AN EXPERIMENTAL STUDY TO ASSESS THE EFFECT OF 
ASTHISAMHARADI CHURNA ON FRACTURE HEALING

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ABSTRACT

A fracture is a break in the continuity of bone or cartilage or both. Bone fracture healing is a spontaneous, natural, complex, biological process. In Ayurvedic classics many drugs are advocated for enhancing fracture healing, Asthisamharadi churna is one of them. An experimental study was planned to evaluate the effect of Asthisamharadi churna on fracture healing in Wister rats. The study was conducted on 30 male albino wister rats (Rattus Norwegicus) age of approximately 12 weeks. Before starting the trial toxicological study was accomplished. After 1 week of adaptation, all rats were given left radial open transverse osteotomy under aseptic conditions. The rats were evenly, randomly divided into three groups. Group I was intervened with trial drug 270 mg/kg body weight twice daily dissolved in godugdh (cow milk) and goghrita given according to body weight of rat through oral route, in group II with double dose of trial drug and in group III no drug was given. Group III animal were administered only godugdh and goghrita twice daily in order to avoid biasing concerning diet and handling stress. The treatment was carried out for four weeks. The blood samples were taken directly from orbital puncture technique for bone specific alkaline phosphatase analysis before and during the trial. Digital X-Ray was taken on 2nd, 3rd and 4th week during trial period. The bone fragments of euthanized rats were taken on the last day of trial for histopathology. Study shows that the levels of bone specific alkaline phosphatase (BsALP) in the osteoblast line cells and bones
are proportional fracture healing. The study conclude that drug “Asthisamharadi Churna” augment the fracture healing process and was found to have slightly better outcome in group II when compared against group I and control group.

**KEYWORDS:** Asthisamharadi churna, Bhagna, Fracture, Fracture healing, BsALP activity.

**INTRODUCTION**
Physical injury contributes to a wide variety of alteration in the bones, joints, and soft tissues. In addition to fracture, dislocation, subluxations and capsular, tendinous, muscular and ligamentous tears, trauma affect the growth plate of the immature skeleton as well as the hyaline cartilaginous and fibro-cartilaginous joint structures.[1]

The fracture is a break in the continuity of bone or cartilage or both.[2] After a fracture, a remarkable series of events occurs that leads to osseous healing in the majority of cases. Bone fracture healing is a complex biological process. Normal fracture healing is generated by increased osteoblastic activity. Osteoblasts secrete large quantities of Alkaline Phosphatase (ALP) which is involved in the process of bone matrix formation and its mineralization[3]. Alkaline Phosphatase (ALP) is believed to either increase the concentration of local inorganic phosphate or neutralize inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal formation[4] (Volpin et al., 1998). Osteoblasts synthesize organic bone matrix (osteoid) and collagen. Osteoblasts represent the final stage of differentiation of pluripotential stem cell in the bone marrow.

Experimental studies show that the levels of bone specific alkaline phosphatase (BsALP) in the osteoblast line cells and bones are proportional to the formation of collagen. Clinical studies also show that the level of BsALP in the serum correlates with the bone formation rate[5]. Various authors have shown that regardless of the treatment method (conservative or surgical or even compressive osteo synthesis) the maximum values of the total ALP occur after the twenty-first day after the injury.[6,7]

For the management of fracture, reduction and immobilization are universally required and are to be done accordingly. But attention must also be paid to avoid or to minimize the coming complications due to fracture itself or immobilization. Role of indigenous drugs on fracture treatment is under scrutiny by the scientists worldwide and use of various indigenous
drugs for fracture healing has been the subject of interest.

In Ayurvedic classics, there are numerous formulations which are prescribed for the management of fracture healing and for early callus formation and to avoid the complication. The scientific evaluation of such drugs along with their fundamental principles is essential for their universal acceptance. Hence in this study an attempt is made to prepare a drug about which there are textural references regarding Asthisamharadi churna\textsuperscript{[8]}. Through experimental trial in the present study, it has been tried to prove the efficacy of the Asthisamharadi churna for early fracture healing process.

**AIM & OBJECTIVES**

**Aim**

To evaluate the efficacy of the Asthisamharadi churna on fracture healing.

**Objectives**

1. To assess acute toxicity study of Asthisamharadi churna according to OECD guidelines.
2. To validate effects of Asthisamharadi churna scientifically on fracture healing.
3. Compare its effects against control group.

**MATERIALS AND METHODS**

In the present experimental study 30 Albino wistar rats were used to assess the effect of Asthisamharadi Churna on fracture healing. The fractures were created in left radius bone by open transverse osteotomy. The experimental study followed the acute toxicity study of trial drugs as per the guidelines of OECD para-423, on dose 2000 mg/kg body weight. The all experimental subjects were divided in three groups.

