Research Article

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BIOCHEMICAL AND HORMONAL EVALUATION OF *CISSUS QUADRANGULARIS* IN ACCELERATING HEALING PROCESS OF BONE FRACTURE: A CLINICAL STUDY

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ABSTRACT

Repair of bone defects secondary to trauma, osteoporosis, osteomyelitis and fracture nonunion poses a significant problem for many orthopedicians. As a part of our continuing screening of biologically active natural fracture healing accelerating agents, drug Asthishrunkhala reported to contain phytoestrogenic steroids, ascorbic acid, carotene, calcium, and anabolic steroids and shown to heal the bone fractures in several *in vivo* studies. Therefore, a random controlled study was conducted with the aim to evaluate the effective remedial therapy to accelerate bone healing so as to rehabilitate the individual as early as possible. Fracture healing was assessed with biochemical parameters like serum calcium, serum phosphorus and a hormonal parameter PTH (parathyroid hormone) and their values were evaluated during fracture healing. From the assessed data it was found that bone healing process was accelerated as serum level of PTH Hormone have shown increasing trend during the period of 31 days and it was at its peak on 21st day from inception of administration of drug; but levels of calcium and phosphorus were remain maintained during period of fracture healing. In control group it was observed that serial values of Serum Calcium, Serum Phosphorus and PTH Hormone were not having significant fluctuations. This indicates that the process of fracture healing or osteoblastic activity was initiated earlier in trial group. Hence study concludes that the drug *Cissus quadrangularis* is having influence on accelerating the fracture healing process and further it helps in reducing period of immobilization and early rehabilitation.

Keywords: Fracture, Asthishrunkhala, PTH hormone, Serum calcium, Phosphorous, Parathyroid hormone

INTRODUCTION

In present days the fracture healing is assessed subjectively along with various objective parameters like radiological, histopathological, mechanical, biochemical and hormonal parameters. Among these radiological, histopathological, mechanical methods are commonly followed by most of the researchers in assessing fracture healing and the other hormonal and biochemical assessment methods are seldom used. Main reason behind selecting the drug Asthishrunkhala (*Cissus quadrangularis*) is based on previous animal study which concluded that *Cissus quadrangularis* has following effects; Early callus formation; More cellularity and vascularity formation; Average 6 % more cartilage formation in 3rd week; Earlier formation of harveshian system and bone matrix and Earlier starts of medullary and cortical repair process. These were assessed on histopathological parameters. Animal study demonstrates that parathyroid hormone accelerates natural fracture healing process in the femoral osteotomy model at Department of Orthopedic Surgery, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa, Japan. This study gives an understanding about the PTH mode of action as it accelerates the natural fracture healing process by shrinking callus size and increasing degree of mineralization of the fracture callus; thereby restoring intrinsic material properties. But, in human beings few clinical studies has been conducted with help of biochemical and hormonal parameters and their changes during fracture healing with use of Ayurvedic formulation. Based on this, a random controlled study was conducted with following aim and objectives

Aim

To evaluate the effective remedial therapy to accelerate bone healing so as to rehabilitate the individual as early as possible.

Objectives

- To correlate the serial values of serum calcium, serum phosphorus, with process of fracture healing.
- To correlate the serial values of parathyroid hormones with process of fracture healing.
- To enhance the present knowledge of mode of action of drug *Cissus quadrangularis*.

MATERIAL AND METHODS

The study designing was based on conventional methodology used for single blind clinical controlled trial. Study was initiated after Ethical clearance granted by Institutional Ethical committee Govt. Ayurved College Nagpur, India and final report approved by the same with reference EC/Ph.D.P./01/13. Patients of uncomplicated bone Fracture attending O.P.D. was the major material for this study. Total 96 patients were screened and 80 patients were included in this study. A separate special case record form was used for the detailed history and the findings

during the course of study. Patients were randomly selected and carefully examined including inspection, palpation and systemic examination. A detail proforma was prepared to note all these observations. After taking proper history the diagnosis of bone fracture was confirmed by X-Ray and necessary investigations were performed to assess the general condition of the patient. Patients were classified into two groups including 40 patients in each group by simple random allocation method.

- Masking: Open label Prospective
- Control: Controlled
- No. of Groups: Two
- Sample Size: 40 in each group (Total = 40 x 2 i.e. 80)

Group A

- Drug of administration- Asthishrunkhala churna in capsule form
- Dose – 10 g in three divide doses
- Time of administration-TDS
- Route of administration - orally
- Vehicle – Luke warm water
- Duration- 30 days

Group B

- Drug of administration- Starch capsule
- Dose – 500 mg (capsule appearance as like trial drug)
- Time of administration- 1 TDS
- Route of administration - orally
- Vehicle – Luke warm water
- Duration- 30 days

General management includes only Immobilization of the part i.e. post cast and POP. Post cast on day 1st, POP on 7th day. Anti-inflammatory analgesic drugs were avoided because drug *Ciccus qudrangularis* has anti-inflammatory and analgesic property. Patients in Group A who required medication for further complications they were excluded from study with proper advice. Patients of Group-B for pain paracetomol /Diclofenac sodium was given if needed. Separate documentation was maintained. Patients were advised to follow-up for 31 days. On 1st, 07th, 21stand 31stday biochemical and hormonal parameters were assessed.

