

**Studies on biocompatibility and biodegradability  
of magnesium based orthopaedic bone  
implants in avian model**

**Thesis**

**Submitted to the**



**Govind Ballabh Pant University of Agriculture & Technology  
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**By**

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(B.V.Sc. & A.H.)

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FOR THE DEGREE OF***

***Master of Veterinary Science***  
**(VETERINARY SURGERY & RADIOLOGY)**

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*Place: Pantnagar  
June, 2019*


  
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# CERTIFICATE

This is to certify that the thesis entitled “**Studies on biocompatibility and biodegradability of magnesium based orthopaedic bone implants in avian model**” submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** with major in **Veterinary Surgery and Radiology**, of the College of Post-Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of bonafide research carried out by **Mr. Abhishek M S.**, Id. No. **52737**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

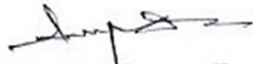
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Pantnagar  
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(**Arup Kumar Das**)  
Chairman  
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We, the undersigned, members of the Advisory Committee of **Mr. Abhishek M S., Id. No. 52737**, a candidate for the degree of Master of **Veterinary Science** with major in **Veterinary Surgery and Radiology** and minor in **Veterinary Gynaecology and Obstetrics** agree that the thesis entitled *“Studies on biocompatibility and biodegradability of magnesium based orthopaedic bone implants in avian model”* may be submitted in partial fulfillment of the requirements for the degree.

  
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## LIST OF ABBREVIATIONS

AD	Anterio-dorsal
ALCAP	Aluminium calcium phosphate
ANOVA	Analysis of variance
AO/ASIF	Association for osteosynthesis/association for study of internal fixation
ATP	Adenosine triphosphate
BP	Bard parker
Ca	Calcium
DAPI	4',6-diamidino-2-phenylindole
EDX-S	Energy dispersive X-ray spectrophotometry
GPa	Giga Pascal
HA	Hydroxyapatite
IIT	Indian Institute of Technology
IM	Intramuscular
IPF	Instructional poultry farm
IV	Intravenous
kVp	peak kilovoltage
K-wire	Kirschner wire
LE	Leading edge
mAs	milliampere second
Mg	Magnesium
mg/dL	milligrams per deciliter
ML	Medio-lateral
mmol/L	millimoles per litre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
P	Phosphorous
SC	Subcutaneous
SE	Standard error



# *Introduction*



The practicing veterinarians sometimes encounter avian orthopaedic cases, but no patient friendly modalities have been developed for the osteosynthesis of avian fractured patients. There are regular newsflash about increased incidence of injuries to birds during natural calamities and sometime during the kite festival like Makar-Sankranti. These injured birds are then treated in charity hospitals with inadequate equipments and knowledge. Most of such injured birds either die or they are bound to spend rest of their life in rehabilitation centers as flightless amputees (Appendix I).

Flight is the most important characteristic of the bird, and the avian species underwent evolutionary journey to attain this function. Avian cardiac, respiratory and skeletal systems synthesize the orchestra of flight which should be considered by the veterinarian in order to solve common clinical conditions (Coles, 2007; Dascalu *et al.*, 2015 and Sakas, 2002). The avian fracture cases ranges from simple to compound fractures, with latter being the most common as bones in birds are covered with minimum soft tissue as compared to other species and thus healing of such fractures becomes cumbersome. Intraoperative risk in avian species is also a major concern because of their high basal metabolic rate (BMR), unique and typical physiology. As the pneumatic bones are directly connected with lungs and several air-sacs, the actual mechanism of healing of such bones remained unknown. Such bones are obviously under the threat of direct infection from the commensals of respiratory tract and environment as well. The ethical issues related to wildlife leaves less scope for experimentation on them for evaluation of osteosynthetic modalities, so this is warranted that some basic guidelines are evaluated, experimentally, for osteosynthesis of pneumatic bones on domestic avian species.

Successful orthopaedic applications of biodegradable bone implants have been used for more than two decades in certain cases of human and veterinary medicine. Traditional orthopaedic implants provide reliable and steady initial postoperative fixation helping early ambulation but they and their permanent support become redundant and may produce chronic harmful effects after consolidation. Biodegradable implants abrogate secondary surgery for retrieval of hardware, often required after healing of fractures with conventional metallic devices. Biopolymer materials were introduced over 60 years ago as surgical sutures and using the same for surgical

implants was anticipated by Kulkarni *et al.*, as early as in 1966. The first biodegradable bone fixation implant was a polyglycolic acid (PGA) based material. Other than polymers viz., bioactive glass and hydroxyapatite also exist but have mainly been used as bone graft substitutes.

Currently the development of magnesium (Mg) and its alloys had drawn an attention of surgeons who are using degradable biomaterials by breaking the paradigm of current biomaterial science due to their low density, high strength to weight ratio, biodegradability and biocompatibility, good heat dissipation, good damping and good electromagnetic shield. In contrast to other alloys, Mg and its alloys have modulus of elasticity around 45 Giga Pascal (GPa), which is closer to the bone (18.6GPa), relatively large amount of Mg is tolerated by the body without ill effects and excess  $Mg^{+2}$  ions are excreted readily through kidneys (Staiger *et al.*, 2006; Haghshenas, 2017). The potential benefits of Mg based orthopaedic implants are osseo-active properties (Staiger *et al.*, 2006; Janning *et al.*, 1995) and elimination of surgery for implant retrieval. Rapid degeneration rate of Mg and its alloys is one of the major drawbacks in using these materials as successful implants (Castellani *et al.*, 2011). Therefore, surface modification of Mg and its alloys could be a possible strategy to control its degradation rate in physiological environment. A compatible implant should be chosen to reduce the negative bodily response, while maintaining requisite function. Biodegradable metallic implants dissolve inside the body with the same rate of the healing of tissues, which ultimately does not leave any residues of implant. A non-toxic oxide is generated through dissolution which is excreted through urine without causing any harmful impacts on the body (Remennik *et al.*, 2011; Friberg, 1986).

Considering above facts this study was undertaken to study the biocompatibility and biodegradability of Mg-based customized spacer [Mg and Mg-Hydroxyapatite (HA) five and 15 per cent] with the hypothesis that (i) all Mg based metallic implants are biodegradable (ii) HA matrix helps in the concentration dependent biodegradation of the implant and the objective is,

**Objective:**

- i. Radiological and clinical evaluation, with respect to biocompatibility and biodegradation, of surgically implanted Mg-based implant in the intramedullary space of avian humerus.



*Review  
of  
Literature*

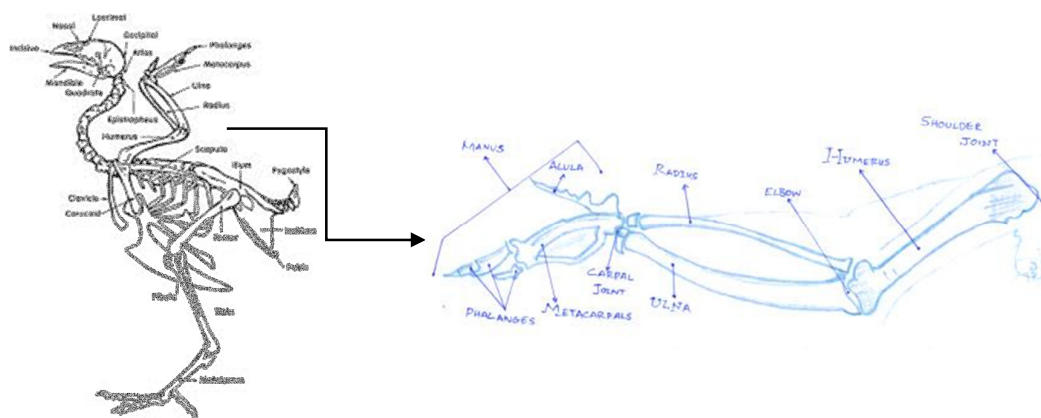


Fracture repair in birds often presents a significant challenge to the veterinary surgeons. Avian bones are described mainly as thin and brittle because of high calcium content with a large medullary cavity and thin cortices that do not hold implants well. Because of little soft tissue coverage and loose attachment with the underlying bone, the avian bone has tendency to fragment or shatter upon impact.

A review of literature relevant to avian fractures and use of biodegradable implants in avian species follows under the subsequent headings of -

- 2.1. Anatomy of humerus of poultry bird
- 2.2. Avian fracture management
- 2.3. Physical and chemical characteristics of Magnesium and Hydroxyapatite
- 2.4. Ceramic materials as bone implants
- 2.5. History of biodegradable Magnesium implants
- 2.6. *In-vivo* Corrosive properties of Magnesium and its alloys
- 2.7. Biocompatibility and Biodegradability
- 2.8. Mg metabolism and its interplay with Ca and P
- 2.9. Evaluation of biomaterial degradation activity

**2.1. ANATOMY OF HUMERUS OF POULTRY BIRD**



**Fig. 1: Skeletal model of bird with highlighted wing anatomy**

**a) Skeletal structure of chicken**

The skeleton of carinates (flying birds) is much lighter than that of mammals, in fact, a large part of their bones are pneumatic in nature with scanty bone marrow. Pneumatic cavities of major bones are communicated with the respiratory system of such birds and act to maintain the buoyancy, makes flight easier. In carinates, long bones possess thin cortex and the medullary cavity contains a network of trabeculae. These trabecula impart the structural stability of avian bones, but at the same time additional fragility and less elasticity than those of mammals. Biomechanics of avian bone also synchronizes with the structure of the wing by possessing the harmonizing ability of elbow and wrist joint flexion or extension (McLelland, 1990).

Avian humerus, when extended for flight and viewed from above the shaft appears gracefully recurved, convex along its upper half, and concave along its lower portion, distally the humeral metaphysis flares abruptly, forming the humeral condyle, which is composed of lateral and medial heads to accommodate the articular portions of ulna and radius respectively. Especially in birds the humerus head is set into a shallow socket formed by the scapula caudally and coracoids cranially, the latter is overlain by a common articular cartilage, and then clavicle gives support to the joint medially. Here humerus gives pneumatic foramen medially below its head. Head of the humerus is very much oval and flat. The cylindrical shaft is bowed and presents four surfaces (Fig.1). The lateral surface is bowing, smooth and presents a musculo spiral groove, which accommodates the brachialis muscle. At the middle of the medial surface there is a tubercle for the insertion of the teres major and latissimus dorsi muscles. This tubercle is known as teres tubercle. Anterior surface is triangular, wide and smooth above and narrow below. Between anterior and lateral surfaces there is a prominence for the insertion of deltoid muscle, termed deltoid tuberosity. The posterior surface is round and smooth. The nutrient foramen is usually situated at the distal third of this surface.

The proximal end is large and presents head, neck, tuberosities and grooves. Head is convex articulates with the glenoid cavity of the scapula. Tuberosities are lateral and medial separated by intertuberal groove. Distal end is articular, expanded and is a modified condyle and is consists of a trochlear, the capitulum, the medial and lateral epicondyles, the olecranon fossa and the radial or coronoid fossa. Medial epicondyles give origin to the flexor muscles and the lateral one to the extensor carpi radialis (Dyce *et al.*, 2002; Farrow, 2008).

## **b) Uttara-fowl: The first poultry breed of Uttarakhand**

In this study a native bird of Uttarakhand was used as the avian model for validation of test materials (Appendix I). Uttara-fowl is, said to be descended from the Red-jungle-fowl, a feathered shank fowl of Central Himalayan Region and this has been recognized as an indigenous fowl of Kumoan region of Uttarakhand. Kumoan is adjoining to the Nepal and Tibet border. Uttara-fowl symbolizes a precious and distinctive germplasm which is well adapted under harsh climatic and unsatisfactory management conditions forms the backbone of the backyard poultry farming in the villages (Kaur *et al.*, 2010).

## **2.2. AVIAN FRACTURE MANAGEMENT**

Over the past few years, great advances have been made in techniques for repairing fractures in human orthopaedics and a few of those developed techniques have been adopted in animals. But the utility of those techniques have neither been translated nor studied in avian fracture management. Earlier, external co-optation was the only method of fracture stabilization routinely recommended for avian patients, still some methods like tie-in fixator (TIF) (Tufail, 2016), a combination of an intramedullary pin (IM) linked to external skeletal fixator pins (ESF) nowadays biodegradable pin implanting has a trustable development in the biomaterial science (Bennet and Kuzma, 1992).

The bulk of an avian long bone is concentrated in a thin, porcelain-like shell which shows little or no organization into classical Haversian systems. The interior of the bone contains a network of struts, or trabeculae, each one of which is orientated to counteract the stress of external forces imposed on the bone at that particular point. The maximum stress on the bone is at the two ends, so that it is here the bone is expanded with the greatest concentration of trabeculae.

## **2.3. PHYSICAL AND CHEMICAL CHARACTERISTICS OF MAGNESIUM AND HYDROXYAPATITE**

Magnesium, a ninth most abundant element in the universe, is a shiny gray-white colored lightweight metal which belongs to Group two (alkaline earth metals) of the periodic table, represented as  $^{12}\text{Mg}_{24}$ .

### 2.3.1. MAGNESIUM

#### a) Physical properties

Density of elemental magnesium is two-third that of aluminium. It tarnishes slightly when exposed to air, unlike the other alkaline earth metals. Thin relatively impermeable layer of oxide ( $MgO_2$ ) make its storage safe in normal environment. Magnesium has the lowest melting (923 K i.e. 1,202°F) and boiling point (1,363 K i.e. 1,994°F) among group two.

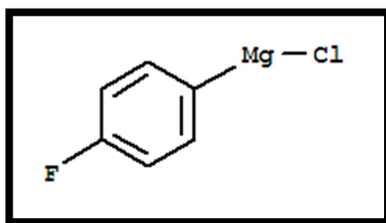
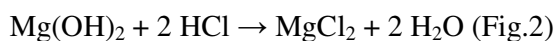
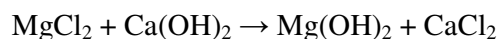
#### b) Chemical properties

##### Flammability

Magnesium is highly flammable, especially when powdered. When burnt in air, magnesium produces a brilliant-white light that includes strong ultraviolet wavelengths. Earlier, Magnesium powder (flash powder) was used for subjecting illumination in photography. Magnesium has a tendency to creep during storage at high-temperatures, which can be eliminated by the addition of scandium and gadolinium. Flammability is greatly reduced by addition of a small amount of calcium in the alloy (Dodson and Brian, 2013 and Hannavy and John, 2013).

##### Occurrence

The extraction of magnesium from seawater is done by adding calcium hydroxide and gets precipitated out as magnesium hydroxide (brucite). This water insoluble precipitate after filtration reacts with hydrochloric acid to produce concentrated magnesium chloride. Meanwhile, electrolysis of magnesium chloride produces elemental magnesium.



**Fig. 2: Chemical structure of magnesium chloride**

### 2.3.2. CHEMICAL AND BIOLOGICAL FUNCTION OF HYDROXYAPATITE

Hydroxyapatite (HA)  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  is a naturally occurring mineral form of calcium apatite. About 50 per cent by volume and 70 per cent by weight of a bone mineral is a modified form of hydroxyapatite (Junqueira *et al.*, 2003). Wet chemical deposition, biomimetic deposition, sol-gel route (wet-chemical precipitation) or electrode position are some chemical techniques of synthesis of HA (Ferraz *et al.*, 2004). The appendages of the peacock mantis shrimp (*Odontodactylus scyllarus*) are made of a tremendously dense type of mineral with superior specific strength and toughness than any synthetic composite material; these properties have led to its investigation for potential synthesis and engineering use. Clinical application of HA includes grafting materials as well as dental prosthetics and repair. Some implants, e.g. hip replacements, dental implants and bone conduction implants are coated with HA (Habibah and Salisbury, 2018 and Weaver *et al.*, 2012).

### 2.4. CERAMIC MATERIALS AS BONE IMPLANTS

The coalitional study of chemical technology and medical sciences had led to the development of new era in materialistic field, namely, the development of bio-glasses, ceramic materials, polymers, metal ions and material composites for making artificial bone implants. However, the chemical composition of metals and synthetic polymers are unsuitable for natural bone as they are not subjected to resorption and in the living organism as they will not replace natural tissue, respectively.

In 1917 Professor L. L. Hench (USA), studying the  $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2-\text{P}_2\text{O}_5$  system showed that it is bio-compatible, i.e. the material is capable of becoming alive in a living organism (Berzinya and Sedmalis, 1990 and Malokov, 1991). Butler *et al.* (2001) studied the morphological characteristics of the fibrous tissue capsule ensuing from the implantation of aluminum calcium phosphate (ALCAP) and HA bioceramic and showed that histo-hematological parameters like, number of neutrophils, macrophages, fibroblasts, degree of vascularity, and thickness of the fibrous tissue matrix was found to be statistically different between the subcutaneous implanted ceramic groups and ALCAP and HA ceramics (I/P) groups.

The experimentation to determine the effect of tricalcium phosphate (TCP), ALCAP and HA sintered ceramic particles on the viability and proliferation rate of cell

culture found that HA and ALCAP materials would affect the cells more compared to TCP ceramics (Benghuzzi, 1998). TCP and HA ceramic devices based ceramic drug delivery system for continuous release of azidothymidine (AZT) studies found to be successful in delivering drugs without ill effects and some results suggest treatment of ceramic devices with oil decreases the release rate and prolongs the delivery of AZT (Bajpai *et al.*, 1976; Bajpai, 1983; Cannon and Bajpai, 1995; Mattie and Bajpai, 1988). The reactive and inflammatory changes at implant sites are not necessarily harmful they are advantageous, because reactions like those allow host tissue to directly interface with the device (Meachim, 1976 and Mileti and Bajpai, 1995). Coleman, King, and Andrade suggest that it is logical to assume that the number of inflammatory cells participating in the foreign body reaction is directly related to the implant's reactivity. Williams noted that the presence of alumina particles in the tissue during ALCAP study have not much clinical significance (Williams, 1981). Foreign body reactions like osteolytic foci formation tends to occur in the deepest parts of the implant channels by accompanying degradation of absorbable fracture fixation implants radiographically for fractures treated with open reduction and internal fixation (ORIF) technique (Bostman, 2000).

## **2.5. HISTORY OF BIODEGRADABLE MAGNESIUM IMPLANTS**

### **2.5.1. HISTORY OF CLINICAL USES OF MAGNESIUM**

Nowadays, more than two hundred years after the discovery of elemental magnesium by Sir Humphrey Davy in 1808, these Mg based implants breaking the paradigm in metallurgy science to develop highly *in vitro* degradation resistant metals. Edward C. Huse used magnesium ligature as haemostatic during the early 1878. During 1892 most influential clinical experiments about resorptional study of Mg implants were conducted by the physician Erwin Payr and thereafter, on early 19<sup>th</sup> century Payr reported the carbon dioxide, the dissolved salts, tissue oxygen and water content, and cellular chemical activities were responsible for the *in vivo* biodegradation of Mg (Payr *et al.*, 1900 and Payr, 1901). Clinical usage of Mg was explored lately as cardiovascular, musculoskeletal and soft tissue surgery (Lambotte, 1909; Verbrugge, 1934 and Verbrugge, 1937). Rapid corrosion rate of Mg has made many cases to experience subcutaneous gas formation but even then improved recovery were found during the postoperative follow-up (Witte *et al.*, 2015). Iron and magnesium alloys are possible candidates for use in cardiovascular intervention and osteosynthesis.

Magnesium alloys have appropriate corrosion rates in physiological media as compared to slow corrosion of iron (Prakasam *et al.*, 2017 and Zberg *et al.*, 2009).

Many studies reported practical applications of commercially available magnesium alloys characteristics, regarding their rigidity, density (1.7–2.0 g cm<sup>3</sup> closer to bone 1.8–2.1 g cm<sup>3</sup>) and strength, for the internal retention of bone fragments in addition to their totally absorbable property (Gray and Luan, 2002; Grobner *et al.*, 2001; McBride, 1938 and Pollock, 2010).

### **2.5.2. Mg and Mg BASED IMPLANTS IN SOFT-TISSUE, MUSCULO SKELETAL AND GENERAL SURGERY**

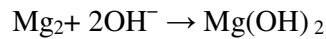
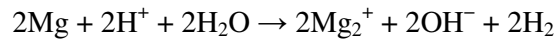
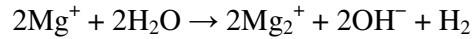
Magnesium as suture material like wires and other designs (tubular thin walled materials) as ligature for bleeding vessels (Huse, 1878 and Payr, 1900); connectors for vessel anastomosis and for intestinal anastomosis (Payr, 1901); Mg implants in musculoskeletal applications like osteosynthetic application, resection and resurfacing arthroplasty to regain joint, includes fixator pins, nails, wires (cerclage), pegs, cramps, Mg sheets and plates (Chen *et al.*, 2014; Rostock, 1937 and Payr, 1901); wires for haemangioma and large-vessel aneurysms treatment (Hoffheinz and Dimitroff, 1928); Mg implants neutralizes the acid environment in the inflammatory tissue and stimulate the formation of bone callus (Verbrugge, 1934 and Verbrugge, 1937); Silicate-substituted hydroxyapatite (SA) scaffold and osseous response of silicon (Si) level over the activity of both bone forming and resorbing cells (Hing *et al.*, 2006). Good *in vivo* surface bioactivity of Ca-P coated Mg implants compared to plain Mg implants (Xin *et al.*, 2011 and Xu *et al.* 2009). Osteogenesis and osteoblasts adhesive property of Mg ions cause new bone formation around the Mg-Ca alloy implant (Zreiqat *et al.*, 2002).

### **2.6. IN-VIVO CORROSIVE PROPERTY OF MAGNESIUM AND ITS ALLOYS**

Controlled *in vivo* corrosion of magnesium alloys might provide a novel development of biomedical application of implants in musculoskeletal surgery. The corrosion process follows electrochemical reactions, to release oxides, hydroxides and H<sub>2</sub> gas species, when the implants come in contact with physiological environment rich in dissolved oxygen, proteins and electrolyte ions (chloride and hydroxide ions) (Agarwal *et al.*, 2016; Ghali, 2010; Kubasek *et al.*, 2013; Rettig and Virtanen, 2008; Song *et al.*, 2012; Witte *et al.*, 2004; Xin *et al.*, 2011 and Zhang *et al.*, 2010). The *in*

*in vitro* corrosion tests for implants are not suitable to predict *in vivo* degradation rates of magnesium and their alloys (Walker *et al.*, 2012 and Witte *et al.*, 2006),

### **Chemical reaction of hydrogen gas formation**



A slow and delayed *in vitro* corrosion and *in vivo* degradation process can be achieved by adding rare earth elements or by coating the Mg implant surface with a corrosion resistant layers like HA, Ca-P, Ca-Zn and other alloys (Imwinkelried *et al.*, 2013 and Thomann *et al.*, 2010; Kuhlmann *et al.*, 2012; Schaller *et al.*, 2016 and Wang *et al.*, 2015).

## **2.7. BIOCOMPATIBILITY AND BIODEGRADABILITY**

The good biocompatibility, biodegradability, mechanical integrity and radiopaque property of magnesium led to an area of research in orthopaedic osteosynthesis and cardiovascular procedures (Chaya *et al.*, 2015 and Marukawa *et al.*, 2015; Windhagen *et al.*, 2013 and Zartner *et al.*, 2005). Porous materials degrade effectively than compact block of same material, likewise, vascularized spongy bone shows faster course than the avascular connective tissue (Bergsma *et al.*, 1995; Suuronen *et al.*, 1998 and Witte *et al.*, 2006).

### **2.7.1. PATHOPHYSIOLOGY AND TOXICOLOGY OF BIODEGRADABLE Mg BASED ORTHOPAEDIC IMPLANTS**

Mg ions may cause systemic and local toxicity in the humans as well as animal models as the result of degradation of Mg and their alloys on peri-implant cells, when their values exceed the serum threshold level. As Mg is the most abundant element in the body, it shows relatively very low toxicity (Raggatt and Partridge, 2010; Sun *et al.*, 1997; Witte *et al.*, 2007a and Witte *et al.*, 2007b).

In general, the healing process of bone consists of three phases; inflammatory (foreign body reaction), reparative and remodeling. The biocompatibility of degradable implants might strongly dependent on inflammatory phase and *in vivo* behavior of the material (Feyerabend *et al.*, 2010). The degradation of synthetic biodegradable

polyesters of orthopaedic use occurs largely by hydrolysis and a lesser extent by enzymatic reaction and elimination through respiration (Williams, 1982 and Williams, 1992).

*In vivo* degradation of metallic implants made of magnesium alloys depends on the composition of the alloying elements and likewise, high magnesium ion concentration could lead to bone cell activation and increased implant bone contact for implants with Mg-HA (Kuwahara *et al.*, 2001; Li *et al.*, 2004; Revell *et al.*, 2004 and Witte *et al.*, 2005)

### **2.7.2. CLINICAL OBSERVATION**

#### **a) General prevalence of adverse tissue reactions**

During mid 1980s after introduction of Mg based biodegradable implants it became obvious that these materials may provoke adverse body response like inflammatory, non-bacterial and non-mutagenic foreign-body reaction (Athanasidou *et al.*, 1996; Bostman *et al.*, 1987 and Hirvensalo, 1989). Rather developing a direct toxic influence, the foreign-body stimulated osteolytic response due to nonspecific activation of the osteoclasts by the hydrolyzed implant debris (Anderson *et al.* 1997).

#### **b) Clinical characteristics of the reactions**

The classical foreign-body response originally presents as a directly emerging painful, erythematous, papule over the implantation site or sometimes acute tissue reactions at intra-articular implantation area (Barfod and Svendsen, 1992 and Takizawa *et al.*, 1998). Even though Mg and their alloys have good biocompatibility these implants were clinically forsaken over stainless steel because of rapid corrosion with excess amount of subcutaneously emphysema formation (McBride, 1938 and McCord, 1942). Biodegradable magnesium alloy not only fulfils the enhanced bone response but also meets the excellent bone implant interface strength (Castellani *et al.*, 2011).

### **2.8. Mg METABOLISM AND ITS INTERPLAY WITH Ca AND P**

Magnesium is an essential element for the metabolism and nutritional intake is 300–400 mg/day. The body contains 0.05 per cent Mg by weight [60 per cent in skeleton, 38 per cent in soft tissue, one to two per cent in extracellular component] and Mg is second abundant intracellular cation and an activator of over 300 enzymes in the

body including phosphatases and ATP involving enzymes (Grubbs and Maguire, 1987; Heaton, 1990; Quamme, 1997; Saris *et al.*, 2000 and Wolf and Cittadini, 2003). Serum magnesium concentration ranges from 1.5 to 5 mg/dL (0.8 to 2.1mmol/L) depending on the species and is less well controlled than that of calcium (Evans and Parsons, 1988).

### **2.8.1. INTESTINAL MAGNESIUM ABSORPTION**

Magnesium absorption occurs as freely diffusing ion principally in the terminal small-intestine, colon of simple stomach animals and fore-stomachs of ruminants. Calcium and magnesium relates indirectly with each other with respect to absorption in intestine with excess dietary supplementation (Flatman, 1991 and Rosol, 1992).

