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STUDY ON FRACTURE HEALING ACTIVITY OF ETHNOMEDICINAL PLANTS IN WESTERN NEPAL

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ABSTRACT

Study on biological use of ethnomedicinal plant is of high interest in modern biological research. Researchers are interested in developing new sources of drug that can be from plant origin, which has been used locally by ethnic groups for treatment of disease state. In this study, effectiveness of the plants extract used locally in the treatment of bone fracture has been compared. Plant extract against calcium supplement was given to Swiss Albino rabbits and result was interpreted with x-ray analysis and measuring blood calcium and alkaline phosphate level. Data were expressed by their mean values ± standard deviation. X-ray observation showed that group fed with 100 mg/kg of formulation had the best healing property which was supported by serum calcium and alkaline phosphate level observation. Serum calcium level after 40 days of treatment was found 8.1 ± 0.59 mg/dl (test) compared to 10.06 ± 0.21 mg/dl (standard), whereas the serum alkaline phosphate level was 113 ± 6.56 IU/l (test) against 94.67 ± 8.5 IU/l (standard). This study showed that ethnomedicinal plant was comparatively effective in the treatment of fracture healing.

KEY WORDS: Fracture healing, ethnomedicinal plant, serum calcium level, serum alkaline phosphate level.

INTRODUCTION

Interest in complementary and alternative medicine (CAM) has grown rapidly in the past decade because of its natural and holistic approach.[1] The global scenario states, large portion of the population are still influenced by traditional system of medicine.[2] Over 60% of the

world's population, 80% in developing countries, depends directly on plants for their medical purposes.[3-5] In Nepal over 90% of the population depends on herbal remedies,[6] for the treatment of injuries,[7] diabetes mellitus,[8] renal disorder,[9] neurodegenerative disorder[10] and carcinogenesis.[11] This system of medicine is still recognized as the primary health care system[12-14] in many rural communities because of its effectiveness, lack of modern medical alternatives, and cultural preferences.[15] The global demand of herbal medicine is growing and its market is expanding at the rate of 20% annually in South Asia.[16,17] The World Bank reports trade in medicinal plants, botanical drug products and raw materials is growing at an annual growth rate between 5 and 15% in major world markets of US and Europe.[18,19] The present study focus on effectiveness of herbal medicine combination for fracture healing using the biological evidences that are used as markers in fracture healing activity.

Bones are the calcified connective tissue composed of a matrix of collagen fibers,[20,21] which are prone for trauma inducing inflammatory response of neutrophils, macrophages and other inflammatory cells.[22] Fracture healing is the regenerative pattern followed by gene expression[23] which are categorized into inflammatory stage, repair stage and remodeling stage.[24] These three stages are regulated by parathyroid hormone, vitamin D, calcitonin and bone morphogenic proteins, platelet derived growth factor, and fibroblast growth factor[25] which ultimately regulates serum calcium level (SCL)[26-28] and alkaline phosphate level (APL).[29] This study has chosen SCL and APL as biological marker for the fracture healing study after the herbal formulation administration on rabbits**.**

Indigenous polyherbal combination used by local healers in Phirphire Village Development Committee (VDC), Tanahun district of Western Nepal using five medicinally valuable plants which are *Terminalia chebula*, *Terminalia bellerica*, *Mimosa rubicaulis*, *Ziziphus mauritiana*, and *Berginia ciliata* were included in this study to observe fracture healing activity.

METHODOLOGY

Collection of Crude Drugs

Bark of *Terminalia chebula* and *Terminalia bellerica* and root of *Mimosa rubicaulis*, *Ziziphus mauritiana*, and *Berginia ciliata* were collected from Phirphire VDC, Tanahun, Nepal by the proper identification under the supervision of local traditional healer.