Before starting the trial toxicological study was performed for any toxic effects/troublesome side effects in subject.

**Group I:** The group was intervened with the Asthisamharadi churna in the dose of 270 mg/kg body along with godugdh and goghrita twice daily.

**Group II:** The group was intervened with the Asthisamharadi churna in the dose of 540 mg/kg body (Double dose) along with godugdh and goghrita twice daily.

**Group III:** The Group III was considered as a control group and trial subjects were given only the godugdha and goghrita in order to avoid biasing and to balance the handling stress with other groups.
**Assessment criteria**

1. Bone specific Alkaline phosphatase taken on before trial, 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) day.
2. Digital radiograph of fractured bone taken just after fracture, 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) days.
3. Histopathological examination done on the end of the trial.

1. Bone specific Serum Alkaline Phosphatase (BsALP):

   Blood samples of the rats of all groups collected from orbital puncture technique. Blood samples were centrifuged and serum was collected. The serum sample was analysed for bone specific serum alkaline phosphatase value (BsALP).

   The standard BsALP level of albino Wister rat was not available so the results of interventions have been compared with average mean BsALP level of each group calculated before starting of the trial and there after mean BsALP level taken on 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) day in the same group.

   The outcome of intervention on BsALP level is also compared with the overall average value of plasma level of BsALP of 30 albino rats taken from the 1\(^{st}\) day of the trial.

2. Radiographic Assessment

   Radiographs were taken in both Cranio-Caudal and lateral planes and graded on a six point union score.

   **Table No. – 1 Showing Six Point Union Score**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Sharp or Sclerotic line seen throughout</td>
</tr>
<tr>
<td>1</td>
<td>Sharp or Sclerotic line in more than 75% of diameter</td>
</tr>
<tr>
<td>2</td>
<td>A well-defined Osteotomy line extending in both projections</td>
</tr>
<tr>
<td>3</td>
<td>Same as two but in one projection only.</td>
</tr>
<tr>
<td>4</td>
<td>Osteotomy faintly seen</td>
</tr>
<tr>
<td>5</td>
<td>Osteotomy not seen.</td>
</tr>
</tbody>
</table>

3. Histopathological Assessment

   Histopathological Assessment of calluses was made on following Eleven Grade scale.

   **Table No. – 2 Showing Eleven Grade scale**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All fibrous tissue</td>
</tr>
<tr>
<td>2</td>
<td>More fibrous tissue than cartilage</td>
</tr>
<tr>
<td>3</td>
<td>Fibrous and cartilaginous tissue in equal proportion</td>
</tr>
<tr>
<td>4</td>
<td>Evidence of fibrous tissue with more cartilaginous tissue than woven bone.</td>
</tr>
<tr>
<td>5</td>
<td>Evidence of fibrous tissue with equal cartilage and woven bone</td>
</tr>
<tr>
<td></td>
<td>Evidence of fibrous tissue with more woven bone than cartilage</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>7</td>
<td>Cartilaginous tissue and woven bone in almost equal proportion</td>
</tr>
<tr>
<td>8</td>
<td>Less cartilage and more woven bone</td>
</tr>
<tr>
<td>9</td>
<td>Entirely woven bone</td>
</tr>
<tr>
<td>10</td>
<td>Woven bone and some mature bone</td>
</tr>
<tr>
<td>11</td>
<td>Lamellar (mature) bone</td>
</tr>
</tbody>
</table>

**Ethical Justification**

Ethical clearance for animal experimentation was sought from Institutional Animal Ethical Committee of Institute of Biomedical and Industrial research, Jaipur (Rajasthan) CPCSEA Approval No: 1737/PO/C/14/CPCSEA prior to study.

IAEC approval number: - IBIR/IAEC/2014-03

**OBSERVATIONS AND RESULTS**

**I. Results of acute toxicity of trial drug**

It includes observational behaviour of animal, haematological test results and histopathology reports of animals which were given 2000 mg/kg dose of trial drug according to OECD guidelines para - 423.

The analysis of the results obtained show that *Ashthisamharadi churna* in the dose up to 2000mg/kg is safe for albino Wister rats (*Rattus Norwegicus*). No mortality or any morbidity was found in the above mentioned dose of the *Ashthisamharadi churna*. No changes were observed in haematological and histopathological observations.