### **2.8.2. MAGNESIUM EXCRETION**

The degraded products processed by multinucleated cells and were dissolved to eliminate largely via the kidneys and fewer through intestine and mammary secretions. Muscle and the bone Mg storage acts as a reservoir to balance during crisis at the same time excrete during surplus (Birch, 1990 and Lothar, 2000).

### **2.8.3. HOMEOSTASIS**

Magnesium homeostasis is a result of the balance between intestinal absorption and renal excretion with additional regulation by the adrenals, thyroids, and parathyroid glands (Abbott and Rude, 1993; Barri *et al.*, 1990; Bilezikian, 1992 and Halse, 1984).

The physiological mechanisms which are responsible for maintaining the plasma concentration at a constant level is yet not fully understood. Both Calcitonin and parathormone may be involved. Recent studies suggest that magnesium depletion may result in impaired synthesis and release of parathyroid hormone in man and animals (Buckle *et al.*, 1968; Littlelike and Arnaud, 1971 and Sallis and Deluca, 1966).

Hypermagnesemia is frequently iatrogenic observed in acute or chronic renal failure, because of exogenous magnesium treatment, e.g. antacids, enemas or parenteral administration. As renal failure progresses excretion of magnesium with fraction of sodium and other solutes will also increase. Clinician rely on measurements of serum as well as extracellular Mg level as an indicator of symptomatic Mg deficiency, as because erythrocyte and muscle Mg concentrations are no more clinical significance (Mordes and Wacker, 1978 and Watson *et al.*, 1979).

## 2.9. EVALUATION OF BIOMATERIAL DEGRADATION ACTIVITY

### 2.9.1. RADIOLOGICAL EVALUATION

A basic tool for evaluating fracture healing both in clinical use and animal studies is radiography; radiographs are able to visualize callus formation after mineralization, studying radio-opaque materials inside bone marrow, affections of different bodily systems (Aronson and Shen, 1994). Usually radiographs are taken immediately after surgery to examine the location of the fracture, location of hardware materials fixed and the quality of fixation. A variety of measurements, such as bone density or bone dimensions, periosteal reaction (callus formation), quality of union and bone remodelling, can be quantitated on radiographs by different scoring systems, after the sacrifice of experimental animal (Bos *et al.*, 1983, Lane and Sandhu 1987 and Tarvainen *et al.*, 1994). To assess the radiographic union, bone alignment and callus remodelling, for implant assessments the radiographs are to be taken postoperatively on the day of surgery and then on the 2nd, 4th, 6th, and 8th week (Bigham *et al.*, 2008). A union developed in the unilateral fractures of the radius and ulna bones in avian subject by radiographic evidence of a mineralized external callus at 5 weeks after internal fixation and at 8 weeks after external fixation (Newton and Zietlin, 1977).

Tunio *et al.* (2015) took serial radiographs at every three weeks till their end-point of three, six, and 12 weeks post-surgery in fracture experimentally induced in a pigeon ulna model. X-ray machine was set with standard avian settings 44 kilo voltage peaks (kVp) and 2 mill ampere seconds (mAs) with an exposure time of 0.04 milliseconds.



*Materials  
and  
Methods*



The present study was conducted in 18 Uttara-fowls (*Gallus gallus*) that were subjected to intramedullary insertion of biodegradable magnesium based implants into the humerus. The period for this study of the experiment was from October, 2018 to April, 2019 (180 days).

### 3.1. BIRDS

After getting the approval from the Institutional Animal Ethical Committee (vide approval number IAEC/ CVASc/ VSR/ 337 dated May 14, 2018) 18 birds were procured from the Instructional Poultry Farm (IPF), Nagla with leg bands for their identification. Only healthy active 8-9 months old birds of either sex with good appetite and weighing between 1.5 and 2 kg were selected. Birds were weighed periodically and kept in the experimental house where they were provided with feed (adult layer feed) and fresh water (fortified with calcium, vitamin-C and glucose) daily till the end of the experiment. Birds were randomly divided into three groups as per the following protocol as stated in table below:

**Table 3.1: Protocol of the study**

Groups	Implant type	n
Group - I	C-ARM guided Intramedullary insertion of Mg- based plain implant	6
Group - II	C-ARM guided Intramedullary insertion of Mg-5 wt % HA implant	6
Group - III	C-ARM guided Intramedullary insertion of Mg-15 wt % HA implant	6

\*n- Number of birds in each group, N (total number of birds) - 18

### 3.2. *IN-VITRO* STUDY OF THE IMPLANT MATERIAL AND FABRICATION OF IMPLANTS

In the current study, Mg-3Zn/HA composite with varying content of HA (0, 5 and 15 weight per cent) was synthesized and extruded in the Department of Metallurgy & Materials, IIT, Roorkee through powder metallurgy route via spark plasma sintering technique (Sunil *et al.*, 2014). [Magnesium powder (purity > 99 per cent, density 1.71 g/cm<sup>3</sup>) with an average particle size 130 ± 15 µm and Zinc powder (purity > 99.8 per

cent, density  $6.61 \text{ g/cm}^3$ ) with particle size  $30 \pm 5 \mu\text{m}$  were used for the extrusion of the test implant. Nano hydroxyapatite powder, which has an average height  $166 \pm 20 \text{ nm}$  and diameter  $94 \pm 8 \text{ nm}$ , was synthesized by wet precipitation method (Singh *et al.*, 2015). HA powders were first dispersed in acetone through probe sonicator followed by addition of 3 weight per cent Zinc and balanced magnesium and then further sonicated for 40 minutes for better dispersion. With varying weight per cent of HA (five and fifteen weight per cent) were incorporated to make the metal matrix composites (MMC). The powders were compacted and sintered using spark plasma sintering. The samples were named as MZ, MZ5H and MZ15H based on the content of HA addition].

### **3.2.1. PHYSICAL CHARACTERIZATION OF POWDERS AND COMPOSITE**

Elemental distribution maps of the inter-metallic particles or the trace elements of the implants proposed were quantitatively measured using optical microscope [Leica, DMI 5000M light optical microscope]. Helium pycnometry [Smart Instrument Co. Pvt. Ltd. Maharashtra] was used to determine the density of sintered samples. Phase structure of the sintered composites were analysed by X-ray diffractometer [XRD; smart lab, Rigaku, Japan] using Cu  $K\alpha$  ( $\lambda = 1.5418 \text{ \AA}$ ) radiation at  $2\theta$  range of  $20^\circ$  to  $80^\circ$  with  $1^\circ/\text{min}$  scan rate. The surface micrographs of degraded composites, after exposure, and composition of corroded products were examined by field emission scanning electron microscopy [FESEM; Carl Zeiss ultra plus, Germany] and energy dispersive spectroscopy (EDS) [FESEM; Carl Zeiss ultra plus, Germany], respectively.

### **3.2.2. IMPLANT TREATMENT**

To remove the industrial dirt implants were preoperatively treated with acetone and for surgical asepsis they were duly autoclaved.

### **3.2.3. BIOCOMPATIBILITY AND TESTS ON CELL CULTURE**

In the present study, the cytotoxicity test of the Mg based intramedullary implants was performed on human foetal osteoblast cells (hFOB) with respect to MTT and DAPI staining assays. This piece of work was conducted in the Division of Nanotechnology, IIT, Roorkee.

#### **a) Human foetal osteoblast cell culture**

Human Foetal Osteoblast Progenitor Cell (hFOB) line, procured from Central Drug Research Institute (CDRI), Lucknow, were incubated for one, three and five days after impregnating three various magnesium implants with variant percentages of HA (0-, 5- and 15- wt % HA) to study the cytotoxic effect of the implants.

#### **b) DAPI staining**

To check the effect of implants on growing cells over their proliferation 4',6-diamidino-2-phenylindole (DAPI) staining was carried out. The cells grown on implants were fixed with 3.7 per cent formaldehyde after three to five days of culture and stained with 4',6-diamidino-2-phenylindole [DAPI, Sigma, USA]. The images were then captured using a fluorescence microscope [Carl Zeiss Axiovert 25, Germany].

#### **c) Cell viability assay (MTT assay)**

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was procured from Himedia, India. The MTT assay was used to estimate the cell viability, based on nicotinamide adenosine diphosphate (NADPH) dependent cellular oxidoreductase mitochondrial enzyme to determine the metabolic activity of the cells after incubating with the test implants.

### **3.3. IN-VIVO SURGICAL IMPLANTATION**

A general small animal surgical pack along with Schanz screw inserting T-handle and a piece of K-wire were autoclaved and used for C-ARM guided implant insertion surgery in all fowls under general anaesthesia (Fig.19: 19a, 19b).

#### **3.3.1. C-ARM IMAGE INTENSIFIER**

A high frequency mobile X-ray image intensifier television system (Vision: Medicaid Equipments Pvt. Ltd., Pune, India) with nine inches diameter image intensifier for operating voltages between 40-50 kV at variable tube currents between 1.5 to 2.5 mA was used. It consists of control panel C-ARM, image intensifier with CCD (charge coupled device) camera and optics, image memory, trolley with two monitors (17 inch), 3 kva servo stabilizer. The main control units with mobile trolley contain an operating console, lifting column, rotational column, trag arm and holder for C-ARM and a high definition image intensifier tube with CCD (Charge Coupled Device) camera.

### **3.3.2. PRE-OPERATIVE MEDICATION AND ANIMAL PREPARATION**

All the birds were kept off-feed and off-water for one hour prior to surgery and were premedicated with Inj. Atropine sulphate<sup>1</sup>, subcutaneously, at the dose rate of 0.04 mg/kg body weight, Inj. Amoxirum Forte [Amoxicillin (200 mg) + Sulbactam (100 mg)<sup>2</sup>] at the dose rate of 125 mg/kg body weight, deep intramuscularly, 20 minutes prior to surgery. The surgical site was defeathered manually and prepared aseptically. Surgical area was scrubbed using chlorhexidine (1.5 per cent v/v) solution and 70 per cent v/v isopropyl alcohol was sprayed subsequently.

### **3.3.3. ANAESTHESIA**

Ketamine<sup>3</sup> (at the dose rate of 35 mg/kg body weight) anaesthesia was induced in all the birds with half of the calculated dose injected intravenously and remaining half was injected subcutaneously using insulin syringe in a contra-lateral wing vein.

### **3.3.4. POSITIONING AND DRAPING OF THE PATIENT**

After achieving adequate depth of anesthesia, the birds were positioned on draped Parflex-plastic table top in lateral recumbency with extended right wing in such a way that antero-dorsal surface of the humerus facing the surgeon over the table attached to the C-ARM system. The right wing was windowed through the slit of the drape and the distal end of wing draped separately.

### **3.3.5. SURGICAL PROCEDURE**

Superficial course at antero-dorsal aspect of humerus was ascertained by palpation and visualization and considered as the surgical site (Fig.16). One mm skin incision (Fig.15b) was made after the bird was properly anaesthetized and positioned in lateral recumbency (Fig.15a).

### **3.3.6. INTRAMEDULLARY INSERTION OF IMPLANT**

All the birds from group I, II and III were subjected to implantation of intramedullary spacers of Mg, Mg-HA (5 per cent) and Mg-HA (15 per cent), respectively. Stab incision over the skin on antero-dorsal surface of right humerus was

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<sup>1</sup>Atropine sulphate – Alps Pharmaceutical, Almora

<sup>2</sup>Amoxirum forte – Virbac Animal Health India Pvt. Ltd. Mumbai

<sup>3</sup>Ketamine– Themis Medicare Limited, BHEL, Haridwar

given using B.P blade # 23. First, a pilot-hole over the surgical site was drilled by smooth spinning of the lanceolet tip of B.P blade # 11 then a drill-bit (2.0 mm) was used to smoothen the tract attached to T-handle. The test implant was introduced in to the medullary cavity after proper reaming with the help of K-wire. The soft tissues were sutured routinely with 3-0 polyglactin 910 (Vicryl, Ethicon). Skin wound closed by the same material using horizontal suture mattress technique.

[K-wire has become a generic name for any type of wire or pin fixation of a fracture. The Kirschner wire was developed by Martin Kirschner (1879-1942), German surgeon, who introduced the use of wire skeletal traction in 1909]

### **3.3.7. POST OPERATIVE CARE**

Post-operatively, the wound was dressed with povidone iodine. The operated wing was bandaged and wrapped to the chest of bird using a paper tape of four inch size. The bandage was changed and antiseptic dressing of the wound was done on alternate days for five days, with scheduled post operative antibiotic regimen. All birds were kept under cage confinement to restrict their movement during the convalescent period.

### **3.3.8. PROCUREMENT OF BONE SAMPLES FOR HISTOPATHOLOGY**

Each formalin stored bone samples were segmented by placing in bench vise using orthopaedic bone saw. While taking a segmental sample (8 mm approximately) from dismembered bone the position/location of the implant lodgment was ascertained by reading the previous radiographs of respective bird.

### **3.3.9. OBSERVATIONS**

#### **3.3.9.1. INTRAOPERATIVE OBSERVATIONS**

During the time of operation, observations like extent of soft tissue manipulation and damage, degree of technical easiness or difficulties in implant application were recorded and time required for the surgery in minutes starting from the skin incision to application of the last skin suture was also noted.

#### **3.3.9.2. CLINICAL OBSERVATION**

All clinical parameters like heart rate, cloacal temperature, respiration rate and inflammatory signs were recorded for each bird before, during and after implant fixation at different intervals.