Identification and Preservation of Crude Drugs

After collection, all the samples were identified and authenticated by botanist, Mr. Namraj Dhami, Department of Pharmaceutical Sciences, School of Health and Allied Sciences, Pokhara University. The samples were then preserved in the Pharmacognosy lab of School of Health and Allied sciences, Pokhara University with voucher number 323 (*Z. mauritiana*), 324 (*T. bellerica*), 325 (*T. chebula*), 326 (*B. ciliata*), and 327 (*M. rubicaulis*).

Extraction of Formulation

All the plant samples were dedusted, shade dried and then the samples were grinded into fine powder. The amount of samples for individual test animal used for research purpose was 2.362 g of *T. chebula*, 2.354 g of *T. bellerica*, 0.506 g of *Z. mauritiana*, 0.0824 g of *B. ciliata*, and 1.132 g of *M. rubicaulis* (as calculated on the basis of ethnomedicinal uses). The total samples required were calculated as 113 g of *T. chebula*, 113 g of *T. bellerica*, 24 g of *Z. mauritiana*, 4 g of *B. ciliata* and 54 g of *M. rubicaulis*. All the samples were weighed accurately and then mixed properly. Then, extraction was performed by the method of double maceration with water (distilled water: crude = 8:1 or 2464 ml : 308 g) and thus obtained extract was filtered with filter paper. The Filtrates obtained were then mixed and evaporated in rotary evaporator and sample was kept in refrigerator for preservation.

Grouping of Animals

Twelve rabbits (Swiss Albino rabbit) weighing 1.5-2 kg were procured for this study. The animals were housed in individual cages and allowed free access to water and standard laboratory diet. The study was conducted after obtaining ethical approval from School of Health and Allied Sciences and was performed in accordance with the Ethical Guidelines for the Care and Use of Animals in Health Research in Nepal, 2005. The animals were randomly divided into 4 groups with three per group i.e. Standard (fed with Intacal Pet 1 ml/kg), Test 1 (fed with 100 mg/kg extract), Test 2 (fed with 200 mg/kg extract) and Control group (fed with distilled water only) and labeled from number 1-12.

Induction of Fracture

Tibial shaft fracture was induced in 12 rabbits by applying mechanical force in the tibia bone after being anaesthetized using 0.2 ml of injection ketamine Hydrochloride (10 mg/ml) under the supervision of experienced veterinarian. Plaster of Paris was used as a supportive care after fracture induction in every rabbit.

Administration of Formulation

After the induction of fracture, Standard group was fed with 1 ml/kg of Intacal Pet (Intas Pharmaceuticals Pvt. Ltd.) as calcium supplement, Test 1 groups were fed with 100 mg/kg of formulation only whereas Test 2 groups were fed with 200 mg/kg of formulation via oral route of administration and Control group was given only the vehicle i.e. distilled water 1 ml daily for fourty days.

Analysis of Bone Fracture Repair

Fracture analysis was done using x-ray report and biochemical markers of the body like serum alkaline phosphatase and serum calcium level.

Data Analysis

The obtained data are expressed in their mean values ±standard deviation (SD).

3 RESULTS

X-ray

After each x-ray report, there was progress in fracture healing in every group. Groups fed with the formulation were found to have better healed than standard group and control group. Comparatively, fracture healing was best shown in Test-1 group of rabbit which was fed with 100 mg/kg of extract whereas control group showed the least progress. Furthermore, fracture healing was better seen in Test-2 group compared to standard group.

Serum Calcium Level

The serum calcium level of the animals were slightly decreased in the initial 10^{th} day in all of the groups which was then back to normal in the 20^{th} day except for the standard group. Serum calcium level was continuously increasing in the animals from 20^{th} day to the 40^{th} day. In the standard group of rabbits, the level was continuously increasing till the end of the study within the normal range (5.5-12.5 mg/dl). Table 1 shown below represents the observations of change in serum calcium level in group of rabbits given two different doses of extract and calcium supplement. Similarly, Figure 1 represents the graph for change in serum calcium level after the administration of extract and calcium supplement.

Table 1: Calcium Level (mg/dl) of Test Animals.