Investigations

Routine investigations like CBC, Hb %, ESR, BSL were done whenever necessary to assess general condition of patient, serum parathyroid hormone, serum calcium and serum phosphorus, Radiography (X-Ray)

Inclusion Criteria

- Patients of single bone fracture with good general condition.
- Males or non pregnant females aged 12 to 70 years.
- Able to communicate adequately with the investigator and to comply with the requirements for the entire study.
- Capable of and freely willing to provide written informed consent prior to participating in the study.

Exclusion Criteria

- Patients of systemic disorders like DM, PTB, malignancy
- Patients of thyroid dysfunction history
- Patients of calcium disorder
- Patients of open fracture and fracture with dislocation and who requires acute medical care and surgical intervention
- Patients below 12 years and above 70 years.
- Immuno-compromised patients
- Highly Displaced fracture which needs anesthesia to reduce
- Pregnant / Lactating women.
- Patient on steroids, oral contraceptive pills or estrogen replacement therapy.
- Alcoholics and/or drug abusers.
- Patients suffering from major systemic illness necessitating long term drug treatment (Rheumatoid arthritis, Psycho-Neuro-Endocrinal disorders, etc.)
- Hypersensitivity to any of the trial drugs or their ingredients.
- Patients who have completed participation in any other clinical trial during the past six (06) months.
- Any other condition which the investigator thinks may jeopardize the study.

Assessment parameters

- Biochemical – serum calcium, serum phosphorus is assessed on day 1st, 7th, 21st and 31st day.
- Hormonal – parathyroid hormone was assessed on day 1st, 7th, 21st and 31st day.

For analysis following Kits were used

- DIA source hPTH- ELISA kit Manufactured by –DIA source immunoassay S.A. Rue du bosquet, 2, B-1348 Louvain-la-neuve, Belgium for PTH analysis.
- Calcium and phosphous kit manufactured by Crest ecosystems Goa 403202 supplied by Tinu treders pvt. Ltd. Nagpur, India

The data obtained during the study were subjected to statistics analysis by repeated measure ANOVA test and wilcoxon Rank sum test.

RESULTS

The data that has generated during the clinical study is described under two headings

- Demographic Analysis
- Clinical efficacy and safety of drug under study

Demographic Analysis

Table 1 showed that, Age wise classification of patient revealed, the maximum 18 (22.5 %) number of patients were in 31-40 years age Group, thereafter 16 (20 %) patients in age group 41-50 years and patients in age group 51-60 years were 16 (20 %). Patients in above 60 years age group were 09 (11.25 %) and patients in 21-30 years age group were 11 (13.75 %) and 10 (12.5 %) patients were in 12-20 years age group.

Table 2 showed gender wise distribution of patients under study. Out of 80 patients, 46 (57.5 %) patients were male and 34 (42.5 %) patients were female.

Table 3 showed that 67 (83.75 %) patients were of Hindu religion. 05 (6.25 %) patients were of Muslim religion. 08 (10 %) patients were of Buddhist religion.

Table 4 showed that 27 (33.75 %) patients had taken education up to primary level. 06 (7.5 %) patients were taken education up to secondary level. 09 (11.25 %) patients education status was up to S.S.C. level. 20 (25 %) patients were taken education up to H.S.C. level. 15 (18.75 %) patients were of Graduate educational status; whereas only 03 (3.75 %) patients were illiterate in this study.

Table 5 showed that 13 (16.25 %) patients were working in private sector, 04 (5 %) patients were working in govt. sector, 11 (13.75 %) patients were working in own business, 05 (6.25 %) patients were labors by occupation. 14 (17.5 %) patients were taking education. 06 (7.5 %) patients were retired and 27 (33.75 %) patients were housewife by occupation.

Table 6 showed that maximum number of 17 patients (21.25 %) were inflicted with fracture of the phalenx followed by fractures of radius – 16 in number (20 %), 05 patients (6.25 %) were inflicted with fracture of the Humerus bone, 09 patients (11.25 %) were inflicted with fracture of the Metatarsals 08 patients (10 %) were inflicted with fracture of the Metacarpals, the patients who were inflicted with fracture of the Tibia were 7 in number (8.75 %), Ulna – 5 in number (6.25 %), Fibula – 2 in number (2.5 %). Calcaneum -2 in number (2.5 %), patella -01 in number (1.25 %), Scaphoid -1 in number (1.25 %), ribs 04 in number (5 %) clavical- 03 in numbers (3.75 %).