- a) **Heart rate:** Heart beat for one minute was recorded by using stethoscope by placing it on the thoracic area of the bird.
- b) **Cloacal temperature:** Temperature was recorded by using the clinical thermometer (in Fahrenheit scale) kept touched in the cloacal wall for two minutes.
- c) **Healing of surgical wound:** Wound healing progression was noted after the removal of bandage and the sutures were removed at 6-7<sup>th</sup> day post operatively.
- d) **Flight test:** Flight test was conducted in all birds at three, six and 12 post operative weeks. The birds were comfortably tossed in the air to facilitate their flight and observations like deviation in flight, flight pattern and landing pattern were recorded and scored. The observations were scored in a scale of 0-3 (Table 3.2).

**Table 3.2: Score for flight test observations**

Score	Observations
0	Lack of flight with crash landing
1	Deviation in the flight towards right with clumsy landing
2	Minor deviation in the flight towards right with proper landing
3	Normal flight with proper landing

### 3.3.10. RADIOLOGICAL EVALUATION

Immediate postoperative radiographs were taken to evaluate the success of implant fixation into the distal intramedullary space of right humerus in birds of each group. The progress of degradation of the implant was evaluated from the radiographs i.e. extent of clearance of gas formation, degradation of implant, callus formation over the hole drilled and complications, like fracture and hematoma, hydrogen gas accumulation (subcutaneous emphysema), wing dropping and general weakness (on the basis of body weight), if any were observed by taking radiographs at different intervals i.e. on first, second, third, fourth, sixth, eighth, 10<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup>, 21<sup>st</sup> and 24<sup>th</sup> post operative week. The medio-lateral (ML) and leading edge views of right wing for

implanted humerus of all the birds were taken with the exposure factors of kVp of 49 and 6.0 to 6.5 mAs and 90 cm as Focal Film Distance. For presentation of data photographs of all the taken radiographs were snapped from a distance of 10-15 cm from the illuminator by using 10 Mega Pixel high magnifications of Gionee-S6s model mobile phone.

### **3.3.11. BIOCHEMICAL STUDY**

A total of 1 milliliter of blood samples were collected from the jugular vein from the each bird by using 26 gauge needle preoperatively and subsequent first, second and third postoperative weeks into the vial containing clot activator. Serum was then extracted by centrifuging at 2500 rotations/minute for five minutes and then preserved in deep freezer (-20°C). The ERBA Mannheim® calcium and phosphorous kit<sup>4</sup> was used for estimation. Estimation of magnesium by laser ablation inductively coupled plasma mass spectrometry, calcium and phosphorus by semi-automated analyzer using Moorehead and Briggs method (1974) and modified Daly and Ertingshausen's method (1972), respectively, was used as a quantitative methods. The data obtained was recorded and statistical analysis was performed from the same.

### **3.3.12. HISTOLOGICAL EVALUATION**

On completion of six months of study period all birds were euthanized by intravenous injection of Thiopentone sodium (at the rate of 125 mg/kg body weight) as recommended by The Animal Welfare Board of India. The explanted test bones were dissected from soft tissue and the segmented bone of interest was located and excised using high-precision saw. During the course of sawing a metallic sound in the center region of some bones (bird numbered T-103) was evident. These engraved segments were procured and fixed in the neutral buffered four per cent formalin solution. While taking a segmental sample from the dismembrated bone the position/location of the implant lodgment was ascertained by reading previous radiographs. Decalcification was performed by Acid decalcification i.e. Nitric Acid Formaldehyde technique [Nitric acid - 10 ml, Formalin -10 ml & Distilled water to make 100 ml]. The samples were dehydrated in the ascending grades of alcohol and the dehydrated tissue were subsequently processed through xylene into paraffin and embedded in paraffin blocks.

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<sup>4</sup> ERBA diagnostics Mannheim GmbH, Mallaustr., Mannheim/Germany.

The blocks were then processed by microtome for sectioning and stained with Hematoxylin and eosin stain and mounted using DPX mount.

### **3.3.13. STATISTICAL ANALYSIS**

The data for surgical time, temperature and serum biochemical parameters were compiled and their mean values with standard error in parentheses are presented. The data was analyzed by two-way Analysis of Variance (ANOVA) according to the method described by Snedecor and Cochran, (1994).



*Results  
and  
Discussion*



In this endeavor biocompatibility and biodegradability evaluation of magnesium based intramedullary implants (viz. plain Mg, five per cent weight Mg apatite and fifteen per cent weight Mg apatite) was attempted in three groups of Uttara-fowl birds with six birds each in a group.

#### **4.1. IN-VITRO STUDY OF THE IMPLANT MATERIAL**

##### **4.1.1. COMPOSITIONAL ANALYSIS**

The quantitative assay on Elemental distribution (EDX) (Fig.3: 3a, 3b, 3c, Fig.4: 4a, 4b and Fig.5: 5a, 5b, 5c, 5d) of the inter-metallic elements of the three test implants were carried out. The analysis shows the peak graph for Mg content and some amount of Ca and P indicative of presence of HA in matrix implant, with traces of Zinc can also be observed. The EDX analysis clearly confirms the sintered Mg ( $67.4\pm 4.3$  per cent weight) in plain implant, HA in different concentrations containing Ca and P at the rate of  $1.3\pm 0.5$  and  $0.5\pm 0.1$  per cent weight and  $3.85\pm 0.2$  and  $1.39\pm 0.1$  per cent weight, in five per cent and in fifteen per cent HA implant, respectively.

##### **4.1.2. BIOCOMPATIBILITY AND TESTS ON CELL CULTURE**

Cytocompatibility of the implants were checked on human fetal osteoblast cells (hFOB), using respective viability (MTT) assays. The MgHA-15 surfaces have shown improvement in hFOB cell viability over Mg specimen (Fig.6: 6a, 6b).

Previous studies from the same group have reported cell viability and growth for human osteosarcoma cells (MG-63) on sol gel as well as electrophoretic coating of HA on Mg alloys. The coating of HA on Mg alloy substrate improved the cell adhesion property. The presence of HA and its solubilisation in nearby environment can positively affect proteins adsorption and bioorganic compounds formation, which stimulates cell proliferation and adhesion. This could be linked to better cytocompatibility of MgHA-15. These observations confirmed that the orthopaedic implant with 15 per cent weight HA is most cytocompatible, providing the cells almost a natural environment for growth throughout the incubation period. It was

observed that most of the cells survived on implants with 5 and 15 per cent weight HA up to five days of incubation. There was larger number of cells adhering on surfaces of composite with HA. This was confirmed through DAPI staining of the cells on composites structures. Visually, significantly higher number of cells was observed in fluorescence images of hFOB cells grown on MgHA-15 implants after third and fifth days of incubation, relative to other implants. The numbers were comparable to the controls groups from both third and fifth days (Huan *et al.*, 2016; Muthupandi *et al.*, 2013 and Singh *et al.*, 2015).

**Justification for no chemical interaction between cleansing agent (acetone) with the implants:**

As the acetone is used during the process of fabrication of implants it does not interfere in any chemical reaction with the Mg, HA and Zn. It can be used as pre-sterilizing agent before autoclaving. Because as acetone was used while preparation of the implants for dispersion procedure, i.e. HA powders were first dispersed in acetone through probe sonicator (PKS-750FM, PCi Analytics, Mumbai) with the frequency of 20 kHz for 20 minutes followed by the addition of three per cent weight Zinc and balanced magnesium and then further sonicated for 40 minutes for better dispersion (Dubey *et al.*, 2018 and Jaiswal *et al.*, 2018). Hence acetone can be used as a cleaning agent for implants.

**4.2. ANAESTHESIA**

Induction of Ketamine anaesthesia was accomplished, to the effect, using injection Ketamine (at the rate of 125 mg/kg body weight, intravenously) under this study. Every bird was duly premedicated with injection atropine sulphate (at the rate of 0.04 mg/kg body weight, subcutaneously) in all the birds for surgical implantation of test implant in the test bone. This anaesthesia was found to induce general anaesthesia smoothly and maintained adequate muscle relaxation for a short period of time.

Though there is no scope for evaluation of the anaesthetic regimen for implantation in this study it is imperative to mention the effect of anaesthesia during the clinical surgical procedures. Head shaking of birds was taken as the indicative of effective anaesthesia. During the induction of anaesthesia in some of the birds half of the calculated dose was ought to be injected intravenously and remaining half subcutaneously as per the response of individual birds.

**Figure 3(a):** EDX-S spectrum corresponding to the area marked in a graph showing elemental distributions of plain Mg implant.

**Figure 3(b):** EDX-S spectrum corresponding to the area marked in a graph showing elemental distributions of five per cent Mg apatite implant.

**Figure 3(c):** EDX-S spectrum corresponding to the area marked in a graph showing elemental distributions of fifteen per cent Mg apatite implant.

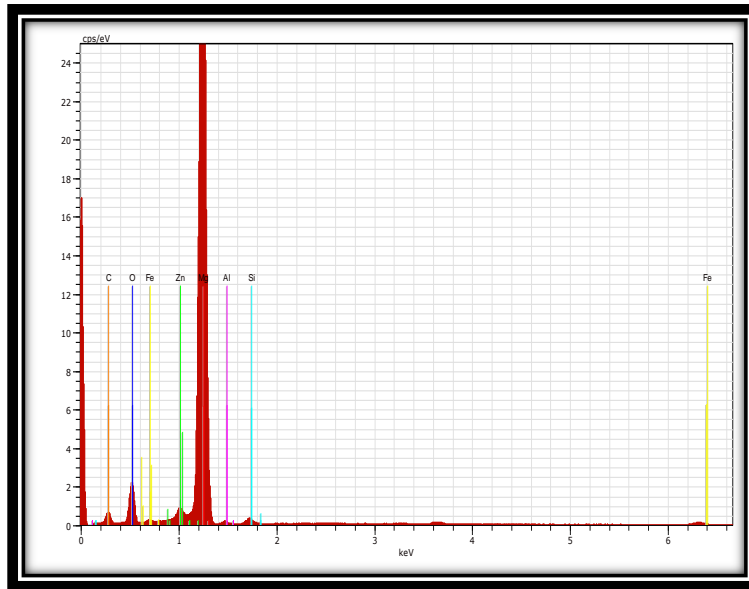
**Figure 4(a):** Representative scanning electron microscopy images of surface micrograph of pure Mg implant.

**Figure 4(b):** Representative scanning electron microscopy images of Mg-apatite surface micrograph evident as white powder in a blackish grey background.

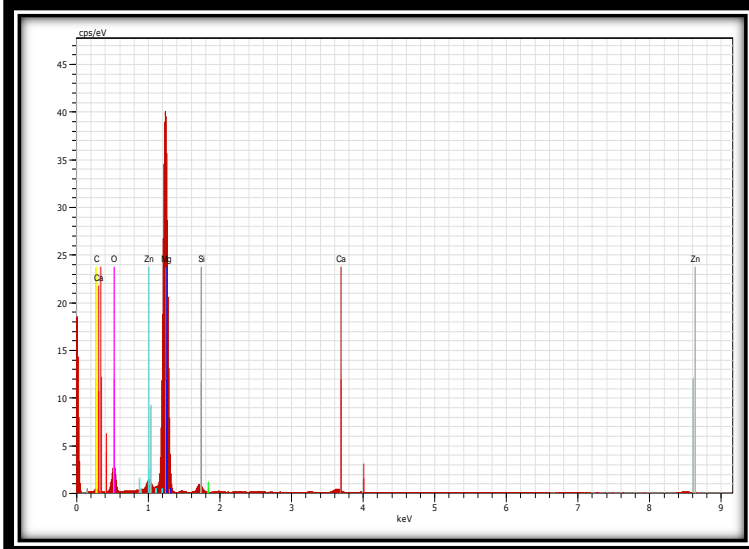
**Figure 5(a):** Representative photograph showing surface view area mapping in scanning electron microscope depicting distribution of magnesium (a- red colour) and calcium (b- blue colour)

**Figure 5(b):** Representative photograph showing surface view area mapping in scanning electron microscope depicting distribution of phosphorous (c- green colour) and zinc (d- sky blue colour)