Groups	0 day	10th day	20th day	30th day	40th day
Test-1	7.3± 0.26	6.8± 0.20	7.27± 0.11	7.73± 0.41	8.1± 0.50
Test-2	7.3± 0.11	6.87± 0.25	7.2± 0.17	7.5± 0.21	7.8± 0.26
Standard	7.33± 0.15	7.77± 0.21	8.47± 0.21	9.23± 0.21	10.06± 0.21
Control	7.27± 0.35	6.97± 0.25	7.2± 0.26	7.43± 0.25	7.46± 0.61

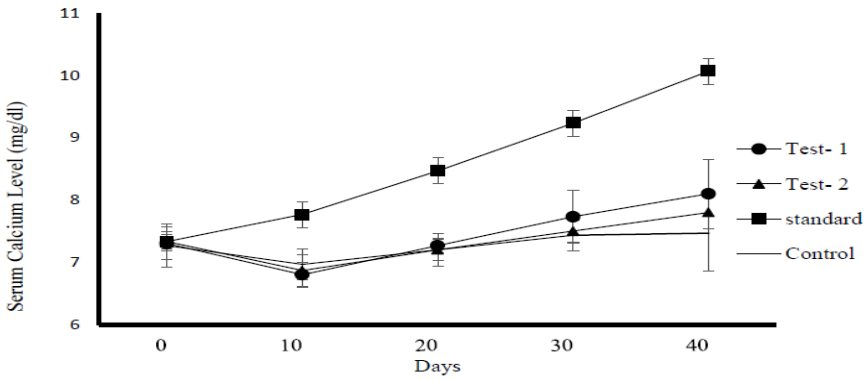

Figure 1: Graph of Serum Calcium Level in Test Animals.

Alkaline Phosphate Level

Serum alkaline phosphatase measured on the initial 10 days was higher than the 0 day normal level (10-96 IU/l) in all of the groups. This level was gradually decreasing in the standard and the control groups. However, in the test groups, the level was still increasing slowly till the day 30th. Only on the 40th day, the level was decreased in the test group. The fluctuating level of serum alkaline phosphatase was found. Table 2 shown below represents the observations of change in serum alkaline phosphatase level in group of rabbits given two different doses of extract and calcium supplement. Similarly, Figure 2 represents the graph for change in serum alkaline phosphatase level after the administration of extract and calcium supplement.

Table 2: Serum Alkaline Phosphatase Level (IU/l) in Rabbits.

Groups	0 day	10th day	20th day	30th day	40th day	
Test-1	79.67± 8.02	101.67± 3.06	110.33± 3.06	117.67± 4.36	113± 6.56	
Test-2	78± 4.36	99± 3.60	103± 2.64	108.67± 2.52	104.33± 4.04	
Standard	79± 5.57	102.67± 6.81	96.33± 3.79	100.33± 2.52	96.67±	3.79
Control	81.67± 6.51	102± 5	95.67± 4.04	99.33± 5.13	94.67±	8.5

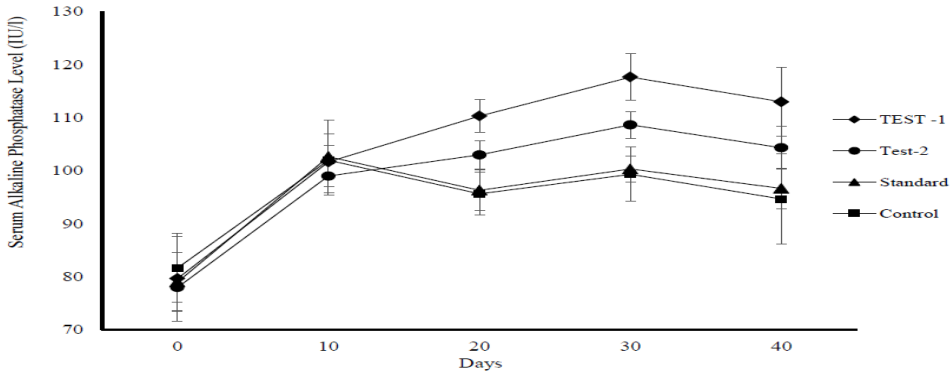

Figure 2: Graph of Level of Serum Alkaline Phosphatase.

