechanism of action. According to herbal medicine *Acacia arabica* is also of great use in dental problems. Vernacularly it is known as a Babool and it possesses anti diabetic properties and often recommended.  

*In-vitro* methods reduce the use of animals and some evidence exists that in-vitro studies are capable or potentially capable of providing more rapid, precise, and relevant information than do some animal studies. The primary advantage of *in vitro* work is that it permits an enormous level of simplification of the system under study, so that the investigator can focus on a small number of components.  

The aqueous and methanolic extracts of *Acacia arabaica* caused a significant increase in the calcium content in the diaphyseal and metaphyseal tissues in *in vitro* bone-culture experiments. From the drug concentration response curve we can say that effective range for the *Acacia Arabica* and *Terminalia Arjuna* is 10-100 μg/ml. The effect could be due to the steroidal content present in drug extracts. So *Acacia Arabaica* can be used as an anti-osteoporotic agent. 

In conclusion, the results obtained in the present study provide suggestion that *Acacia arabica* contributes significantly to the prevention or treatment of the osteoporosis. It is clear that drug has positive effects on bone culture and also displaying effects on the calcium content, an important factor in the treatment of osteoporosis. Furthermore, *acacia arabaica* extracts available widely in most of the area and it can be prepared easily and conveniently and is cost-effective as an anti-osteoporotic agent. This drug may have the potential for further development as an effective anti-osteoporotic drug and it may be used either alone or in conjunction with other available treatment and beneficial for postmenopausal women.

REFERENCES

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>EC 50 VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia arabica</em> aqueous (metaphyseal)</td>
<td>22.14</td>
</tr>
<tr>
<td><em>Acacia arabica</em> aqueous (Diaphyseal)</td>
<td>24.53</td>
</tr>
<tr>
<td><em>Acacia arabica</em> methanol (metaphyseal)</td>
<td>11.97</td>
</tr>
<tr>
<td><em>Acacia arabica</em> methanol (Diaphyseal)</td>
<td>12.46</td>
</tr>
</tbody>
</table>
INTRODUCTION

Osteoporosis is a disease characterized by low bone mass, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk, it is a major cause of morbidity and mortality and medical expense worldwide. In India, it is found that 29.9% of women and 24.3% of men aged between 20 and 79 year had low bone mass. Furthermore about 50% women and 36% of men over 50 years of age were noted to have low bone mass. The wound healing time of the vertical incision group was slightly shorter than those of the transverse incision group. But didn't differ significantly. None of the distributions of wound length and healing percentage per day showed significant variation between these two groups. Variation of the wound length is not differing between two groups

Keywords: Osteoporosis, transverse surgery, vertical surgery
Experimental animal models play an important role in improving the knowledge of the etiology, pathophysiology, and diagnosis, as well as on preventive and therapeutical techniques, regarding osteoporosis.

Rats are currently principal laboratory animals, used to investigate this disease, since they are inexpensive to maintain, grow rapidly, have a relatively short lifespan and are widely available [4, 5]. There are also various methods of obtaining a standardized pattern of osteoporosis, such as, for example, low calcium diet, LHRH agonists or ovariectomy [5, 6]. The latter one is considered to be the procedure that gives reliable model of osteoporosis [7, 8]. Ovariectomy itself can be performed in some different ways. The choice of operative method is very important, particularly, when it is necessary to operate of a few dozen animals in a short time. There are several types of incision for doing ovariectomy in rats, such as single midline dorsal skin incision two dorso-lateral incisions [8] single dorso- lateral incision, and single transverse lateral incision. The aim of the study was to present and compare two operative methods, regarding the duration of procedure, the degree of difficulty of operative technique and access to gonads.

MATERIALS AND METHODS

Animals:

Twenty female Sprague-Dawley rats with three month old age and mean weight 220 ± 20.35 were enrolled in this study. They were kept at constant room temperature (28±2°C) under a 12/12 h light/dark cycle at least 10 days prior to surgery in animal house of Parul Institute of Pharmacy; the animals were accessed to a standard rat chow pellet and tap water freely. The animals were randomly divided in to two groups (Group 1 include 10 and Group 2 include 10 rats).