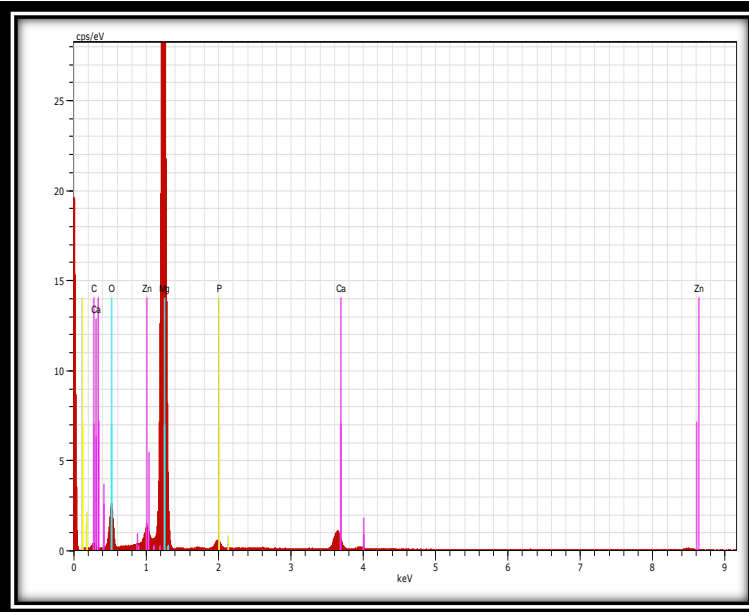
**Figure 3(a)**



**Figure 3(b)**



**Figure 3(c)**



## Surface micrograph and area mapping study on SEM

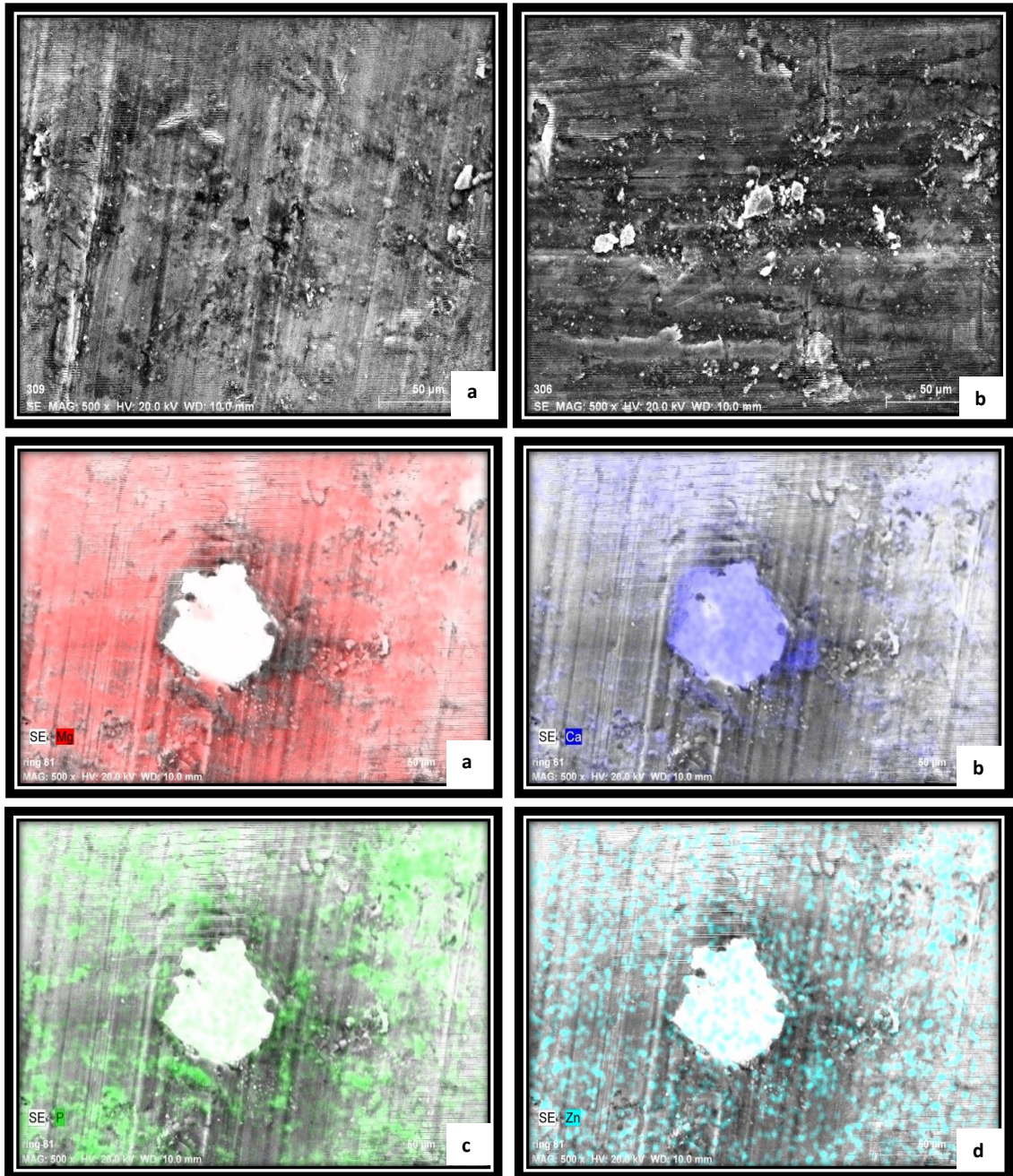
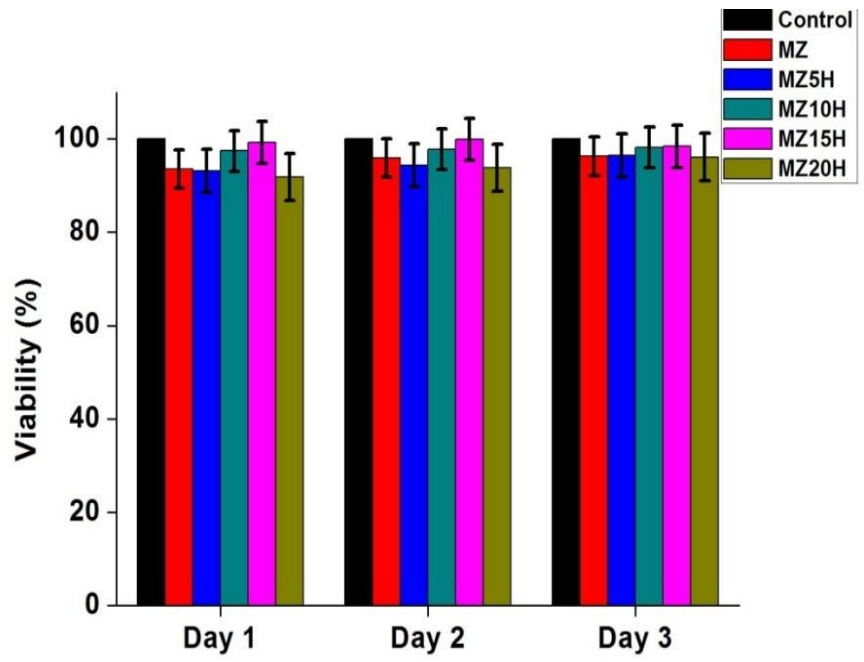


Figure 4 (a, b) and 5 (a to d)

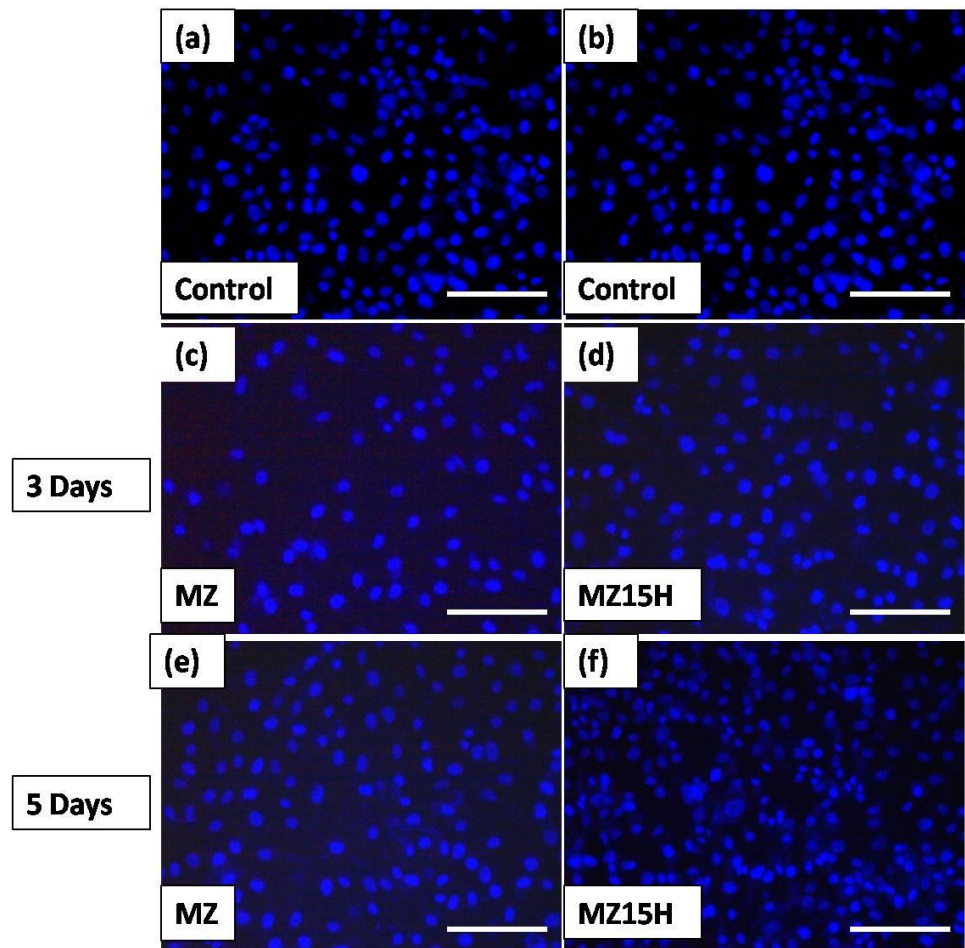
**Figure 6(a):** Quantitative representations of MTT assay as per cent cell survival of hFOB cells grown on different orthopaedic implants on 1, 3 and 5 days relative to control cells grown on regular tissue culture petridish. Data represents mean  $\pm$  standard deviation [MZ, MZH 5, 10, 15, 20, represents Mg, MGHA 5, 10, 15, and 20, respectively].

**Figure 6(b):** Fluorescence images of hFOB cells stained with DAPI on third and fifth days after seeding on (a and b) Control (tissue culture dish), (c and e) MZ, (d and f) M3Z15H surfaces. Images are representative of 3 experiments.

**In-vitro MTT assay and DAPI staining**



**Figure 6(a)**



**Figure 6(b)**

### 4.3. OBSERVATIONS

#### 4.3.1. INTRAOPERATIVE OBSERVATIONS

Different Intraoperative observations such as extent of soft tissue manipulation and damage, degree of technical easiness or difficulties in implant application were evaluated and scored into three grades i.e. slight (+), moderate (++) and severe (+++) (Table 4.1). Furthermore, duration of surgery in minutes starting from the skin incision to application of the last skin suture was also recorded.

**Table 4.1: Intraoperative observations during surgical intramedullary implant fixation in different groups**

Parameter	Group I	Group II	Group III
Average duration of surgery (minutes)	16.83±3.22	14.16±1.3	9.33±1.25
Degree of soft tissue manipulation and damage	+++	+++	+++
Technical easiness	+	+	+

It took only 16.83±3.22 minutes, 14.16±1.3 minutes and 9.33±1.25 minutes to introduce implant, into the distal aspect of intramedullary cavity in group I, group II and group III, respectively from skin incision, it was a simple procedure as it only involves placement of implant by drilling a pilot hole over antero-dorsal aspect of humerus bone. Extent and degree of soft tissue injury is very minimal and mild in all groups as the humerus bone is covered by minimal soft tissue and the operation does not involve large area for implant placement.

#### 4.3.2. CLINICAL OBSERVATION

##### **Justification against selection of humerus as test bone:**

Anatomically, the arm is the most proximal segment of the forelimb, the part that extends from the shoulder to the elbow. Especially in birds the humerus binds with coracoids in addition to scapula. Here humerus gives pneumatic foramen medially below the head. Head is very much oval and body is twisted much. Avian humerus is pneumatic has connection with the interclavicular air sac involve in respiration and hence contributes for maintaining the light weight. Moreover it is situated superficially under the skin in antero-dorsal aspect.

Fracture repair in birds often presents a significant challenge to the veterinary surgeons. As avian bones described commonly as thin and brittle due to the rich inorganic substances (calcium phosphate) i.e. hydroxyapatite content with a large medullary cavity and thin cortices that do not hold implants well. Because of little soft tissue coverage and loose attachment with the underlying bone, the avian bone has tendency to fragment or shatter upon impact. Long bones contain a network of trabeculae in the medullary cavity, with a very thin cortex, which increases the strength of the bone. These factors make the bones harder, but more fragile and less elastic than those of mammals (McLelland, 1990 and Rangnath *et al.*, 2009).

Three birds were lost during the study period out of which one during intra-operatively due to anaesthetic complications and out of remaining one (sixth post-operative week) due to visceral gout and other (eighth post-operative week) due to winter stress. Post mortem findings of dead birds revealed different findings viz. bird numbered T-103 (group II) had swollen and edematous lung filled with water due to winter stress pneumonia and the bird numbered T-111 (group II) was found to be due to visceral gout, a metabolic disease, where post-mortem changes like uric acid crystals deposition, found on all the organs includes liver, heart and spleen, and the kidneys were highly affected with one hypertrophied and another fully atrophied having fragile and smooth consistency. Furthermore, fracture of the humerus noticed in four birds two bird from group III, one bird each from group I and group II, which was observed peri and postoperatively as a consequence of drilling (Fig.17).

#### **Justification and rectification in the methodology while drilling a pilot hole:**

Methodology of drilling a pilot hole was evolved newly by using BP blade #11 and drill bit 2.0 mm diameter, initially a pit was created with the help of BP blade to make easier entry of drill bit and then by using drill bit a slightly curved pilot hole was created. Then intramedullary hole reaming was completed using K wire of size 1.5 mm. During this course, one midshaft humerus fracture was evident (Fig.17) and there were another midshaft humerus fracture developed during early postoperative hours (during 24 to 36 hour). The drilling pilot hole might have created stress point in the cortex and there after any muscular contraction might have aggravated the progression of the fracture cleavage. Such intra-operative and immediate post operative incidence of fracture may have developed due to the fragile nature of avian bone.

The avian skeleton is much lighter than that of mammals, in fact, a large part of their bones contain air (pneumatization) instead of bone marrow (Fig.14c). These cavities are communicated with the respiratory system and act to decrease weight, so flying make easier. Bones which are not pneumatized include most vertebrae, and those distal to the humerus and pelvis. Avian bones are richer in inorganic substances (calcium phosphate). Long bones have a very thin cortex and the medullary cavity contains a network of trabeculae, which increases the strength of the bone. These factors mean that avian bones are harder, but at the same time more fragile and less elastic than those of mammals (McLelland, 1990).