Clinical efficacy and safety of drug under study

Clinical efficacy

Effects of therapy on hormonal parameter - hPTH

Table 7 showed that mean serum hPTH in Group A (Treated Group) was 26.78 ± 9.06, 29.30 ± 9.44, 51.60 ± 9.28 and it was 34.90 ± 7.43 at baseline, 7th, 21st and 31st day respectively. Mean Serum hPTH in Group B (Control Group) was 30.78 ± 7.51, 37.15 ± 14.57, 32.95 ± 8.95 and 29.20 ± 7.47 at baseline, 7th, 21st and 31st day respectively.

Table 8 showed that in Group A it was observed that serum PTH was significantly different at different time points (f- = 98.89, P > 0.0001). No significant change in serum PTH was observed at day 7th from baseline (P > 0.05) but it was significantly increased on day 21st (p < 0.001) and also at day 31st (P < 0.001) which is highly significant. In Group B serum hPTH level was significantly increased at day 7th (p < 0.001, HS) but no significant change was noted at day 21st (p > 0.05, NS) and day 31st (p > 0.05, NS).

Table 9 showed that mean serum parathyroid hormone on day 7th in Group A was -2.52 ± 11.95 and -6.37 ± 12.03 in Group B. No significant difference was found between two groups (p-value is 0.155). While mean change in serum parathyroid hormone on day 21st from baseline in Group A was -24.82 ± 9.23 and -2.17± 6.18 in Group B. Serum PTH was significantly increased in group A as compared to group B (p-value is < 0.0001, HS). On day 31st mean change in serum parathyroid Hormone in Group A was -8.12 ± 9.22 and 1.57 ± 4.77 in Group B. Serum PTH was significantly increased in group A as compared to group B (p-value is < 0.0001, HS).

Effect of therapy on biochemical parameter i.e. Serum calcium

Table 10 showed that at baseline mean serum calcium in Group A (Treated Group) was 9.59 ± 0.609.58 ± 0.53, 9.49 ± 0.54 and 9.61 ± 0.57 at baseline, 7th, 21st and 31st day respectively. So serum calcium levels did not significantly differ at each time point in group A. (P > 0.05, NS). In Group B (Control Group) mean serum calcium at base line was 9.48 ± 0.57, at day 7th it was 9.49 ± 0.62, at day 21st it was 9.63 ± 0.58 and 9.34 ± 1.78 on day 31st. After performing repeated measure in ANOVA test it was observed that mean serum calcium level was not significantly differ at different time point (P = 0.6171, NS).

Table 11 showed that in Group A, p value was 0.8204, which is not significant whereas f value was 0.3068. In Group B, p value was 0.6171, which is also not significant, f value was 0.5987.

Table 12 showed that mean change in serum calcium in Group A was 0.0075 ± 0.75 and -0.0075 ± 0.81 in group B. On day 7th this difference was not statistically significant where p value was 0.9314. On day 21st mean change in serum calcium in Group A was 0.092 ± 0.84 and -0.14 ± 0.80 in group B. There has no significant change observed between two groups where p value was 0.2017. On day 31st mean change in serum calcium in Group A was -0.022 ± 0.83 and 0.14 ± 1.84 in Group B. No significant difference was observed in two groups where p value was 0.6075.

Effects of therapy on biochemical parameter i.e. Serum phosphorus

Table 13 showed that mean serum phosphorus in Group A (Treated Group) was 3.85 ± 0.80, 3.72 ± 0.76, 3.96 ± 0.73 and 3.66 ± 0.62 at baseline, 7th, 21st and 31st day respectively. So serum Phosphorus levels did not significantly differ at each time point in group A. (P > 0.2876, NS). In Group B (Control Group) mean serum calcium at baseline was 3.89 ± 0.62, 3.94 ± 0.87, 3.99 ± 0.82 and 3.95 ± 0.81 at baseline, 7th, 21st and 31st day respectively. After performing repeated measure in ANOVA test it was observed that mean serum calcium level was not significantly differ at different time point (P = 0.9595, NS).

Table 14 showed that in Group A, p value was 0.2876, where f value was 1.271 which was not significant. In Group B p value was 0.9595, which was also not significant where f value was 0.8776.