Surgical Procedure:

The same surgeon performs the whole procedure in both the groups. The weight of rats were measured before the surgery and aneasthetized animal with a ketamine (50 mg/ kg) intraperitoneally (IP). In both groups operation was made after placing an animal on its ventral surface. Ovariectomy was preceded by a midline dorsal skin incision (Vertical incision: group: 1), 3 cm long, approximately half way between the middle of the back and the base of the tail or to middle part of abdomen (Transverse Incision: group: 2). Incision was a minimum length to allow the extrusion of ovaries. After peritoneal cavity was accessed, the ovary was found,
surrounded by a variable amount of fat. Ligation of the blood vessels was necessary. The connection between
the Fallopian tube and the uterine horn was cut and the ovary moved out. Because of muscle bleeding, its
incision required suturing. Three single catgut stitches were placed on the skin. High degree of aseptic
procedure was maintained throughout the operation. After operation, animal was covered with paper in order to
avoiding hypothermia.

**Evaluation of Wound Healing:**

To evaluate wound healing, duration of healing, absolute and normalized length area of the wound were
used. The maximum length area was measured on the second day after surgery thereafter; this measurement was
carried out every two day until full healing occurred. The healing percentage or the normalized values were
calculated by dividing the maximum length of the wounds by that measured on the 2 day after surgery. The
"duration of wound healing" was the time taken for full contraction of the wound. Wound healing percentage
was calculated using the equation 
\[
\left( \frac{L - L}{L} \right) \times 100
\]
where L and L are the maximum wound lengths on the second and any other day, respectively [9].

**Statistical Analysis:**

Data are presented as mean and standard deviation. The data were analyzed using the two-tailed
Student's t-test to compare the two groups of rats. Correlations between these parameters were analyzed using
the Pearson test. The statistical significance level was set at 0.05.

**Results**

Animals in group one (vertical incision, n=10) had a mean weight 220 ± 20.35 of and those in group two
(transverse incision, n=10) weighted 222 ± 20.35 g. There was no significant differences in body weight, wound
healing time and interval between administration of anesthetic drug to deep anesthesia between two groups
through the study (p>0.05), but there was a significant differences between two groups in surgery time
(p<0.001) The wound healing time of the vertical incision group two was slightly shorter than those of the
transverse incision group. (10.46 ± 0.973 vs 10.78 ± 0.698 days, respectively) but didn't differ significantly.
None of the distributions of wound length and healing percentage per day showed significant variation between
these two groups. Variation of the wound length is not differing between two groups and it is shown in table.
Table 1: Body Weight in Vertical incision Group one and transverse Incision Group two

<table>
<thead>
<tr>
<th>Groups</th>
<th>Manner of the Incision</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vertical incision</td>
<td>220 ± 20.35</td>
</tr>
<tr>
<td>2</td>
<td>Transverse incision</td>
<td>222 ± 20.35</td>
</tr>
</tbody>
</table>

Table 2: Surgery Duration (min) and Wound Healing Time (day) in Vertical incision Group one and transverse Incision Group two

<table>
<thead>
<tr>
<th>Groups</th>
<th>Manner of the Incision</th>
<th>Surgery Duration (min)</th>
<th>Wound Healing Time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vertical incision</td>
<td>10.67 ± 1.35**</td>
<td>10.46 ± 0.973</td>
</tr>
<tr>
<td>2</td>
<td>Transverse incision</td>
<td>15.22 ± 2.68 **</td>
<td>10.78 ± 0.698</td>
</tr>
</tbody>
</table>

Discussion

The success of a surgery procedure performed through an abdominal incision depends on careful selection of the incision site and proper closure of the wound. The surgeon needs to consider multiple factors before making an abdominal incision. These factors include the area that needs to be exposed, the disease process, body habits, operative exposure, simplicity, previous scars, cosmesis, the need for quick entry into the abdominal cavity (the elective or emergency nature of the operation) and personal preference.
REFERENCES: