Original Article

Evaluation of Osteogenic property of methanolic extract of the root, leaf and whole plant of Tridax procumbens linn in Wistar albino rats

Sri Vignesh R[1], Vivek V[2], Methun F[3], Dasaprakash A[4], Caroline Sindhu P[5], Ruckmani A.[6*]

[1]Postgraduate, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

[2]Assistant Professor, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

[3]Postgraduate, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

[4]Postgraduate, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

[5]Postgraduate, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

[6]Former Professor, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chengalpattu District-603103, Tamilnadu, India.

DOI: 10.47750/pnr.2023.14.S02.239

Abstract

The objective was to evaluate the osteogenic property of methanolic extract of Tridax procumbens linn (TP) in Wistar albino rats using radiological, histopathological and histomorphometric analysis. 24 Wistar albino rats were divided into 4 groups with 6 rats in each group. Rats were anesthetized with 10% ketamine and 2% xylazine and bone defect of 3 mm size was induced in all the animal's right tibia using dental drill. Rats in group 1 were not given any treatment and served as control, rats in group 2 received methanolic root extract of TP 200 mg/kg PO, rats in group 3 received methanolic leaf extract of TP 200 mg/kg PO, and rats in group 4 were received methanolic whole plant extract of TP 200 mg/kg PO. All the animals were sacrificed on day 28, the treatment was continued till the animals were sacrificed. It was noted that the root, leaves and whole plant methanolic extracts of TP have caused bone repair of the defects induced in Wistar albino rat's tibia in terms of mineralization and new bone formation. Among the three extracts, the whole plant extract has more bone regeneration effect. No mortality was seen in any of the groups. Hence the whole plant extract of TP can be a promising prospective therapeutic agent for bone repair.

Keywords: Bone defect, Tridax procumbens linn, efficacy, whole plant extract.

INTRODUCTION

The extremely dynamic human bone is kept in homeostasis by a careful balance between osteoblasts which make bone and osteoclasts which break down bone. Bone remodelling is the outcome of the balanced function of these two types of cells [1,2]. The osteoblasts, osteoclasts and osteocytes form the primary bone cells. Osteoblasts are non-proliferative bone-forming cells that develop from osteoblast progenitor cells. The term "osteocyte" refers to a mature osteoblast. Osteocytes are no longer able to split and produce matrix substances. The osteoclasts take part in bone resorption, which eventually results in reduction of bone mass. The clinician encounters a challenging issue with bone loss and it causes an ongoing rise in the number of days spent in the hospital and the chance of complications. Bone lesions impact people financially and socially [3]. According to estimates, the prevalence of fractures could rise even higher in the upcoming years due to an increase in the worldwide elderly population [4]. By definition, a bone defect is the absence of bone tissue in an area of the body where bone would ordinarily be present [5]. Bone defects may be caused by trauma, tumor, or infection (osteomyelitis). Planning the treatment for bone loss includes assessing a number of variables, including the status of the vascular supply, the soft tissue flap, and the presence or absence of an infection. Autologous and heterologous bone grafting, or using bone matrices to fill the space are the therapy options for bone loss [6]. The growth factors and osteogenic cells, or both, may be used in combination with the latter [7].Different surgical techniques can be used to repair bone defects. The Ilizarov method, decortication, excision and fixation, and cancellous bone grafting are a few of the major methods for reconstructing bone defects. Recent studies on novel

methodologies have yielded a variety of outcomes, many of which involved growth promoters or growth enhancers. Recombinant bone morphogenetic proteins (rBMP) and autologous platelet rich plasma (PRP) in the form of activated platelet gel are examples of such biological enhancers. Though novel methods are available for treating the bone defects, one must take into account the fact that the currently available procedures frequently require periodical outpatient care and may need recurrent surgical procedures, which require lengthy hospital stays and cause financial burden to the economically marginalized patients. Natural products have emerged in this context as a promising alternative to conventional surgical treatments since they are less expensive, and facilitate quick and effective repair process. Many traditional plants utilized for the treatment of bone defects and they include are Chenopodium ambroisoides [8], Epimedium sagittatum [9], Nigella sativa [10], Sambucus Williamsii hance [11], Ulmus davidiana [12], Spinacia oleracea [13], Salvia miltiorrhiza [14], Peperomia pellucida [15], Daebergia sissoo [16], Maranfodes pumilum [17], Cassia occidentalis [18], Tridax procumbens [19].

Among these plants Tridax procumbens is taken up for this study as it is locally available and reported to have multiple pharmacological actions which could facilitate bone regeneration. Tridax procumbens extracts are known for their traditional medicinal properties to increase osteogenic properties and osteoblasts differentiation in mesenchymal stem cells [20,21,22].

Materials and Methods:

Selection of Tridax procumbens:

The Tridax procumbens plant was collected from a garden near Chettinad Hospital and Research Institute, Chennai. The species was identified and authenticated by botanist, Chettinad Hospital and Research Institute, Chennai.

Sample preparation:

The various components of TP plant, including its leaves, root, whole plant were carefully removed, washed thoroughly and air-dried. The dried parts were reduced to coarse powder by using electric blender. Eighty grams of the powdered sample was soaked in a beaker containing 400 ml of 70% methanol for a period of 48 hours with periodic stirring and then filtered with a Whatman filter paper. The filtrate was then concentrated using a rotary evaporator for 36 hours. 3 gms of extracts were yielded. The extracts were stored at 4°C for further use. Concentration of the extract, used in this study is 200 mg/ml and the dose is selected from the previous animal acute and sub-acute toxicity study [23].

Animals:

12 male and 12 female Wistar albino rats were received from Biogen Laboratory animal facility, Bangalore. The study was initiated after receiving the approval of Institutional animal ethics committee from Chettinad Hospital and Research Institute, Chennai. IAEC approval number – IAEC 2 /Proposal:61/A.Lr:44/Dt:10.08.2021

The animals were handled as per the guidelines prescribed by the CPCSEA. They were kept in clean cages, maintained at temperature of 23-25oC, humidity 50-60% in alternate light - dark cycle with water and food.