Table 15 showed that mean change in serum phosphorus in Group A was 0.12 ± 1.17 and -0.04 ± 1.08 was in group B on day 7th this difference was not statistically significant (P = 0.5110 NS). On day 21st mean change in serum Phosphorus in Group A was -0.11 ± 1.16 and -0.09 ± 7 1.15 in group B. There has been no significant change observed in between two groups. (P = 0.9372, NS). On day 31st mean change in serum phosphorus in Group A

was 0.18 ± 1.16 and -0.05 ± 0.86 was in group B. No significant change was observed in two Groups. (P = 0.3065, NS)

Need of concomitant medication

Table 16 showed that – 27 (33.75 %) patients in treated Group required concomitant medication while in controlled Group 39-(48.75 %) patients required concomitant medication. Average concomitant medication dose required in Group A was 2.37 ± 1.27 and in Group B it was 6.10 ± 1.95. This difference was found to be statistically significant (P < 0.0001, HS).

VAS Score for pain

Table 17 showed that mean ± SD of VAS in Group A (Treated Group) was 10, 5.05 ± 0.2.76 ± 0.97 and 0.12 ± 0.33 at baseline, 7th, 21st and 31st day respectively. In Group B (Control Group) it was 10, 5.57 ± 1.41, 3.57 ± 0.59 and 0.72 ± 0.45 at baseline, 7th, 21st and 31st Day respectively.

Table 18 showed that in Group At f = 30.32 p value was < 0.0001, which was highly significant. In Group B f = 10.72 and p value was < 0.0001, which was also highly significant.

Table 19 showed that at day 7th mean change of VAS in Group A was 4.95 ± 0.31 and 4.42 ± 1.41 in group B. This difference did not have statistical significance. (P = 0.5110, NS). On day 21st mean change of VAS in Group A was 7.32 ± 0.97 and in group B it was 6.42 ± 0.59. It reveals that there was significant reduction in pain as per VAS in group A, as compared to Group B (P < 0.0001, HS i.e. highly significant). On day 31st mean change of VAS in Group A was 9.87 ± 0.33 and in group B it was 9.27 ± 0.45 which indicates that there was significant reduction in pain as per VAS in group A, as compared to Group B (p = < 0.0001, HS i.e. highly significant).

Safety assessment

Table 20 showed that in Group A, there were no adverse event observed but in Group B (17.5 %) patients had common adverse event i.e. mild abdominal pain /Epigastric pain due to use of excessive concomitant drugs. Adverse events were more common in Group B as compared to Group A (Chi2 = 16.96, p < 0.0001, HS i.e. highly significant).

Incidence of Study drug related adverse events

It was observed that no incidences of drug related adverse events were seen in both group.

Incidence of painful conditions exacerbations

None in both Groups

Changes in vital signs or clinically significant changes as Safety of assessment at the end of study period compared with baseline showed no significance.

DISCUSSION

Influence of accidents and musculoskeletal injuries adversely affects the national economy and has a major impact on society due to their resultant work disability and absence. They are second only to respiratory disorders as a cause of short-term sickness absence.[1] Bhagna or fracture can be defined as the group of musculoskeletal diseases of traumatic origin where post traumatic squeal manifests resulting to vitiation of vata and rakta dosha leading to generalized features of to Ruja (pain), Shopha (Swelling), Pedanasahatwa (tenderness) etc inflammatory consequences. Asthhishrunkhala as the name suggests acts on asthi dhatu by virtue of loka purush samya siddhant. It has tissue specific targeted action (gamitwa) on asthi dhatu hence can be utilized in diseased condition. *Cissus quadrangularis* has madhur kashay rasa, madhur vipaka, ushna virya and laghu, ruksha sara guna. Pharmacological action of drug posses madhur rasa, madhur vipaka and ushana virya helped for alleviation of vitiated vata and rakta thereby relieving pain, swelling and tenderness.[2] Madhur rasa in the drug which has the property of sthairyakara, sandhankar and sarva dhatu vivardhana, act upon each dhatu (dhatwagni) thereby strengthening the process of transformation of dhatu. While kashay rasa, laghu ruksha guna have shoshan lekhana ropana property that acts as shothhara. According to the principal of ashrya ashryi bhava described by Vagbhata shaman of vata dosha leads to asthi vridhi.[3] Madhura rasa, madhura vipaka sara guna and raktashodhana property of Asthishrunkhala have the action on local circulation with increasing cellularity and vascularity thus the promotes process of healing resulting in to early union of fractured fragments of bone. Total 96 patients were screened for study. Out of them 12 patients were excluded with proper referral as they did not fit in inclusion criteria, whereas 04 patients withdrawn from study due to protocol voidance with irregular follow-up. They were advised for alternative treatment. 80 patients were enrolled and taken up for the trial assessment in the study. They were divided into two groups with simple random allocation method, Group A (Treated Group) treated with Asthishrunkhala capsule orally. Group B-(Control group) treated with placebo starch capsule. Observations were made during and after the treatment, to find out the fracture healing acceleration property of drug *Ciccus quadrangularis* in terms of serum parathyroid hormone, serum calcium and serum phosphorus fluctuation.