DISCUSSIONS

Bone fracture is one of the serious problem in the world. Throughout history, and even today in much of the world, traditional herbal medicine has been the mainstay of medical practice. This long tradition of herbal wisdom has employed various herbs to speed fracture healing. The present study has confirmed the positive effect of polyherbal formulation on fracture healing depending on the results obtained from x-ray analysis, serum alkaline phosphatase and serum calcium level.

The fracture self-repair process is spontaneous and natural. The stage we set for healing greatly influences the speed, comfort, and completeness of the bone renewal process. Further, life-supporting changes made in response to a fracture can strengthen our entire skeleton and reduce the likelihood of future fractures. Bone fracture, however, might be affected by various factors like age, health, occupation, type of bone and the nature of impact on the bone. The potential of bone to heal is influenced by various biochemical, cellular, hormonal, biomechanical, and pathological mechanisms. Fracture healing is a multistage repair process that involves complex, well-managed steps of bone deposition, resorption, and remodeling for repair and restoration of function of bone. The process of fracture-healing is considered to be biologically optimal however the ability to accelerate the repair process would be highly beneficial.[30]

Various efforts have been made to accelerate the rate of fracture healing process. This includes the use of recombinant bone morphogenetic proteins (rh-BMP), low intensity ultrasound, etc.[31] Also, novel approaches like use of biodegradable matrices; cell based approaches supplemented with osteogenic factors and genetic therapy are currently being investigated for the augmentation and acceleration of fracture healing. In addition, the

discovery and cloning of several proteins (bone morphogenetic proteins) that have the ability to induce bone formation, have contributed to the investigation of novel approaches to augment fracture healing. Use of genetic therapy for the augmentation of fracture healing has also recently gained strong interest.[32] However, these modern techniques are quite expensive and unaffordable to be used by all patients. These advanced techniques are not practiced in all health care centers which make these techniques unavailable for all patients at all hospitals.

Calcium and phosphorus are the main minerals in bone, present in the form of calcium hydroxyapatite crystals, that plays an important role in regulating the elastic stiffness and tensile strength of bone.[33] Calcium adequacy at the Recommended Dietary Allowance (RDA) level is important, but unusually high intakes do not appear to speed fracture healing. As calcium absorption is dependent on vitamin D, these nutrients work synergistically. Human studies, in fact, suggest that for best fracture healing both calcium and vitamin D should be obtained in optimum daily levels.[34] Most of us consume plenty of phosphorus and often too much if the diet is high in processed foods and colas. However, the elderly, dieters, and those on low protein diets often do not consume enough phosphorus to meet the needs of new bone formation.

In the present study, the findings on serum calcium level and serum alkaline phosphatase were comparable with the standard drugs. The level of alkaline phosphatase was fluctuating in control and standard groups whereas in test group the level was increased up to day 30th which suggest that the formulation may have played a role in the cell differentiation in the process of new bone formation. ALP is a key enzyme produced by osteoblasts and is recognized as a biochemical marker of bone formation and elevated plasma ALP, prolonged by formulation treatment may indicate that the formulation promoted the systemic formation of osteoblasts which are essential for fracture healing.[35]

CONCLUSION

In conclusion, the present study demonstrated the effect of polyherbal formulation in fracture healing. The X-ray analysis report showed better fracture healing in test-1 group rabbits while the control group rabbits had the least healing. Furthermore, the level of serum alkaline phosphatase was significantly higher in test groups, having highest level in test-1 group. The level of serum calcium was found to be highest in test-1 after the standard group fed with calcium supplement. The high level of serum calcium and alkaline phosphatase in the test

animals showed the positive effect of the formulation in fracture healing. This study has aimed to provide scientific evidence for the use of polyherbal in fracture treatment and utilization of locally available natural resources which can be cost effective.

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