Experimental design:

24 Wistar albino rats of both sexes of age 6-8 weeks with body weight between 200-250 grams were selected and allocated to 4 different groups with 6 rats (3 male and 3 female) in each group. The animals were housed and quarantined individually in cages for one week before starting the experiment. The day of induction of bone defect was taken as day 0. On day 0, before induction of bone defect the body weight and spontaneous activity were measured for all the animals. Bone defect was induced in all the animals including control group and the study group details are as follows:

Groups	**Treatment**
Group 1	No treatment (Control)

Group 2	Methanolic root extract of Tridax procumbens 200 mg/kg PO
Group 3	Methanolic leaf extract of Tridax procumbens 200 mg/kg PO
Group 4	Methanolic whole plant extract of Tridax procumbens 200 mg/kg PO

All the extracts were given orally once a day in the morning.

Induction of bone defect:

Rats were anesthetized with 10% ketamine and 2% xylazine (1:1, 0.1 ml/100 g body weight, intraperitoneal route). The right lower limb was cleaned with spirit. The hair present over the incision site is removed with razor for 1cm. Skin is disinfected using betadine solution. A sterile surgery field is maintained by the placement of a sterile drape over non-sterile parts of the animal and surrounding area. 1cm skin incision is made and tibia was identified and subjected to perforation by using a dental drill (diameter 3 mm) under constant saline irrigation (0.9% NaCl). A 3mm defect is made. The muscle layer was sutured with resorbable 4.0 vicryl and the skin was sutured with interrupted 4.0 silk suture (Ethicon/Johnson & Johnson) [24].

At the end of 4 weeks of treatment, rats in all the groups were sacrificed using high dose of halothane, histomorphometry and histopathology of the right tibia was done after dissection. Tibia was radiographed before initiating treatment on day 0 and at the end of the experiment on day 28.

Postoperative Care:

The animals were housed individually in cages. They were fed and watered ad libitum. There was no postoperative restriction on activity and no supportive orthotic devices used. Postoperatively Inj. Meloxicam 0.5 mg/kg was given subcutaneously once a day for 2 days to relieve pain. Rats were directly observed every one hour for the first four hours and then twice a day till the end of the experiment.

Radiographic analysis:

Radiographic changes were assessed on the basis of radiographs taken with Siemens Heliphos - D X-ray machine.

Histopathological analysis of bone defect:

All the animals were sacrificed by high dose of halothane. Hind limbs were detached and fixed in 10% buffered formalin. Decalcification was done using 5% formic acid and processed for paraffin embedding. The tissues were sectioned to 5 μm thickness and stained with hematoxylin & eosin.

Bone histomorphometric analysis:

Bone histomorphometric analysis was used to assess the bone volume/tissue volume (BV/TV) is the amount of mineralized bone in a given volume of the sample, mineralizing surface/bone surface (MS/BS) is the process mineralization of the bone matrix for the formation of bone, mineral apposition rate (MAR) represents the average rate of mineralization of each osteoid seam and bone formation rate (BFR), amount of new bone that is formed per unit of bone surface in time. The specimens were dissected, the tibia was removed, and they were then preserved for a further three days in 2% phosphate-buffered paraformaldehyde before being decalcified for about 40 days in 7% EDTA with 0.5% par a formaldehyde. Transverse slices of the tibia at two separate locations were obtained after decalcification: Slices were cut from the callus centre at 1.0 mm thick, whereas samples for histomorphometric analysis were taken from the proximal midshaft at 0.5 mm thickness.

All of the animals tibial midshafts were examined in sections. Images were captured.

Statistical analysis:

Analysis of variance (ANOVA) followed by a unpaired t test. P values less than 0.05 will be considered significant. The data are presented as mean ± standard deviation values of independent replicates.

Results:

Clinical examination:

All animals tolerated the surgery and showed no signs of infection. One day following the surgery, the animals resume regular behaviour in their cages. Throughout the course of the study, no indications of aberrant behaviour and wound infection were seen.

Radiographic assessment:

At 4 weeks after surgery, the plain radiographs revealed mild mineralization at the defect area for the control group, but relatable volume of mineralization for the root and leaf extract groups. Whole plant extract group showed much more mineralization than control and other treatment groups. All three groups showed much greater mineralization at 4 weeks than control group. The radiographic evaluation revealed that the regenerated bone was significantly higher in Whole plant extract group in comparison with the other treatment and control group (Fig: 1).

Histological findings:

Hematoxylin and eosin (H&E) staining of the four groups was shown in (Fig: 5). After 28 days, in the control group, a limited bone regeneration is seen in the defect. In defect region of leaf and root extract group, newly formed bones was observed. Bone shows marrow with 100% hematopoiesis. There is a good number of rimming with osteoblast and osteoclast giant cells.

In the whole plant extract group, significantly more new bone cells were observed in the defect, compared to the other three groups. Fibromuscular fatty tissue with bone and cartilage is seen. Enchondral ossification also seen. Bone is woven with osteoblasts rimming with numerous osteoclast giant cell. Linear array of osteoclast giant cell underneath the woven bone is seen. Marrow shows hematopoiesis.

In the majority of areas, osteoblast seams were seen lining the trabeculae. Of the four groups, the control group had the least amount of bone formation. The collagen sponges were replaced by newly formed bone trabeculae in whole plant extract treated group. The bone trabeculae induced by whole plant extract were typically thicker and solid than those induced by other groups. Additionally, the structure of the newly formed bone tissue was nearly identical to that of the normal bone tissue surrounding the defect.