**a. Heart rate**

Bird’s heart beat very fast, as the circulatory system of birds is very active to meet the flight needs. In this study during the operation it was unable to record heart rate in all the birds as per the strategy made, but in some of the bird heart rate was recorded as 255±5 beats per minute.

**b. Cloacal temperature**

Cloacal temperature (Mean ± SE) recorded in the birds of different groups at various intervals have been presented in table 4.2. There was a transient increase in cloacal temperature significantly (p≤0.01) in early post operative period, which later return into normal within a couple of days in birds of all the groups. The cloacal temperature was found to be no significantly different between the groups while by fourth day all the groups showed a significant (p≤0.01) decline from the date of operation. This indicated an improvement in the activity and general condition of the birds postoperatively.

**Table 4.2: Cloacal temperature (in Fahrenheit scale) recorded in birds of different groups (Mean ± SE)**

Group	Postoperative days								
	0	1	2	3	4	5	6	7	14
I	106.9 ±0.14 <sup>x</sup>	107 ±0.24 <sup>x</sup>	106.8±0. 13 <sup>y</sup>	106.6 ±0.14 <sup>x</sup>	106.3 ±0.33 <sup>x</sup>	106.3 ±0.18 <sup>x</sup>	106.4 ±0.22 <sup>x</sup>	106.1 ±0.26 <sup>x</sup>	106.8 ±0.08 <sup>x</sup>
II	105.9 ±0.37 <sup>y</sup>	106.1 ±0.22 <sup>y</sup>	106.9±0. 12 <sup>y</sup>	106.8 ±0.16 <sup>x</sup>	106.7 ±0.29 <sup>x</sup>	106.4 ±0.23 <sup>x</sup>	106.3 ±0.24 <sup>x</sup>	106.4 ±0.19 <sup>x</sup>	106.9 ±0.07 <sup>x</sup>
III	106.7 ±0.35 <sup>x</sup>	106.5 ±0.17 <sup>y</sup>	107±0.2 1 <sup>x</sup>	106.6 ±0.2 <sup>x</sup>	106.6 ±0.18 <sup>x</sup>	106.8 ±0.1 <sup>x</sup>	106.5 ±0.33 <sup>x</sup>	106.7 ±0.17 <sup>x</sup>	107 ±0.07 <sup>x</sup>

\*The values with different superscripts differ significantly within a column (between groups) at p≤0.01.

### c. Healing of surgical wound

The outcome of skin incision healing (first or second intention) in each group has been described in table 4.3 below:

**Table 4.3: Healing of surgical wound**

<b>Group</b>	<b>First intention healing (number of birds)</b>	<b>Second intention healing (number of birds)</b>
<b>I</b>	5	1
<b>II</b>	6	0
<b>III</b>	6	0

All the birds showed healing of wound by first intention, however, some of the birds depicted complications like inflammatory swelling and pain, subcutaneous emphysema, hematoma over the drilled area which was then healed by second intention. Emphysema over the dorsal aspect of the humerus was observed during the first week of implantation in the birds of the group II and III, numbered T-103 and T-109, respectively (Fig.18: 18a, 18b), which meanwhile subsides by second week postoperatively. During this time, the birds showed neither an abnormal behavior nor did body weight reduction indicating any pain, no inflammation and no sterile sinuses eliminating debris were observed during the postoperative follow-up. However, the developed complications like wing dropping, inflammatory swelling completely disappeared by sixth week postoperatively. The implanted biodegradable magnesium scaffolds caused no significant harm to their neighboring tissues. After 1.5 months postoperatively, the scaffolds were largely degraded and most of the original magnesium alloy had disappeared.

### d. Flight test

Flight test was conducted in all birds at third, sixth and 12<sup>th</sup> postoperative weeks. The birds were comfortably tossed in the air to facilitate their flight and observations were recorded and scored; lack of flight with crash landing (score=0), deviation in the flight towards right with clumsy landing (score=1), minor deviation in the flight towards right with proper landing (score=2) and normal flight with proper landing (score=3) (Table: 4.4).

**Table 4.4: Flight test score (Average) at 3<sup>rd</sup>, 6<sup>th</sup>, 12<sup>th</sup> postoperative weeks**

Group	Flight test score(Average)		
	3 weeks	6 weeks	12 weeks
<b>I</b>	2	2	3
<b>II</b>	2	3	3
<b>III</b>	2	3	3

It was concluded from the readings (Table: 4.4), the birds belong to the group I, II and III had the good flight on sixth week onwards; they showed optimal flight and landing pattern. However, they showed mild right deviated flight during early postoperative weeks due to surgical pain and inflammation. These findings were in accordance with the findings in the study of Bennet and Kuzma, 1992.

**Table 4.5: Wing dropping test at 2<sup>nd</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 12<sup>th</sup> postoperative weeks**

	Postoperative weeks											
	Group I				Group II				Group III			
Dropping of wings	2	3	6	12	2	3	6	12	2	3	6	12
		++	+	-	-	+	-	-	-	++	+	-

- =Absent      + =Moderately present      ++ =Severely present

Change in the shape of wing with respect to dropping of wings was recorded on second, third, sixth and 12<sup>th</sup> postoperative weeks. Which was recorded as absent (-), moderately present (+) and severely present (++) (Table: 4.5). Though there is less scope for measuring flight test in this study because the implants were placed intramedullary cavity for degradation measurement not for fracture fixation but it is imperative to mention the flight pattern as because sometime the drilling of bone and soft tissue handling may affects the flight, hence there was no need of measuring significance (Tufail, 2016).

### 4.3.3. RADIOLOGICAL EVALUATION OF IMPLANT DEGRADATION

In osteosynthesis, biodegradable implants were taken as an attractive alternative to their permanent counterparts, particularly in wild, pet and domestic bird cases where surgical interventions are intended to be minimized. In this context the study was undertaken showed a good biodegradation which could be able to find out in radiographs

successfully. All Mg based implants degraded during the implantation period in the bird humerus. The degradation rate was less uniform in the plain Mg implants compared to the Mg apatite implants (five and fifteen per cent HA). Medio-lateral and leading edge (Hanging drop) views of test bone at implanted site were taken in all birds at scheduled time of on immediate post-operative, first, second, third, fourth, sixth, eighth, 10<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup>, 21<sup>st</sup> and 24<sup>th</sup> post operative weeks (Fig. 7, 8, 9 and 10 with different slide No.). These radiographs were scrutinized under radiographic illuminator for observing the radio-disappearance of implants, formation of callus over the pilot hole, excretion of gas produced as a result of *in vivo* corrosion of Mg implants and for other complications. Plain Mg implants shows the more inflammatory and periosteal reaction compared to implants with Mg apatite complex. Sequestration of implant material towards lower third of the humerus is much more distinguishable feature of the plain Mg implants compared to Mg apatite complex materials. Endosteal cortical thickening and obliteration of medullary cavity by the cortical reaction is the major radiographical findings of plain implant of group I (Wilkins, 2005). Group II and III birds shows little periosteal and endosteal reaction and minimal gas production with faster remodeling of bone suggestive of good biocompatibility of HA complex with the bioenvironment. Gas produced might have absorbed through respiratory system via air sacs (suggestive of accumulation of probably hydrogen gas as stated by Song *et al.*, (2012). The mechanical stability and radiological integrity of implants was maintained up to two week in group I, three week in group II and two week in group III, this findings demonstrates the activity of HA in physiological environment. HA content can positively affects the protein absorption, cell adhesion and bioorganic compound formation as per the *in vitro* study results (Muthupandi *et al.*, 2013 and Singh *et al.*, 2015). No articular and metaphyseal changes suggestive of absence of deleterious reactions that altering neither the haematogenous nor cellular architecture of the bone was noticed. These results were in some accordance with the findings of study by Kraus *et al.*, 2012 and Witte *et al.*, 2006.

#### **Justification for *in-vivo* biocompatibility and fate of magnesium hydroxyapatite:**

In this study, obliteration of medullary cavity with bone neoformation at the site of implantations was very common radiological feature observed during third to fourth week. Radiographic evaluation has revealed the new bone formation proximal, distal

and around the implant site, which was noted based on the increased radio-density, on the inner cortex of the test bone (Fig.22) that is an indicative evidence for good osteoactivity and osteoinductivity of plain Mg and the same was enhanced in Mg with HA complex. This facilitation of new bone formation might be associated with the release of magnesium ions during the degradation process. Gradual reduction in serum magnesium concentration in all magnesium based implants suggested that dominant distribution of body magnesium, located in bone, serum and supplementing magnesium resulting from degradation, resulted in a significant increase in bone density and bone strength by forming trabeculae pattern. The released and formed, Hydroxyapatite nuclei, by consuming calcium and phosphate ions from the surrounding tissue, might help in cortex thickening. The disintegrated magnesium particles might be endocytosed in macrophages or giant cells, and further biodegrade in the microenvironment inside these cells (Hanzi *et al.*, 2010 and Pourbaix, 1974).

In orthopaedic research the terminologies- ‘Implant’ and ‘spacer’ have been coined with definite definitions, according to Association for Osteosynthesis/Association for Study of Internal Fixation (AO/ASIF) an implant is a man made medical device used to replace a missing biological structure, to provide an support to existing or damaged biological structure, whereas spacer is one which is also an medical device used for enhancing the space between the bones for therapeutic purposes. Because of the sake of our convenience and to maintain the fluidity in the text these terminologies are used in substitution to one another during the course of study period.

Radiographs obtained immediately after surgery showed excellent fixation of implants on distal intramedullary space of right humerus in all the groups and subsequent radiographs are evident for visualization of *in vivo* biodegradation of implant. Elaborative observations of radiographs were presented in table 4.6.

**Table 4.6: Elaborative observations of radiographs and their features observed in different groups of birds at different interval of time period with their implant identification number**

Implant ID	Radiographic observation
Mg-1	<p>Implant has developed full length cleavage/Longitudinal splitting (suggestive of pitting corrosion) and a gas density is discernible at the distal border of the implant and accumulation of gas in the soft tissue near the proximal border of the implant, incision has also been delineated. Increased radio-density was discernible on second week suggestive of endosteal proliferation and there is positive periosteal reaction evident at the insertion port.</p> <p>At third week intramedullary gas has decreased but soft tissue gas has still been preserved (may be it has been expelled by the pneumatic foramen). On the same week consolidation of intramedullary space increased and that reached maximum in the sixth week and on same sixth week the implant was sequestered by the osteogenic activity.</p> <p>On eighth week establishment of intramedullary channel through the sequestered part was observed and that reaction continued till 21<sup>st</sup> week.</p> <p>The remnant of the implant material was noticeable radiographically still on 24<sup>th</sup> week.</p>
Mg-2	<p>The fracture was developed on first day postoperatively as a consequence of activity of the bird during handling.</p> <p>Increased soft tissue density was discernible around fracture site suggestive of inflammatory reaction and hematoma formation. Gas accumulation distal to implant material and at the fracture site due to escape of intramedullary air was noticed.</p>
Mg-3	<p>Inflammatory reaction was evident on reamed insertion port and implanted area noted as decreased radio-transparency during the first two weeks of implantation.</p> <p>Cleavage of the implant is visible but there is no appreciable gas accumulation, i.e. Longitudinal splitting of the implant material was noticed in the first week radiography.</p> <p>Implant sequestration on lower third of the humerus was discernible on fourth week and it reaches maximum on eighth week thereafter inclined towards fully clearance on 21<sup>st</sup> week (as in Fig.21).</p> <p>Very high endosteal reaction and thickening was noticed on 10<sup>th</sup> week (as in Fig.22 and 24) and thereafter, Mg take up was starts at 12<sup>th</sup> week and its clearance continued till 15<sup>th</sup> week reached maximum clearance on 18<sup>th</sup> week followed by effective remodeling of the bone over the 24<sup>th</sup> week.</p>

Mg-4	The bird was died during peri-operative procedure as a result of anaesthetic complication.
Mg-5	Inflammatory reaction was evident on reamed area during first week of implantation but meanwhile it was reduced by second week onwards with gas accumulation on lower aspect of implant material. Periosteal reaction was evident on fourth week over endosteal region of pilot hole. There was no appreciable splitting of implant but sequestration of implant on lower third of bone was appreciable after fourth week until 12 <sup>th</sup> week (as in Fig.21).
Mg-6	Gas accumulation on the distal and proximal end of the implant with escape of gas to subcutaneous tissue via insertion port. Especially from the proximal end of implant gas escape is delineated later on the distal end at two week interval, On third week also gas entrapment near to the port of insertion of the implant is visible.
MgHA-1	There was a split fracture of the test bone occurred while drilling a pilot hole and reaming of the intramedullary cavity. Meanwhile after insertion of implant during first week soft tissue gas accumulation was evident that might be due to escape of air from broken bone.
MgHA-2	Persistence of radio dense implant was discernible up to third week postoperatively with minimal gas production and minimal periosteal reaction. The bird was died on 7 <sup>th</sup> postoperative week on post mortem revealed swollen and edematous lung filled with water due to winter stress pneumonia.
MgHA-3	Very less periosteal and endosteal reaction was discernible with full bone remodeling. There was a minimal sequestration of implant was noticed on fourth week then subsequently disappeared over sixth week. There was a minimal gas accumulation with cloudiness was observed. The bird was died on 16 <sup>th</sup> postoperative week on post mortem revealed deposition of uric acid crystals all over the visceral organs and the death was confirmed as a result of visceral gout.
MgHA-4	Minimal periosteal and endosteal inflammatory reaction and thickening was noticed. There was a minimal sequestration of implant was noticed on fourth week then subsequently disappeared over sixth week. There was a minimal gas accumulation with cloudiness was observed.
MgHA-5	Initiation of reduction in radio density of an implant material was noticed between third to sixth weeks. On sixth week the implant got entrapped little bit in the endosteal proliferation and focal consolidation of the medullary cavity at the site of implantation was noticed. On eighth week onwards implant density starts disappearing and emits the proliferative endosteal reaction with appearance of thick trabeculation.