From the data presented in Table 1 to 6, it can be inferred that most patients suffered from injuries of the forearm; which are prone to be injured in a trauma, whilst trying to support body against the fall. Hormone PTH (parathyroid hormone), biochemical parameters like serum calcium, phosphorus activity during fracture healing were analyzed and evaluated in both groups, serum analysis was performed as per the standard procedure on 1st day (baseline), 7th, 21st and 31st day.

As per the data presented in Table 7 to 9,analysis showed that serum PTH hormone in group A was significantly elevated on 21st and 31st day as compared to baseline and 7th day But shows regular increasing pattern up to day 21st and from day 31st it start to lowering. Such pattern was not found in control group and in this group hPTH elevated only on day 7th and further it remains insignificant at level up to day 31st. This shows significant differences in Means of group A than group B. It denotes drug *Ciccus quadrangularis* has property to fluctuate

serum PTH and also suggests that it may early initiate and continuously stimulates osteoblastic activity during fracture healing period hence early bone healing and mineralization (callus hardening) may be possible.[5] This activity helps to reduce bone maturation period and so reduces the immobilization period and ultimately early rehabilitation can be possible.

Statistical analysis of observation details presented in Table 10 to 15, regarding the efficacy assessment parameters serum Calcium and phosphorus shows insignificant fluctuations which are within normal physiological limits but statistically insignificant fluctuation, it was observed that in treated group (Group A) serum calcium had some marked elevation between 3rd and 4th week which is the crucial period for bone mineralization and maturation. In group B at this crucial point of time serum calcium was dropped, hence it can be said that drug Asthishrunkhala may help to early mineralization and maturation of fractured bone by raising serum calcium level in crucial period of bone healing. This is quite unusual that serum levels of calcium and phosphorus are not changed with the fluctuation of PTH hormone especially in group A. It suggests that the drug Asthishrunkhala may help to inhibit osteoclastic activity during the fracture healing. The serum calcium level and fracture healing could not be correlated in the present study which confirmed the finding of earlier workers Singh *et al*. (1976).[6] These findings are also in agreement with the earlier workers viz., Lauren and Kelly (1969), Pandey and Udapa (1981), Rao (1991) and Vasantha (1991). This hPTH fluctuation may be because drug *Ciccus quadrangularis* property and therefore this fluctuation pattern was seen in only group A. This may be due to *Cissus quadrangularis* causes less amount of tissue reaction in the fractured region leading to optimum decalcification in the early stage with minimum of callus formation. Hence deposition of calcium is just enough to join the two broken segments of bone so that it's remodeling takes much faster in the treated group as compare to controls. *Cissus quadrangularis* may builds up the chemical composition of the fractured bone e.g. mucopolysaccharides, collagen, calcium, phosphorus etc. as well as its functional efficiency. Early completion of calcification process and earlier remodeling phenomenon may lead to early recovery of patients.

From the data presented in Table 17 to 19 it can be suggested that relief from pain was seen relatively earlier in Group A (Treated group) than in group B (Control group) in spite minimal use of concomitant medication. This was due to the drug *Cissus quadrangularis* which has potent analgesic anti-inflammatory property.[7]

Data presented in Table 16, showed that group A patients required considerably less concomitant medication than patients in group B. This may be because of drug *Cissus quadrangularis* which has an analgesic property anti-inflammatory. Thus with use of *Ciccus quadrangularis* one can reduce the analgesic medication load and also one can keep away the patient from adverse effects of analgesic drugs. Incidence of any adverse events reveals that in Group A, no adverse event was observed but in Group B 17.5 % patients had common adverse event i.e. mild abdominal pain / Epigastric pain due to excessive use of concomitant drugs. Adverse events were more common in group B as compared to group A (Chi2 = 16.96, $p < 0.0001$, HS i.e. highly significant). The under trial drug *Cissus quadrangularis* has a gastroprotective property so the incidences of epigastric pain or abdominal pain were less or none in treated group as compared to control group.[8] *Cissus quadrangularis* contains mostly carbonates and to a smaller extent phosphates of sodium, potassium, magnesium and calcium. Presence of potassium tartarate is also reported.[9] This makes drug more alkaline. Incidence of study drug related adverse events and incidence of painful conditions exacerbations shows that there was no incidence of drug related adverse events and incidence of painful conditions in both group. This shows drug *Cissus quadrangularis* can be used safely without adverse effects. It was observed that to overcome acute inflammatory sequences in study subjects during study, anti-inflammatory drug was used. Patients in control group (Group B) required more medication than treated group (Group A) as group A was treated with drug Asthishrunkhala which has anti-inflammatory activity. Other than efficacy assessment protocol were assessed for the effect on local swelling, nearest joint movement and tenderness during follow-ups which reveals that there was no significant difference in these inflammatory sequences except pain in both groups. Assessment of Radiographs at baseline and after treatment shows the adequate callus formation in both groups. However it excludes nonunion of fracture. The literature suggests that radiographic bone healing can only be seen after 8th week. As in present study duration of subject participation was for 31 days hence callus formation was the only criteria for radiographic assessment. Animal study revealed that drug *Cissus quadrangularis* forms callus in 3rd week based on histopathological parameters. Present study also reveals that serum parathyroid hormone level was in peak at 21st day of fracture healing hence osteoblastic activity was also maximum at the end of third week.