Bone histomorphometric analysis:

The average new bone formation in control group (C) was 0.45 µm3/µm2/d, 1.30 µm3/µm2/d in the whole plant extract (WPE), 0.77 µm3/µm2/d in the leaf extract group (LE), and 0.53 µm3/µm2/d in the root extract group (RE). The average new bone volume for control group was 29.83%, 73% for the whole plant extract group, 43% in the leaf extract group, and 47.33% in the root extract group. These groups biopsies revealed mature, cellularized, and vascularized bone and connective tissue. Specifically for whole plant extract group, the increase in bone surface percentage was 74.33 ± 4.27%, which is significantly higher than that of leaf extract group (55.67 ± 10.15%) and root extract group (53 ± 10.64%) as well as the control group (25.50 ± 5.58%). It is apparent that when compared to the control group, all three of the treated groups achieved greater bone regeneration. P-value for bone surface in C vs WPE is 0.0001, C vs LE is 0.0008, C vs RE is 0.00023.

Adverse events:

There were no adverse events seen in any of the groups till end of the study.

Radiological changes in bone defect

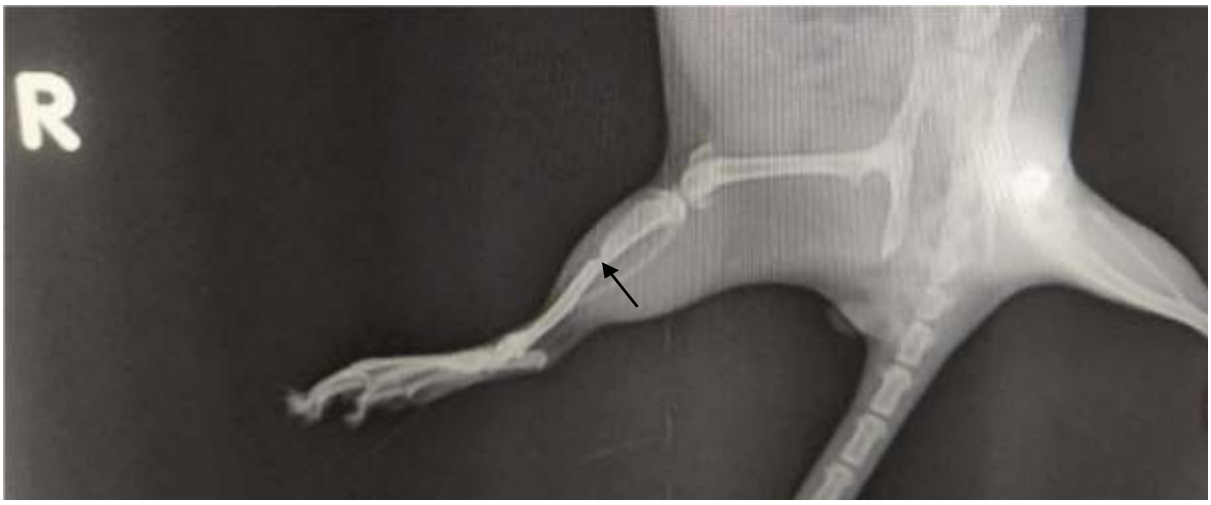


Figure: 1 Digital x-ray of bone defect on the day of induction in rattibia. Arrow shows site of bone defect.

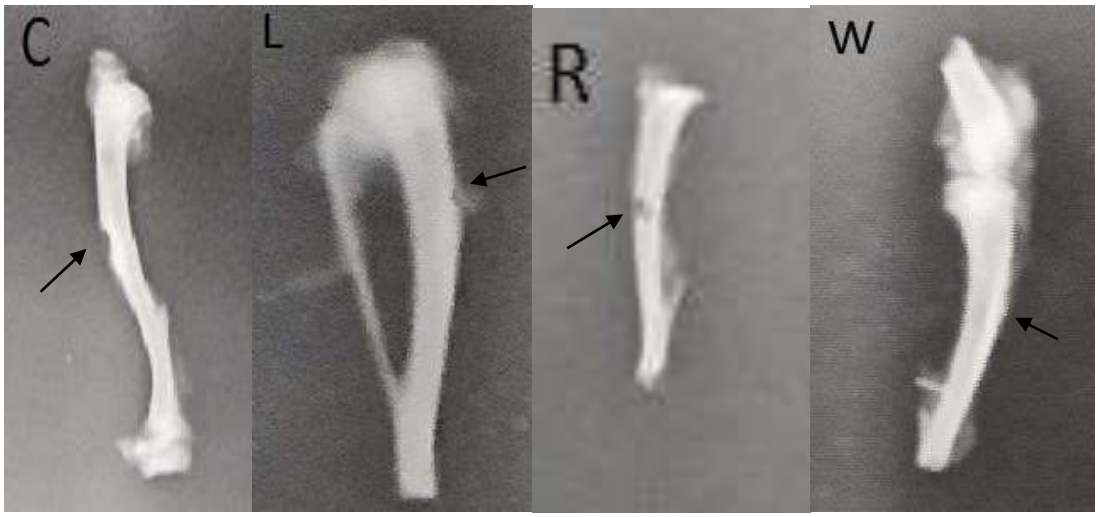
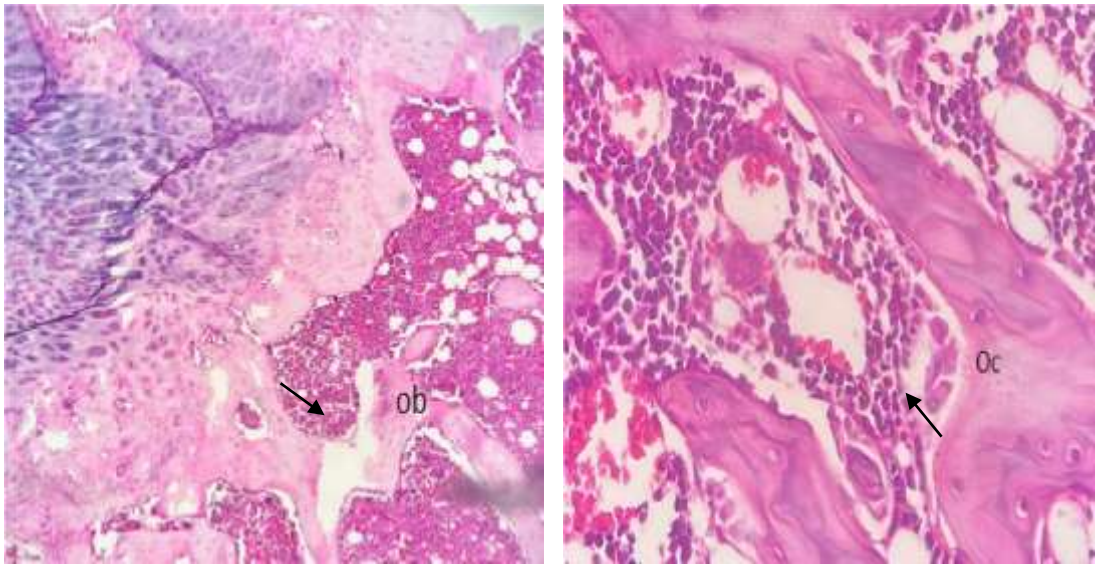
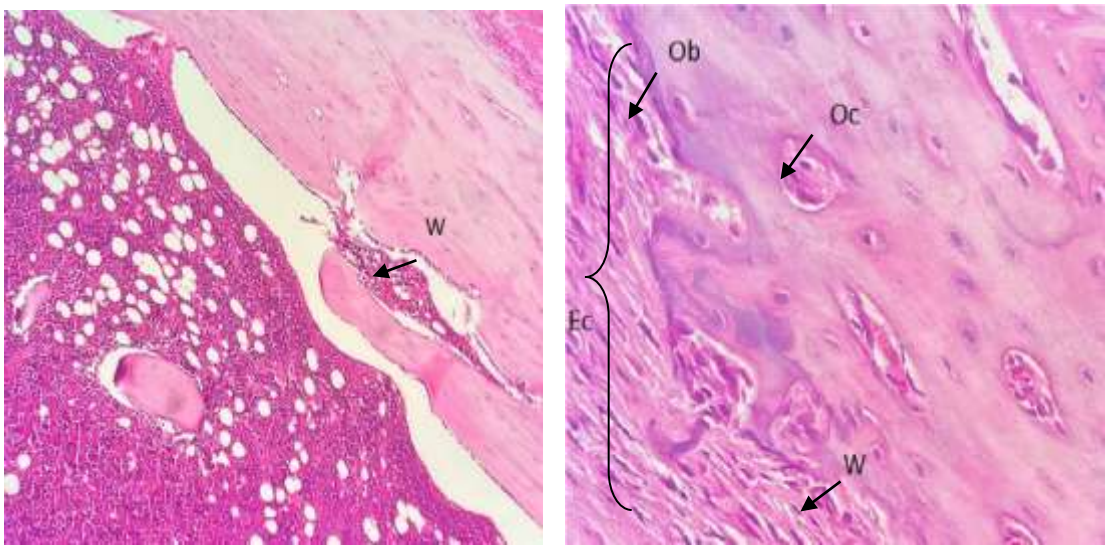