**II. Result of experimental trial**

[A]. Observation data and result of bone specific alkaline phosphatise (BsALP) value of all groups. The outcome is compared with the group wise average mean value of plasma level of BsALP of 10 albino rats taken individually before the beginning of the trial. The group wise average value of BsALP in group I was 26.847µg/l, in group II 25.863µg/l and group III 28.936µg/l before the beginning of the trial.
Table No. – 3 Showing Mean Difference Value

<table>
<thead>
<tr>
<th>Mean Difference Value Of 0 Day And 28th Day BsALP Values</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Difference</td>
<td>1.695</td>
<td>2.613</td>
<td>0.537</td>
</tr>
</tbody>
</table>

Above table data show that mean difference of the BsALP level (between the 0 day and 28th day) in Group - I 1.695, in Group - II 2.613 and in Group - III 0.537.

1. The results show that the Group - II has better outcome in comparison to Group – III which show that double dose of Ashthisamharadi churna is responsible for better increase in the BsALP level in contrast to control group.

2. The results show that the Group - I has superior outcome in comparison to Group – III which show that single dose of Ashthisamharadi churna could be held responsible for the better increase in the BsALP level in contrast to control group.

3. The results show that the Group - II has better outcome in comparison to Group – I which show that double dose of Ashthisamharadi churna could be accredited for the better increase in the BsALP level in contrast to single dose of the drug.

When the outcome is compared with the overall mean value of BsALP of 30 albino rats taken before beginning of the trial, the overall average value of BsALP was observed around 27.22µg/l. The control group has performed better in comparison to trial groups.
Graph No. – 2 Showing BsALP Mean Value Graph

0 day* - Average value of BsALP of all the 30 rats of trial (27.22 µg/l).

Statistical analysis of effect of drug on BsALP

Table No. – 4. Showing In between comparison (Paired t – test results)

<table>
<thead>
<tr>
<th></th>
<th>Mean value</th>
<th>Mean diff.</th>
<th>Diff. S.D.</th>
<th>Diff. S.E.</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day BT</td>
<td>28\textsuperscript{th} Day AT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GROUP-I</td>
<td>26.847</td>
<td>28.542</td>
<td>1.695</td>
<td>1.443</td>
<td>0.4565</td>
</tr>
<tr>
<td>GROUP-II</td>
<td>25.863</td>
<td>28.476</td>
<td>2.613</td>
<td>3.471</td>
<td>1.098</td>
</tr>
<tr>
<td>GROUP-III</td>
<td>28.936</td>
<td>29.473</td>
<td>0.537</td>
<td>2.770</td>
<td>0.8760</td>
</tr>
</tbody>
</table>

1. It is evident that in the group I, the mean difference value of the variable BsALP is 1.695 µg/L and variation between data of BT and AT is statistically significant**.
2. It is evident that in the group II, the mean difference value of the variable BsALP is 2.613 µg/L and variation between data of BT and AT is statistically significant*.
3. It is evident that in the group III, the mean difference value of the variable BsALP is 0.537 µg/L and variation between data of BT and AT is statistically non-significant.

Table No. – 5 Showing Inter group comparison of all three groups One way anova test result

<table>
<thead>
<tr>
<th></th>
<th>Group I Mean±S.E.M.</th>
<th>Group II Mean±S.E.M.</th>
<th>Group III Mean±S.E.M.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BsALP</td>
<td>1.695± 0.4565</td>
<td>2.613± 1.098</td>
<td>0.537±0.8760</td>
<td>0.2436</td>
</tr>
</tbody>
</table>

Non-significant p value is present in intergroup comparison of Group - II vs. Group -III and Non- significant p value is present in intergroup comparison of Group - I vs. Group - III.
Observation data and result of Radiological assessment of all groups:

Graph No. - 3 Radiological Grading Mean Graph

Table No. - 6 Radiological Assessment Mean Difference Value

<table>
<thead>
<tr>
<th>Mean Difference Value Of 0 Day and 28th Day</th>
<th>Group – I</th>
<th>Group – II</th>
<th>Group – III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Difference</td>
<td>2.6</td>
<td>3.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Above table data show that Mean difference of the radiological assessment grading (between the 0 day and 28th day) in Group - I 2.6, in Group - II 3.4 and in Group - III 2.1.

1. The results shows that the Group - II has better outcome in comparison to Group – III which shows that double dose of *Ashthisamharadi churna* better amplify the fracture healing in contrast to control group.

2. The results shows that the Group - I has superior outcome in comparison to Group – III which illustrate that single dose of *Ashthisamharadi churna* also amplify the fracture healing in contrast to control group to certain extent.

3. The results shows that the Group - II has better outcome in comparison to Group – I which is prove that double dose of *Ashthisamharadi churna* more significantly augment the fracture healing process in contrast to Group – I.