MgHA-6	Initiation of degradation started on third week of implantation with splitting of implant material first week postoperatively. Minimal gas formation and accumulation with sequestration of implant noticed over distal third of implant. Complete bone remodeling with trabecular stimulation was noticed on 24 <sup>th</sup> week.
MgHAA-1	Dusty intramedullary reaction at the site of implant and non-visibility of the implant was discernible at first week postoperatively. It does not shown similarity with the implant reaction that are discernible on group I birds. Sequestration of implant and endosteal thickening was noticed on eighth week.
MgHAA-2	The wedge fracture was developed perioperatively as a consequence of drilling of hole during intramedullary implantation, but the implant was localized inside the fracture fragment and subsequently immobilization was performed and furthermore radiographs were unable to interpret as difficulty due to overlapped segments. On 18 <sup>th</sup> and 24 <sup>th</sup> week bone showed complete healing with minimal soft tissue damage with no gas accumulation.
MgHAA-3	Minimal reaction on reamed area of intramedullary tract with no periosteal and endosteal reaction observed. Less significant gas accumulation on distal aspect of implant. Establishment of sequestration of implant on sixth week and maximal on eighth week with full clearance on tenth week was discernible.
MgHAA-4	Disappearance of the implant on first week of implantation was noticed with dusty reaction at implantation site with minimal gas production distal to implant. No periosteal inflammatory reaction. Cortical thickening was minimal on eighth week and persistence of sequestration of implant noticed from sixth week to eighth week postoperatively.
MgHAA-5	Periosteal bone reaction was noticed over the drilled area on sixth week. Obliteration of intramedullary cavity due to endosteal reaction because of sequestration of implant within the lower half of the medullary space and its clearance from 12 <sup>th</sup> week onwards with reopening of the intramedullary space due to endosteal osteogenic reaction mediated remodeling processes.
MgHAA-6	The bird was developed a secondary fracture as a consequence of activity while handling on second postoperative week. Fractured fragments remained distracted inside the swollen inflamed soft tissue and the same was maintained till 24 <sup>th</sup> week without ill definable effects.

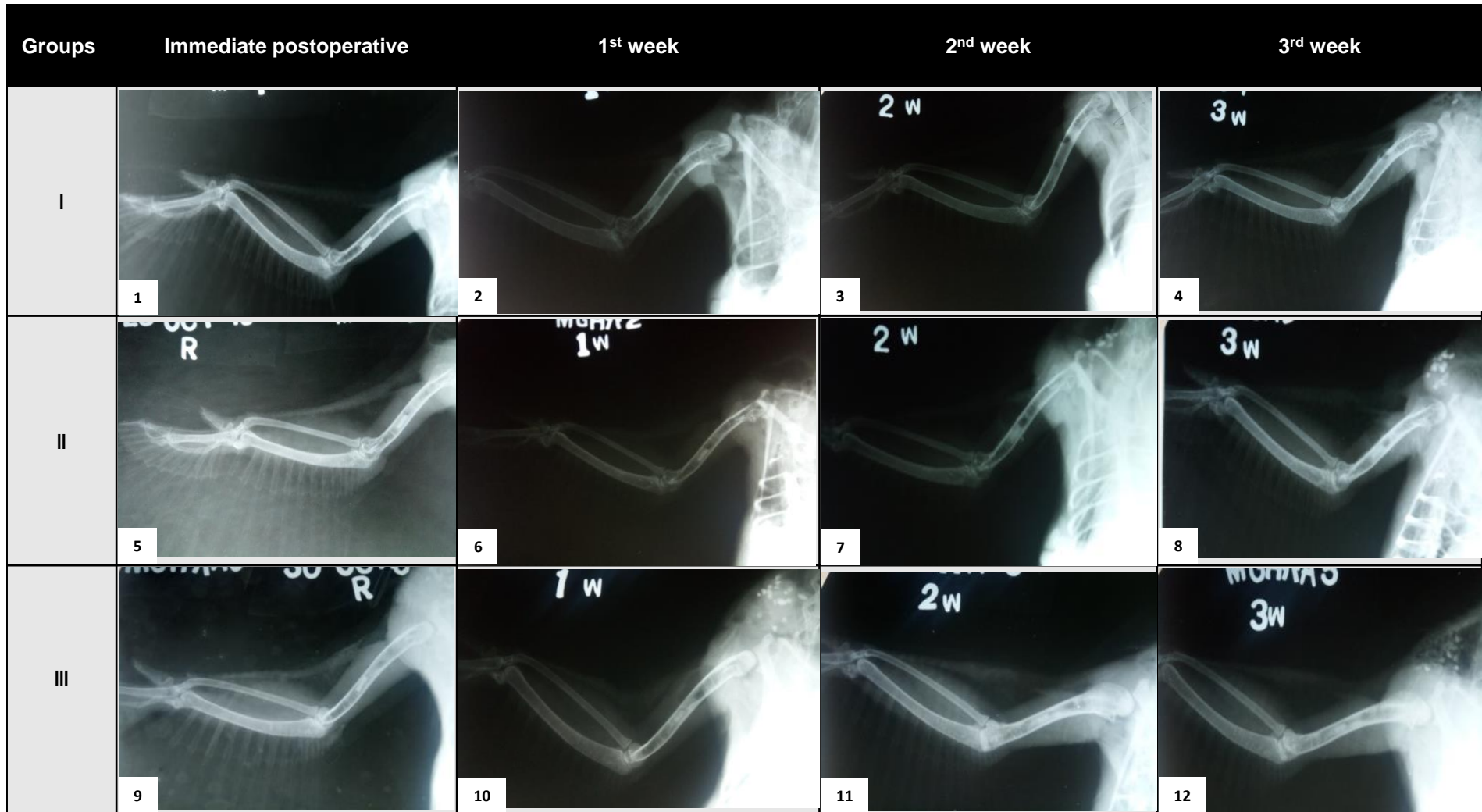


Figure 7: Photographs showing radiographic changes (medio-lateral view) over the postoperative period (from immediate postoperative up to 3<sup>rd</sup> week) after surgical implantation of Mg/Mg-HA (5% and 15%) orthopaedic spacer in the medullary cavity of humerus of fowl.

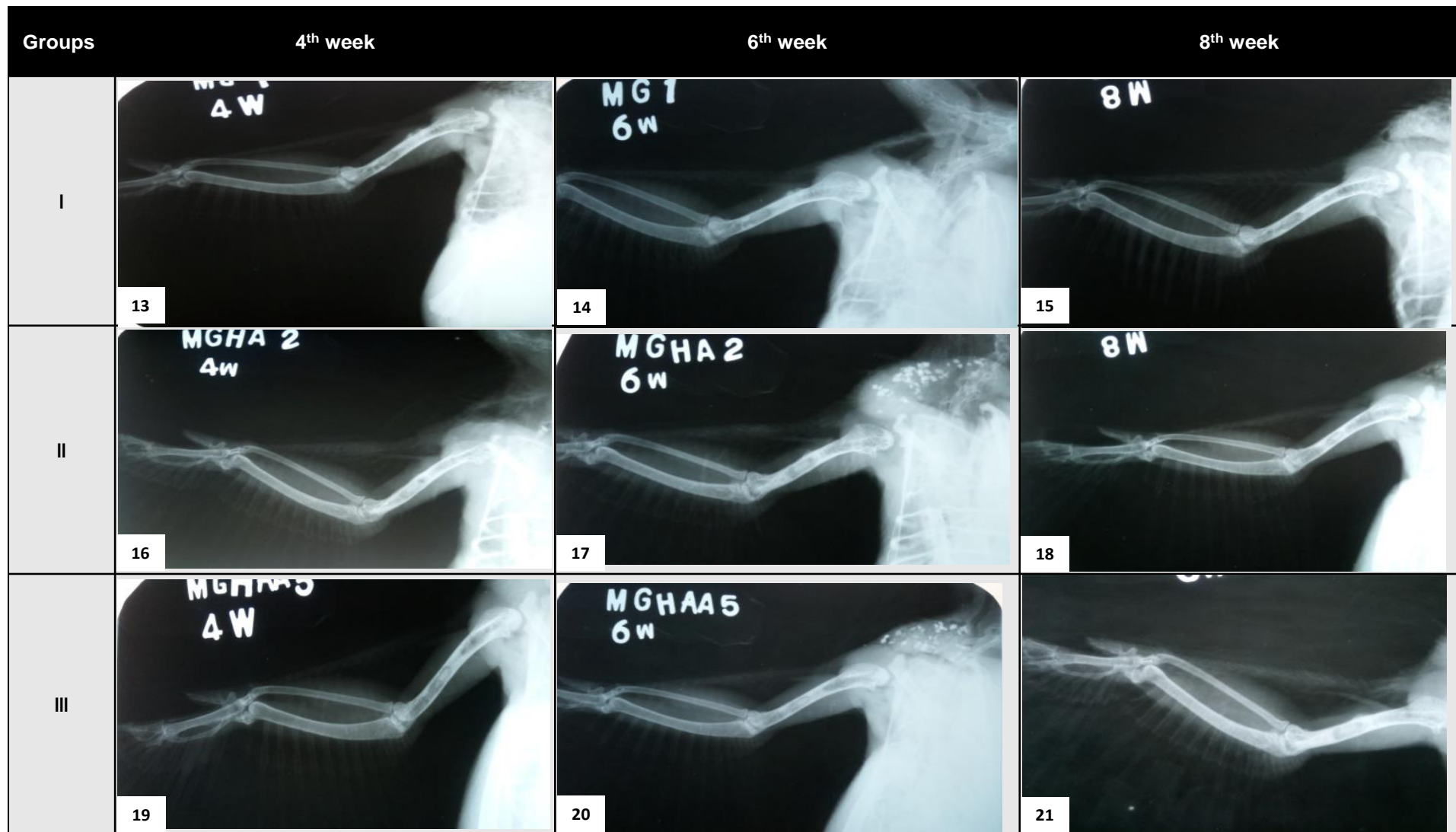


Figure 8: Biodegradation trend of radiopaque implants are visible in the lower third of the medullary cavity (medio-lateral view) on 4<sup>th</sup> week (No. 13, 16 and 19); up to the 8<sup>th</sup> week (No. 15, 18 and 21).

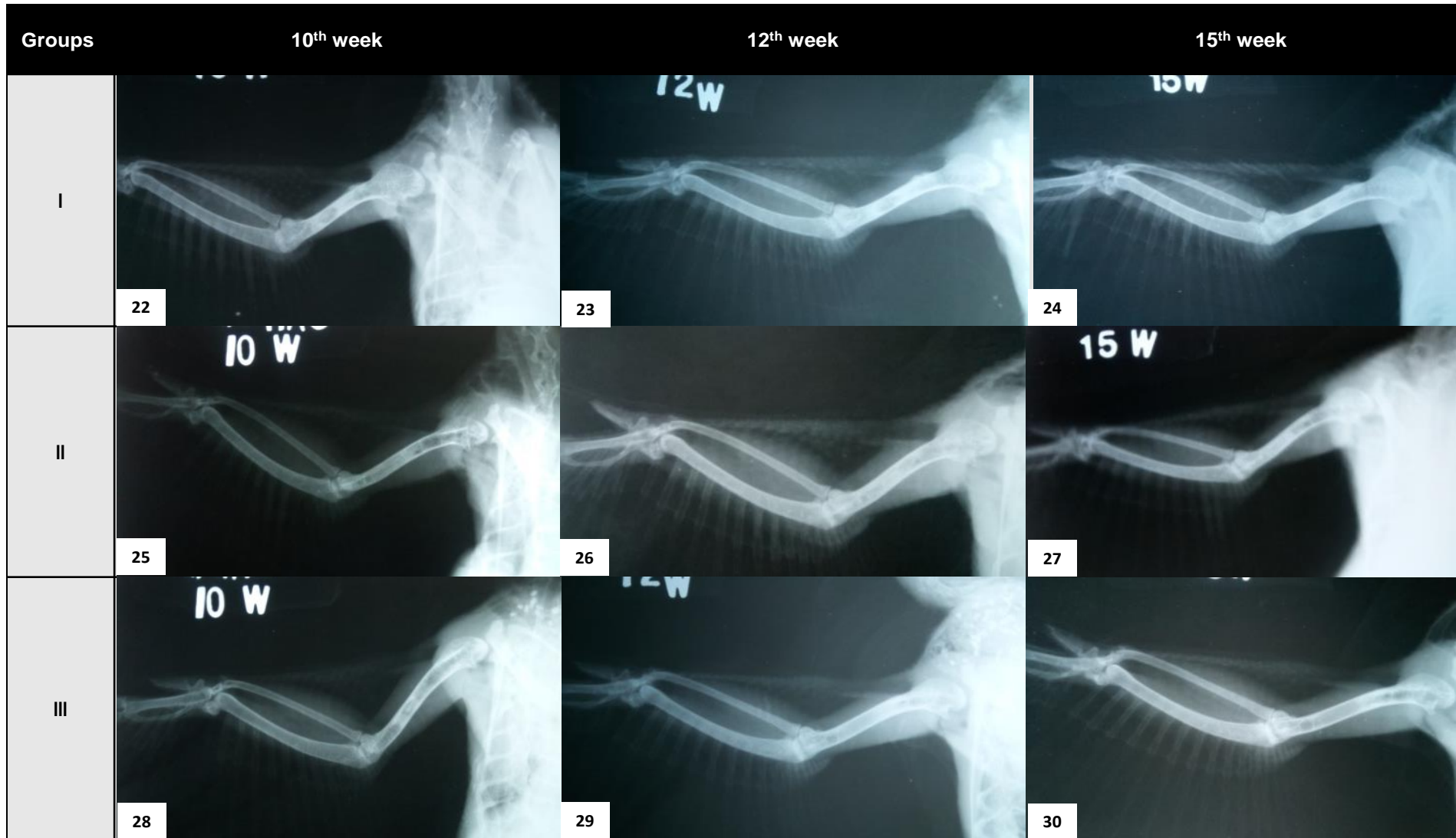


Figure 9: Radiopaque implants of reduced radio density are visible in the lower third of the medullary cavity (medio-lateral view) on 10<sup>th</sup> week (No. 22, 25 and 28); up to the 15<sup>th</sup> week (No. 24, 27 and 30). Radiographic disappearance can be noted in slides of 15<sup>th</sup> week.