Figure: 2 x-ray of bone defect site in rat tibia 28 days postoperatively for the control (C), leaf (L), root (R) and whole plant (W) extract groups



(a) Control **(b) Root extract**



(c) Leaf extract **(d) Whole plant extract**

Figure: 3 Histopathological changes of bone defect

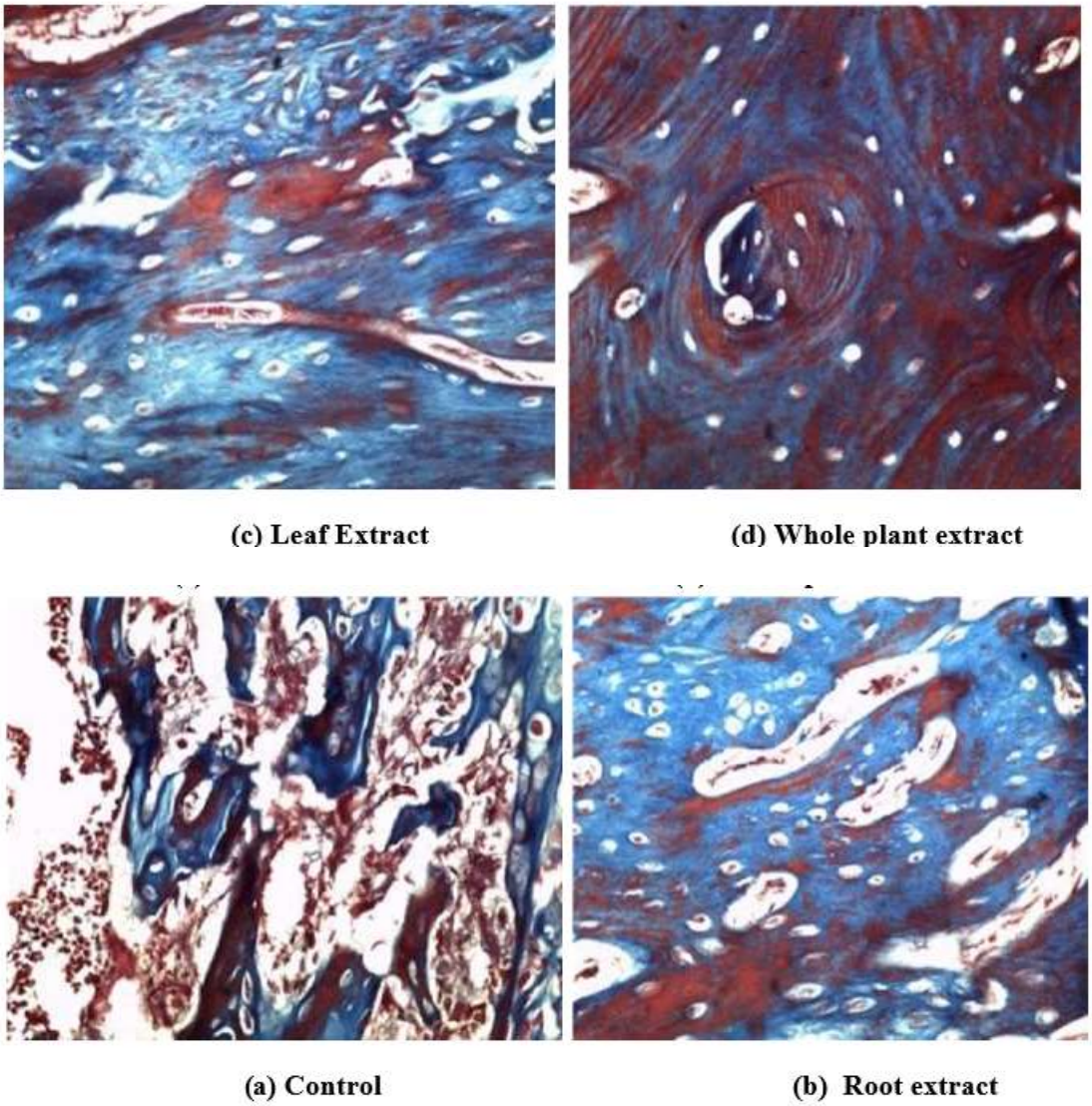


Figure: 4 Bone histomorphometry

Bone specimens of experimental groups stained with Masson TrichromeImmature collagen (blue color), Matured collagen (red color) staining (magnification, X400) Discussion:

This study was conducted to evaluate the osteogenic property of methanolic extract of

TP in Wistar albino rats. The results have showed changes in the following parameters

- Radiological examination
- Histopathological examination
- Histomorphometric analysis

Bone defects are a major problem and a challenge to therapy. Since bone grafting techniques have significant drawbacks, other methods are required to heal bone defects. In the present study it was aimed to evaluate the osteogenic property of

methanolic extract of TP on bone regeneration. The study results provided substantiation for the effectiveness of TP on bone defect repair and this includes the improvement produced by TP in the defective bone, observed in radiographic, histopathological and histomorphometric examinations.

Md Abdullah et al. (2015) reported that Tridax procumbens flavonoids (TPF) stimulated osteoblast differentiation by up-regulating ALP staining of osteoblasts derived from newborn mouse calvaria. The calvarial osteoblasts were cultured with TPF's. TPF promoted osteoblast differentiation and bone in cultured mouse primary osteoblast [25].

Al Mamun et al. (2015) analysed the TPF on in-vivo bone formation. They injected the TPF subcutaneously (20mg/kg) at base of the tail in low calcium diet mice and found that it stimulated the trabecular bone formation [26].

ALP staining was carried out on osteoblasts produced from newborn mice calvaria, and the results showed that the TPF treated osteoblasts had increased ALP staining and activity. Moreover, cell viability analysis revealed that primary calvarial osteoblasts exposed TPF did not exhibit any harmful effects or death as a result of the exposure, this was assessed by Al Mamun et al. (2017) [27].

In a study published in 2020, by Md. Muzammal Haque et al. investigated the in- vitro molecular mechanism of TPF's effects on osteoclast differentiation and actin ring formation caused by lipopolysaccharides (LPS). Osteoclast activity was evaluated by performing pit formation assays, and mature osteoclasts were quantified as the number of multinucleated cells that were tartrate-resistant acid phosphatase (TRAP) positive. Together, the data showed that TPF inhibits osteoclast differentiation and activity. TPF may therefore represent a promising and developing therapeutic candidate for the treatment of bone conditions like osteoporosis [28].

In order to improve bone repair, BMP-2 is frequently employed as an efficient growth factor. BMP-2's ability to stimulate bone regeneration is strongly dependent on the large doses needed to counteract its short half-life. Treatment that combine BMP-2 with bioactive substances that limit the quantity of BMP-2 have been developed. Mamun et al. (2019) conducted a study, he used male C57BL/6 mice as a model for calvarial defects. On the defect sites, TPF in conjunction with BMP-2 was implanted. On day 28 following implantation, the mice were sacrificied. To evaluate the healing of the defects, radiographic and histomorphometric investigations were carried out. When compared to the BMP-2 only group, the X-ray imaging of the defect revealed that the TPF with BMP-2 group led to more bone repair. Histomorphometric investigations revealed that using TPF with BMP-2 greatly increasing the bone regeneration parameters. According to data, BMP-2 induced bone repair in mice with critical-sized defects may be stimulated more by TPF [29].

This is the first study to demonstrate the oral administration of TP, which stimulated bone regeneration.

Histomorphometric analysis showed increased bone formation in the area of bone defect in treatment groups especially in the whole plant extract group. This indicate stimulatory effect of Tridax procumbens. The results of this study are on par with earlier study done by Al Mamun.et.al, [25].

Generally, no signs of local inflammation or infections were observed in any of the samples in histological examination as this plant has wound healing and antibacterial property [30,31]. In the peripheral areas of the defects, growth of the newly formed woven bone was observed. The newly formed bone is highly vascularized. Multinucleated giant cells were found in leaf and whole plant extract groups. The Control group displayed less extent of bone formation. At 4 weeks of healing, newly formed bone was observed in all groups. New bone cells was observed throughout the defect in whole plant extract group irrespective of sex. New bone in the central region of whole plant group was composed mainly of woven bone.

In this study radiographic evaluation revealed that the whole plant extract has produced significant reversal of bone defect compared to control group. Whole plant extract group showed much more mineralization than control and other treatment groups.

In summary, TP in bone defects produced beneficial effects - evidenced by (i) significant narrowing of bone defect space and soft tissue swelling and (ii) increase in the bone volume, mineralizing surface, bone formation and mineral apposition rate. It was also well tolerated by all the animals in the experimental group.

The mechanism by which the TP induces bone formation is that the TPF significantly suppressed the RANKL-induced osteocytes differentiation and bone resorption. The TPF also promoted osteoblasts differentiation and bone formation by increasing bone formation markers.

Considering the results of the current study and the available evidences for the Osteoinduction effects, TP can be further evaluated for its use in humans.