Table No. – 7 Showing Inter group comparison of all three groups (One way anova kruskal-wallis test result)

<table>
<thead>
<tr>
<th></th>
<th>Group – I Mean±S.E.M.</th>
<th>Group - II Mean±S.E.M.</th>
<th>Group – III Mean±S.E.M.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 day</td>
<td>0.7±0.1528</td>
<td>1.3±0.1528</td>
<td>0.8±0.2494</td>
<td>0.0786</td>
</tr>
<tr>
<td>21 day</td>
<td>1.8±0.2000</td>
<td>2.1±0.1795</td>
<td>1.4±0.1633</td>
<td>0.0413*</td>
</tr>
<tr>
<td>28 day</td>
<td>2.6±0.2667</td>
<td>3.4±0.2667</td>
<td>2.1±0.1795</td>
<td>0.0046**</td>
</tr>
</tbody>
</table>
14th day: - The p>0.05 which is statistically non significant.
21st day: - The p<0.05 which is statistically significant.

*Significant p value is present in between comparison of Group - II vs. Group - III and non- significant in between comparison of Group - I vs. Group - III.
28th day: - The p<0.005 which is statistically significant.
** Significant p value is present in between comparison of Group - II vs. Group - III and non- significant in between comparison of Group - I vs. Group - III.

[C]. Observation data and result of Histopathological examination (HPE) assessment of all groups

Table No. – 8 Showing Inter group comparison of all three groups

<table>
<thead>
<tr>
<th>One way anova kruskal-wallis test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Mean±S.E.M.</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>28th Day</td>
</tr>
</tbody>
</table>

28th day: - The p <0.05 which is statistically significant.
Above table data show that Mean of the Histopathological assessment of callus grading was in Group – I 4.9, in Group - II 5.4 and in Group - III 4.3.
Statistical analysis reveals that comparison of Group - II with Group - III shows significant results (p<0.05) and comparison of Group - I with Group - III shows non- significant results.
The results shows that the Group - II has better outcome in comparison to Group – III and group I which shows that double dose of Asthishamharadi churna has better enhanced the fracture healing in contrast to other groups.

DISCUSSION

1. Discussion on acute toxicity study of Asthishamharadi churna
The analysis of the results obtained show that Asthishamharadi churna in the dose up to 2000mg/kg is safe for albino Wister rats (Rattus Norwegicus). No mortality or any morbidity was found in the above mentioned dose of the Asthishamharadi churna. No changes were observed in haematological and histopathological observations.

2. Discussion on the plasma levels of bone specific serum alkaline phosphatise (BsALP)
All the three groups had shown the increase in the plasma level of BsALP. Various author had already ascertained the fact that regard less of the treatment method (conservative or
surgical, or even compressive osteosynthesis) the maximum value of the total ALP occur after the twenty-first day after the onset of fracture.

On thorough statistical analysis of plasma level of BsALP values which were done group wise, the group II has performed better in comparison to control group and group I. This could be attributed to the trial drug and in particular to the amount in which it was administered to the subjects of trial Group II. Group I also performed better improvement in comparison to control group owing to the effect of trial drug.

But the control group has performed better in comparison to trial groups when the outcome is compared with the overall average value of plasma level of BsALP of 30 albino rats taken from the 1st day of the trial. This outcome might be having multifaceted explanations. This could be due to the reason that the authentic baseline value of BsALP for the Wister rat is not available. So for this experimental study the baseline value of BsALP has been calculated by taking mean of the entire 30 experimental subject. This could also be purely a co-incidental finding owing to the small number of trial subject or in particular related with the variety and species of the experimental model.

3. Discussion on radiographic assessment results
In the radiographic investigation it was observed that results are much better in group II in comparison to group I and to the control group. This could be attributed to the callus formation promoting potentials of the trial drug and in particular to its dose which has been given in double amount in comparison to group I. This is also statistically evident from the highly significant outcome from inter group comparison in between group II and control group. Statistically Inter group comparison between group I and control group shows non-significant results. The difference between these statistical outcomes could clearly be accredited to the dose of the trial drug. Clinically the difficulty was encountered while grading the callus formation in the experimental model owing to the less radio opaque nature of bone, which casts poor shadow on radiographs.

4. Discussion on the Histopathological study of callus tissue
The Histopathological study (conducted at Institute of Biomedical and Industrial research, Jaipur Rajasthan) of all groups reveals that the callus tissues of group-II were containing more part of woven bone (Immature bone) than cartilage and fibrous tissue. The results were statistically significant in comparison to control group. Whereas the study of control group
was shows non-significant results. Hence it is proved beyond doubt that the drug *Asthisamharadi churna* accelerates fracture healing.

**CONCLUSION**

On the basis of experimental study it can be concluded that drug “*Asthisamharadi churna*” augment the fracture healing process without any acute toxicity and was found to have slightly better outcome when the trial drug is intervened with the double dose. The further comprehensive study could be planned in humans to establish the effects of *Asthisamharadi churna* in the management of fracture.

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