CONCLUSION

Our study concluded that *Cissus quadrangularis* is a pharmacological agent for acceleration of bone healing. *Cissus quadrangularis* treated group shows elevation pattern of serum hPTH, early initiation of bone resorption with early initiation of osteoblastic activity and continuous bone maturation so that the hardening of callus at fractured site may be quicker and this may help to cause reduction in healing period or immobilization period which may lead to early rehabilitation. Serum calcium and serum phosphorus dose not fluctuated significantly but they remained in maintained level even in bone resorption phage suggests that the drug Asthishrunkhala may helps to inhibit osteoclastic activity during the fracture healing. Encapsulated Drug *Cissus quadrangularis* has analgesic property so it can reduce the load of analgesic drugs used in fracture management. Drug *Cissus quadrangularis* accelerates bone fracture healing. It can be easily prepared and can be safely administered orally without any serious or adverse effects. Assessment of PTH Hormone and biochemical parameters calcium and phosphorus alone is still debatable.

Table 1: Age Group involved in study

S. No.	Age group	Group A	Group B	Total	Percentage
1	12-20 years	05	05	10	12.5 %
2	21-30 years	06	05	11	13.75 %
3	31-40 years	07	11	18	22.5 %
4	41-50 years	10	06	16	20.00 %
5	51-60 years	08	08	16	20.00 %
6	Above 60 years	04	05	09	11.25 %

Table 2: Genders involved in study

S. No.	Sex	Group A	Group B	Total	Percentage
1	Male	23	23	46	57.5
2	Female	17	17	34	42.5
	Total	40	40	80	100 %

Table 3: Religion of subjects involved in study

S. No.	Religion	Group A	Group B	Total	Percentage
1	Hindu	33	34	67	83.75 %
2	Muslim	04	01	05	06.25 %
3	Buddhist	03	05	08	10 %
	Total	40	40	80	100 %

Table 4: Education level of subjects involved in study

S. No.	Education	Group A	Group B	Total	Percentage
1	Primary	13	14	27	33.75 %
2	Secondary	03	03	06	7.5 %
3	SSC	06	03	09	11.25 %
4	HSC	07	13	20	25 %
5	Graduation	09	06	15	18.75 %
6	Illiterate	02	01	03	3.75 %
7	Total	40	40	80	100 %

Table 5: Occupations of subjects involved in study

S. No.	Occupation	Group A	Group B	Total	Percentage
1	Private service	04	09	13	16.25 %
2	Student	08	06	14	17.5 %
3	House wife	15	12	27	33.75 %
4	Govt. service	03	01	04	5 %
5	Retired	01	05	06	7.5 %
6	Business	05	06	11	13.75 %
7	Labour	04	01	05	6.25 %
	Total	40	40	80	100 %

Table 6: Fractured bone distribution in present study

S. No.	Bone	Group A	Group B	Total	Percentage
1	Phalanx	07	10	17	21.25 %
2	Metatacarapel	02	06	08	10 %
3	Radius	13	03	16	20 %
4	Ulna	04	01	05	6.25 %
5	Humerus	03	02	05	6.25 %
6	Clavical	01	02	03	3.75 %
7	Rib	01	03	04	5 %
8	Metatarsal	03	06	09	11.25 %
9	Tibia	04	03	07	8.75 %
10	Fibula	00	02	02	2.5 %
11	Patella	00	01	01	1.25 %
12	Calcaneum	02	00	02	2.5 %
13	scaphoid	00	01	01	1.25 %
	Total	40	40	80	100 %

Table 7: Serum parathyroid hormone in 2 groups at different time points

Group	Baseline	Day 7	Day 21	Day 31
A	26.78 ± 9.06	29.30 ± 9.44	51.60 ± 9.28	34.90 ± 7.43
B	30.78 ± 7.51	37.15 ± 14.57	32.95 ± 8.95	29.20 ± 7.47