Conclusion:

The present study suggests that the root, leaves and whole plant methanolic extracts of Tridax procumbens have caused bone repair of the defects induced in Wistar albino rats tibia. Among the three extracts, the whole plant extract has more bone regeneration effect. Hence the whole plant extract can be a promising prospective therapeutic agent for bone repair. The findings stated in this study need to be substantiated by further research and clinical trials.

Funding: Self

Conflict of interest: Nil

Table: 1 Extracts effect on bone volume

Groups	Treatment	Mean ± SD	P Value
1	Control	29.83 ± 5.38	NA
2	Whole Plant Extract	73.00 ± 3.74	0.000000001*
3	Leaf Extract	43.00 ±10.94	0.0244803*
4	Root Extract	47.33 ±12.82	0.011567416*

Values expressed as Mean ± SD, n=4, * p-value <0.05 was considered statistically significant

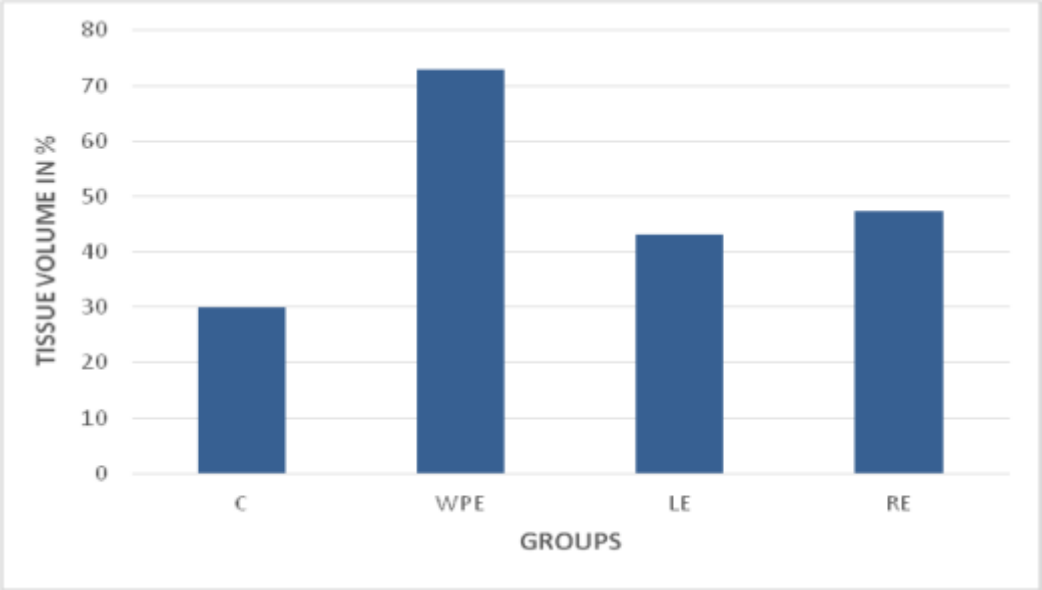


Figure: 5 Bone volume (BV) / Tissue volume (TV)

Table: 2 Extracts effect on bone surface

Groups	Treatment	Mean ± SD	P Value
1	Control	25.50 ± 5.58	NA
2	Whole Plant Extract	74.33 ± 4.27	0.000000001*
3	Leaf Extract	55.67 ± 10.15	0.0000804*
4	Root Extract	53.00 ± 10.64	0.000225307*

Values expressed as Mean ± SD, n=4, * p-value <0.05 was considered statistically significant

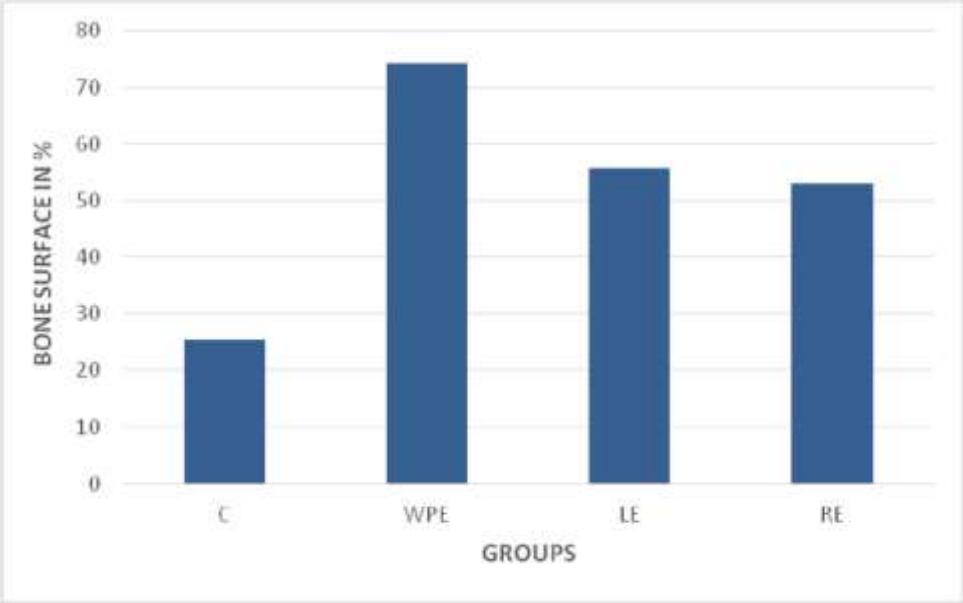


Figure: 6 Bone surface (BS) / Mineralizing surface (MS)

Table: 3 Extracts effect on bone formation rate

Groups	Treatment	Mean ± SD	P Value
1	Control	0.45 ± 0.16	NA
2	Whole Plant Extract	1.30 ± 0.21	0.0000145*
3	Leaf Extract	0.77 ± 0.24	0.024306393*
4	Root Extract	0.53 ± 0.18	0.374729453

Values expressed as Mean ± SD, n=4, * p-value <0.05 was considered statistically significant

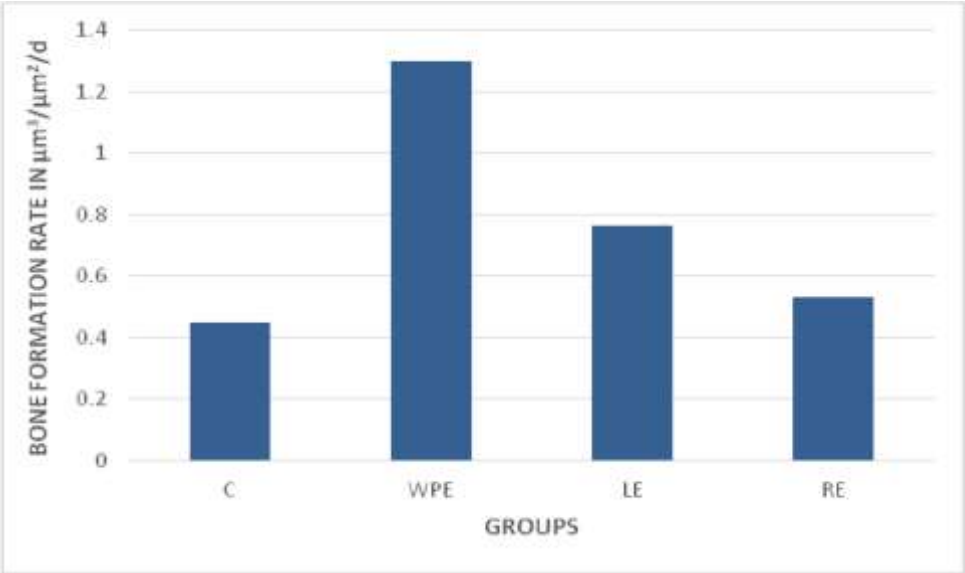


Figure: 7 Bone formation rate

Table: 4 Extracts effect on mineral apposition rate

Groups	Treatment	Mean ± SD	P Value
1	Control	1.83 ± 0.75	NA
2	Whole Plant Extract	4.50 ± 2.74	0.044266493*
3	Leaf Extract	4.50 ± 1.87	0.008882867*
4	Root Extract	4.00 ± 1.79	0.021027798*

Values expressed as Mean ± SD, n=4, * p-value <0.05 was considered statistically significant