Table 8: Comparison of mean serum parathyroid hormone at different time point in each group (Repeated measure ANOVA)

			Multiple comparison		
	f value	p value	Baseline vs Day 7	Basline vs Day 21	Day 21 vs Day31
Group A	98.89	< 0.0001, HS	p > 0.05, NS	< 0.001, HS	< 0.001, HS
Group B	6.693	< 0.0001, HS	< 0.001, HS	> 0.05, NS	> 0.05, HS

Table 9: Comparison of change in serum parathyroid hormone at day 7, day 21 and day 3 from baseline between 2 groups (Wilcoxon Rank sum test)

Time point	Group A	Group B	p value
Day 7	-2.52 ± 11.95	-6.37 ± 12.03	0.1552, NS
Day 21	-24.82 ± 9.23	-2.17 ± 6.18	< 0.0001, HS
Day 31	-8.12 ± 9.22	1.57 ± 4.77	< 0.0001, HS

Table 10: Serum calcium in 2 groups at different time points

Group	Baseline	Day 7	Day 21	Day 31
A	9.59 ± 0.60	9.58 ± 0.53	9.49 ± 0.54	9.61 ± 0.57
B	9.48 ± 0.57	9.49 ± 0.62	9.63 ± 0.58	9.34 ± 1.78

Table 11: Comparison of mean serum calcium at different time point in each group

	f value	p value
Group A	0.3068	0.8204, NS
Group B	0.5987	0.6171, NS

Table 12: Comparison of change in serum calcium at day 7, Day 21 and Day31 from baseline between 2 groups

Time point	Group A	Group B	p value
Day 7	0.0075 ± 0.75	-0.0075 ± 0.81	0.9314, NS
Day 21	0.092 ± 0.84	-0.14 ± 0.80	0.2017, NS
Day 31	-0.022 ± 0.83	0.14 ± 1.84	0.6075, NS

Table 13: Serum phosphorous in 2 group at different time points

Group	Baseline	Day 7	Day 21	Day 31
A	3.85 ± 0.80	3.72 ± 0.76	3.96 ± 0.73	3.66 ± 0.62
B	3.89 ± 0.62	3.94 ± 0.87	3.99 ± 0.82	3.95 ± 0.81

Table 14: Comparison of mean serum phosphorous at different time point in each group

	f value	p value
Group A	1.271	0.2876, NS
Group B	0.8776	0.9595, NS

Table 15: Comparison of change in serum phosphorous at day 7, day 21 and day31 from baseline between 2 groups

Time point	Group A	Group B	p value
Day 7	0.12 ± 1.17	-0.04 ± 1.08	0.5110, NS
Day 21	-0.11 ± 1.16	-0.09 ± 7 1.15	0.9372, NS
Day 31	0.18 ± 1.16	-0.05 ± 0.86	0.3065, NS

Table 16: Concomitant medications required for study subject

S. No.	Concomitant Medication	Group A	Group B
1	Yes	27(33.75)	39(48.75 %)
2	No	13(16.25)	01(1.25 %)
3	Total	40(100%)	40(100 %)
	Mean± SD	2.37 ± 1.27(n = 27)	6.10 ± 1.95(n = 39)
	p	P < 0.0001, HS	

Table 17: VAS in 2 group at different time points

Group	Baseline	Day 7	Day 21	Day 31
A	10	5.05 ± 0.31	2.76 ± 0.97	0.12 ± 0.33
B	10	5.57 ± 1.41	3.57 ± 0.59	0.72 ± 0.45