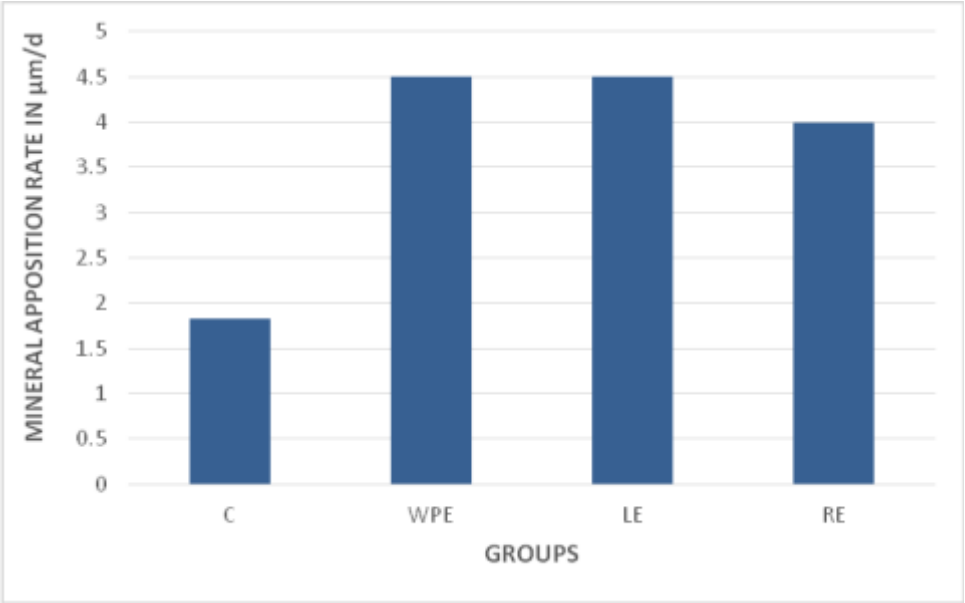


Figure: 8 Mineral apposition rate

REFERENCES

1. Boyle W J , Lacey DL Osteoclast differentiation and activation. Nature.2003; 423:337- 342 . doi: 10.1038/nature 01658
2. Teitebaum SL.Bone resorption by osteoclasts. Science.2000;289; 1504-1508 . doi: 10.1126/science.289.5484.1504
3. F. Loi, L.A. Cordova, J. Pajarinen, T.H. Lin, Z. Yao, and S.B. goodman, " Inflammation, fracture and bone repair" , Bone, vol.86,pp.119-130,2016.
4. H.E. Rosberg and L. B. Dahlin, " An increasing number of hand injuries in an elderly population- a retrospective study over a 30- year period," BMC Geriatrics, vol. 18, no. 1, p . 68, 2018.
5. Schemitsch EH. Size matters: defining critical in bone defect size. Journal of orthopaedic trauma. 2017 Oct 1;31:S20-2.
6. Maurer S.G., Baitner A.C., Di Cesare P.E.: Reconstruction of the failed femoral component and proximal femoral bone loss in revision hip surgery. J Am Acad Orthop Surg 2000; 8: pp. 354-363
7. Cancedda R., Giannoni P., Mastrogiacomo M.: A tissue engineering approach to bone repair in large animal models and in clinical practice. Biomaterials 2007; 28: pp.4240-4250.
8. V.F. Pinheiro Neto, R. M. Ribeiro, C. S. Morais et al., "Chenopodium ambroisioides in the repair of fractures in rabbits," International Journal of Pharmacology, vol. 11, pp.732–737, 2015.
9. R. A. Burim, D. I. Sendyk, L. S. Hernandes, D. F. M. de Souza, L. Correa, and M. C. Z. Deboni, "Repair of critical calvarias defects with systemic Epimedium sagittatum extract," Journal of Craniofacial Surgery, vol. 27, no. 3, pp. 799–804, 2016.
10. S. Ezirganli, H. O. Kazancioglu, H. Ozdemir, D. S. Inan, and M. Tek, "The effects of Nigella sativa seed extract on bone healing in an experimental model," Journal of Craniofacial Surgery, vol. 27, no. 7, pp. 1905–1909, 2016
11. B. Yang, X. Lin, J. Tan, X. She, Y. Liu, and H. Kuang, "Root bark of Sambucus Williamsii Hance promotes rat femoral fracture healing by the BMP-2/Runx2 signaling pathway," Journal of Ethnopharmacology, vol. 191, pp. 107–114, 2016.
12. X. Zhuang, C. Fu, W. Liu et al., "Ulmus davidiana extract improves lumbar vertebral parameters in ovariectomized osteopenic rats," American Journal of Translational Research, vol. 8, no. 2, pp. 298–313, 2016.
13. S. Adhikary, D. Choudhary, N. Ahmad et al., "Dried and free flowing granules of Spinacia oleracea accelerate bone regeneration and alleviate postmenopausal osteoporosis," Menopause, vol. 24, no. 6, pp. 686–698, 2017.
14. G. Chen, X. Zhang, H. Lin, G. Huang, Y. Chen, and L. Cui, "Tanshinol alleviates osteoporosis and myopathy in glucocorticoid-treated rats," Planta Medica, vol. 83, no. 16, pp.1264–1273, 2017.
15. N.T. Florence, S. T. S. Huguette, D. J. Hubert et al., "Aqueous extract of Peperomia pellucida (L.) HBK accelerates fracture healing in Wistar rats," BMC Complementary and Alternative Medicine, vol. 17, no. 1, p. 188, 2017.
16. A. Karvande, V. Khedgikar, P. Kushwaha et al., "Heartwood extract from Dalbergia sissoo promotes fracture healing and its application in ovariectomy-induced osteoporotic rats," Journal of Pharmacy and Pharmacology, vol. 69, no. 10, pp. 1381–1397, 2017.
17. T. R. Giaze, A. N. Shuid, I. N. Soelaiman et al., "Maranfodes pumilum leaves promote repair of osteoporotic fracture in postmenopausal Sprague-Dawley rats," International Journal of Pharmacology, vol. 14, no. 7, pp. 973–980, 2018.
18. S. Pal, P. Kumar, E. Ramakrishna et al., "Extract and fraction of Cassia occidentalis L. (a synonym of Senna occidentalis) have osteogenic effect and prevent glucocorticoid-induced osteopenia," Journal of Ethnopharmacology, vol. 235, pp. 8–18, 2019.
19. Al Mamun M, Hosen MJ, Islam K, Khatun A, Alam MM, Al-Bari M, Alim A. Tridax procumbens flavonoids promote osteoblast differentiation and bone formation. Biological Research. 2015 Dec;48(1):1-8.
20. Dhar U, Singh UK, Uddin A (2003) Ethanobotany of Bhuyans and Juangs of Orrisa. Medicinal Plants 7:200.
21. Warrier PK, Nambiar VPK, Ramankutty C (2003) Indian Medicinal Plants; A compendium of 500 species. Orient Long-man 1:368-372.
22. Sharma B, Kumar P (2009) Extraction and pharmacological evaluation of some extracts of Tridax procumbens and Capparis deciduas. International Journal of Applied Research in Natural Products 1:5-12.
23. Abubakar A, Ogbadoyi E. O, Okogun J. I., Gbodi T. I, Tifin U.F.Acute and Sub Chronic Toxicity Of Tridax Procumbens In Experimental Animals; IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT) ; ISSN: 2319-2402, ISBN: 2319-2399. Volume 1, Issue 6 (Nov. - Dec. 2012), PP 19-27;
24. Azeena S, Subhapradha N, Selvamurugan N, Narayan S, Srinivasan N, Murugesan R, Chung TW, Moorthi A. Antibacterial activity of agricultural waste derived wollastonite doped with copper for bone tissue engineering. Materials Science and Engineering: C. 2017 Feb 1;71:1156-65.
25. Al Mamun M, Hosen MJ, Islam K, Khatun A, Alam MM, Al-Bari M, Alim A. Tridax procumbens flavonoids promote osteoblast differentiation and bone formation. Biological Research. 2015 Dec;48(1):1-8.
26. Al-Bari MA, Hossain S, Mia U, Al Mamun MA. Therapeutic and mechanistic approaches of Tridax procumbens flavonoids for the treatment of osteoporosis. Current Drug Targets. 2020 Sep 1;21(16):1687-702.
27. Al Mamun M, Hosen MJ, Khatun A, Alam MM, Al-Bari M, Alim A. Tridax procumbens flavonoids: a prospective bioactive compound increased osteoblast differentiation and trabecular bone formation. Biological Research. 2017 Dec;50(1):1-0.
28. Al Mamun MA, Asim MM, Sahin MA, Al-Bari MA. Flavonoids compounds from Tridax procumbens inhibit osteoclast differentiation by down-regulating c-Fos activation. Journal of Cellular and Molecular Medicine. 2020 Feb; 24(4):2542-51.
29. Mamun MA, Asim MM, Sahin MA, Alam MM, Al Bari MA. Tridax Procumbens flavonoids stimulated synergistic effects on BMP-2-induced bone regeneration in critical-sized of calvarial defect. J Dent Oral Health. 2019; 6(204):1-8.
30. R.B.Mahato, R.P. Chaudhary,"Ethnomedicinal study and antibacterial activities of selected plants of Palpa district", Nepal. Scientific World,Vol. 3, Issue. 3, pp. 26- 31, 2005.
31. R.S Bhat, J. Shankrappa, H. G. Shivakumar, "Formulation and evaluation of polyherbal wound treatments", Asian Journal of Pharmaceutical Sciences, Vol. 2, Issue. 1, pp. 11-17, 2007.