Table 18: Comparison of VAS at different time point in each group

	f value	p value
Group A	30.32	<0.0001,HS
Group B	1072	<0.0001,HS

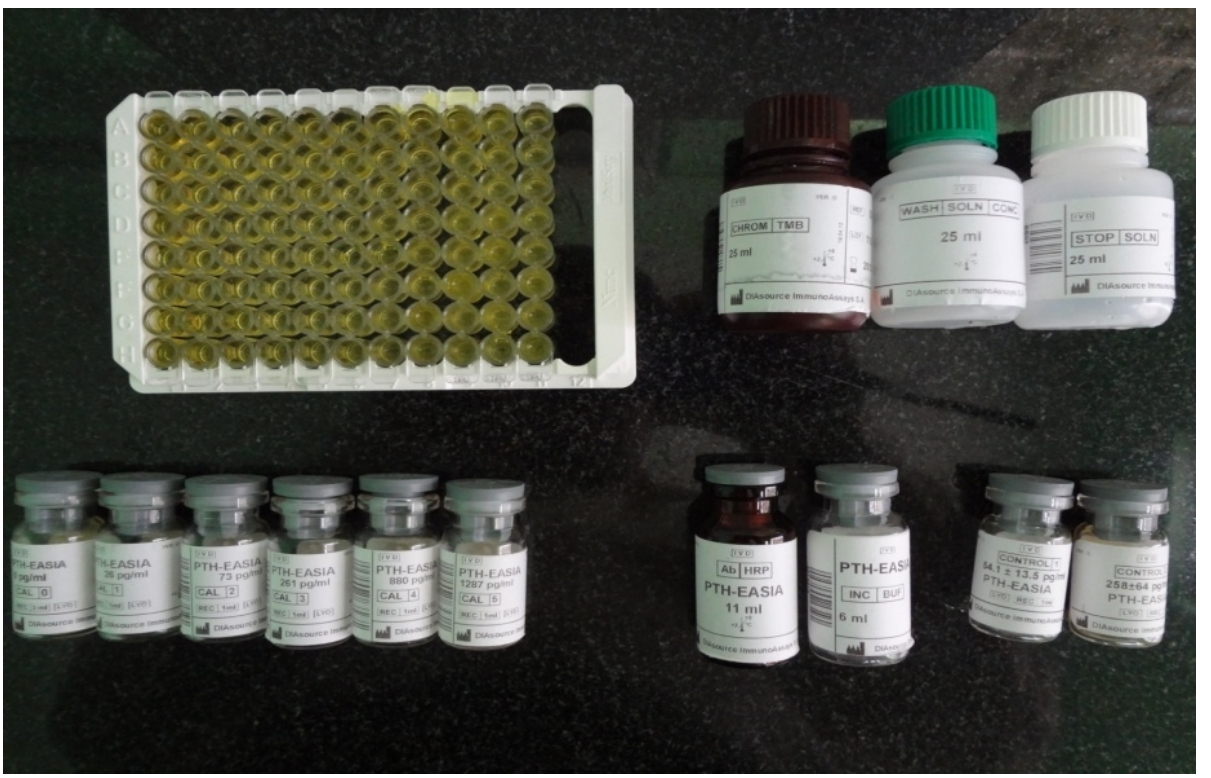
Table 19: Comparison of VAS at day 7, day 21 and day31 from baseline between 2 groups

Time point	Group -A	Group-B	p value
Day 7	4.95 ± 0.31	4.42 ± 1.41	0.5110, NS
Day 21	7.32 ± 0.97	6.42 ± 0.59	< 0.0001, HS
Day 31	9.87 ± 0.33	9.27 ± 0.45	< 0.0001, HS

Table 20: Percentage of adverse effects reported during study

S. No.	Group	AE percentage
1	Group A	00
2	Group B	17.5 %

Photos of collected steams of Asthishrunkhala:



Serum PTH hormone Analysis end step wells gained yellow color (bound enzyme labeled)

These results can be strengthened by involving other assessment parameters like bone marrow density, computed tomography and micro angiography of fractured site with hormo-biochemical parameters on each follow up and by increasing sample size and weekly follow-up until 8th week including other assessment parameters hence further research work is recommended, so that it may be proved useful and an effective approach in the management of Bone fractures.

REFERENCES

1. Influence of accidents and musculoskeletal injuries; 2013.
2. Vagbhata, Ashtang Hridya, Vidyotini Tika commentary of Kaviraj Atridev Gupta edited by Yadunandan Upadhyaya, reprint edition chaukhamba prakashana Varanasi sutrasthana 10/ 7, 8, 9; 2005. p. 110.
3. Vagbhata, Ashtang Hridya, Vidyotini Tika commentary of Kaviraj Atridev Gupta edited by Yadunandan Upadhyay, reprint edition chaukhamba prakashana Varanasi sutrasthana 11/ 26, 27, 28; 2005. p. 117-118.
4. Indian Council of Medical Research Task-Force Project Report; 2012, prevalence of fracture and musculoskeletal injuries; 2013.
5. Garima Mishra *et al* Int. J. Pharm Tech Res 2010; 2(2): 1301-02, Animal study at Department of Orthopedic Surgery, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa, Japan.
6. Singh LM and Udupa KN. Studies of *C. quadrangularis* in fracture by using phosphorus, Ind. J. Med. Sci 1962; 76: 926-931.
7. Priyanka Vijay *et al.* Journal of Pharmaceutical Science and Technology 2010; 2(1): 111-118.
8. Mallika J, Shyamala Devi CS. Gastroprotective action of *Cissus quadrangularis* extract against NSAID induced gastric ulcer: role of pro inflammatory cytokines and oxidative damage, Biol Interact 2006; 161: 262–70. http://dx.doi.org/10.1016/j.cbi.2006.04.011
9. Kritikar KR, Basu BD. In: Basu LM, editor. India Medicinal Plants (Third revised and enlarged ed.). India: Allahabad; 2000. p. 841–3.

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