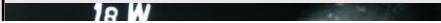


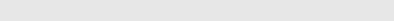

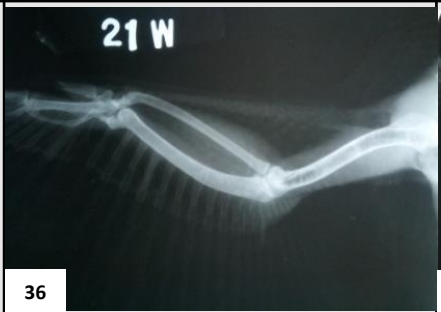






Groups	18 <sup>th</sup> week	21 <sup>st</sup> week	24 <sup>th</sup> week	Leading edge view
				
II				
III				

Figure 10: Completely dissolved implants with radio-transparency in the lower third of the medullary cavity can be noted on 18<sup>th</sup> week (No. 31, 35 and 39); up to the 24<sup>th</sup> week (No. 33, 37 and 41). Slide No. 34, 38 and 42 showing the hanging drop projection of wing for differentiating densities of cortical bone at implanted area to other site and to rule out hair line fracture possibilities due to cortical lysis.

#### 4.3.4. BIOCHEMICAL STUDY

It was reported that the presence of magnesium on orthopedic implants could enhance the adhesion of osteoblastic cells and promote optimal osteogenesis. Therefore, magnesium might play an important role in the growth of bone tissue (Zreiqat *et al.*, 2002). Hence study of serum Mg, Ca and P concentration is at most important for studying the biodegradation of degradable implants.

In the present work, significant statistical difference in serum magnesium, calcium and phosphorous concentration were found in between the groups and at different interval of time from that of preoperative concentration. It was interesting to find that the serum magnesium of all the groups were decreased significantly (at  $p \leq 0.01$ ) by the first, second postoperative weeks compared to that of preoperation and on third week onward the value again increased significantly (at  $p \leq 0.01$ ) in all the groups and remained normal till the observation period. This might be controlled by the self-regulation mechanism of birds and the surplus Mg may have excreted from the urine (Vormann, 2003). In contrast to decreased Mg concentration measured during first two weeks postoperatively (Table.4.7), the molar Ca/P ratio increased by 50 percent (Table.4.8 and 4.9). After third week postoperatively as Mg concentration reached a normal level (Fig.11), Ca/P ratio (Fig.12 and 13) also down to 0.5. Reduced Ca/P ratio levels after third week indicates a remineralization process where Ca was partially substituted by Mg. the tolerance level of Mg ion release are usually not exceeded during the degradation period. Gu *et al.* (2009) reported that even at their faster degradation rate Mg-Ca binary alloys could be considered for safe application, as because daily release of Mg would be still at milligram level and far below the daily allowance of Mg uptake (0.7 gm/day). It may be concluded that, at the later stages of implantation period where bone getting mineralization Mg acts along with Ca for increasing mechanical stability of bone by thickening the cortical surface. These results were in accordance with the findings of study by Amerstorfer *et al.*, 2016.

#### **Justification for getting or not getting hydrogen gas shadow in this model:**

As per Pourbaix (1974) descriptions that high pH value ( $>11.5$ ) will promote a stable protective hydrogen layer over the surface of the Mg implants, lower pH value ( $<11.5$ ) will facilitate corrosion of Mg alloys in aqueous solution, thus an accelerated

corrosion at the Mg implant surface can be expected in-vivo, as the local pH can be estimated at 7.4 or lower due to secondary acidosis resulting from metabolic and resorptive processes after surgery. As the degradation of Mg based implants produces the hydrogen gas when they come in contact with the physiological environment, the then released gas tend to accumulates in the subcutaneous tissue but here in the birds because of pneumatization no subcutaneous gas accumulation because of air exchange between bone and respiratory system via air sacs. It was observed that, radiological intramedullary gas formation, disappeared by time with no appreciable subcutaneous emphysema.

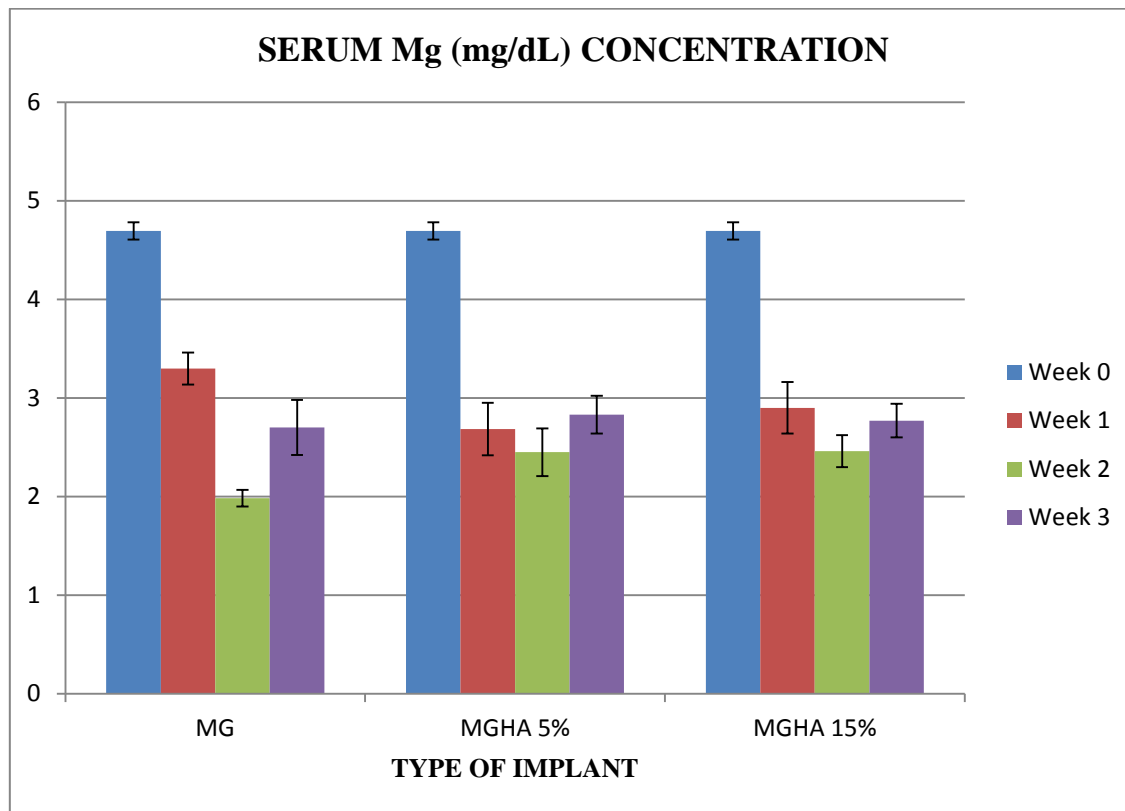
Hydrogen gas was another degradation product and was detected in the early implantation period (Fig.23). This could be explained by the accelerated degradation at the pin surface due to exposure to the physiological environment. Hydrogen gas was found to be self absorbed by clearing through interclavicular air sacs and subsequently via expiration. These findings were in accordance with the findings of study by Hanzi *et al.*, 2010 and Kraus *et al.*, 2012.

**Table 4.7: Serum Mg level (mg/dL) obtained from birds of different groups (Mean ± SE)**

Group	Postoperative weeks			
	0	1	2	3
I	4.69±0.08 <sup>a</sup> <sub>p</sub>	3.29±0.16 <sup>Ab</sup> <sub>Pq</sub>	1.98±0.08 <sup>Cd</sup> <sub>Rs</sub>	2.7±0.27 <sup>Bc</sup> <sub>Rr</sub>
II	4.69±0.08 <sup>a</sup> <sub>p</sub>	2.69±0.26 <sup>Cc</sup> <sub>Rr</sub>	2.45±0.24 <sup>Bd</sup> <sub>Qs</sub>	2.83±0.19 <sup>Ab</sup> <sub>Pq</sub>
III	4.69±0.08 <sup>a</sup> <sub>p</sub>	2.9±0.26 <sup>Bb</sup> <sub>Qq</sub>	2.46±0.16 <sup>Ad</sup> <sub>Ps</sub>	2.77±0.17 <sup>Bc</sup> <sub>QR</sub>

\*The values with different superscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at p≤0.05.

\*The values with different subscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at p≤0.01.



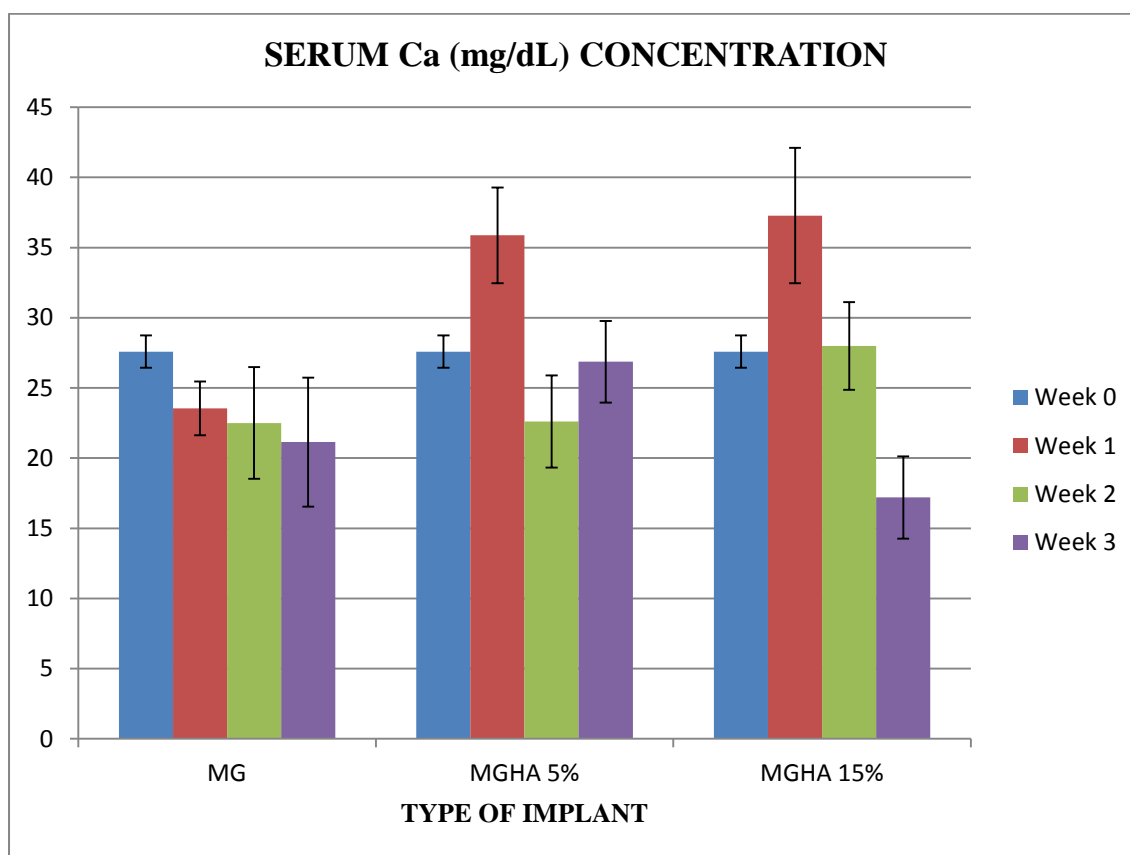
**Figure 11: Changes in serum Mg levels of birds implanted with plain Mg, 5% Mg apatite and 15% Mg-apatite implants after 0<sup>th</sup>, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> postoperative weeks (Mean ± SE)**

**Table 4.8: Serum Ca level (mg/dL) obtained from birds of different groups (Mean ± SE)**

Group	Postoperative weeks			
	0	1	2	3
I	27.59±1.15 <sup>a</sup> <sub>p</sub>	23.54±1.91 <sup>Cb</sup> <sub>Rq</sub>	22.5±3.97 <sup>Bc</sup> <sub>Qr</sub>	21.13±4.59 <sup>Bd</sup> <sub>Qs</sub>
II	27.58±1.15 <sup>b</sup> <sub>q</sub>	35.87±3.4 <sup>Ba</sup> <sub>Qp</sub>	22.60±3.28 <sup>Bd</sup> <sub>Qs</sub>	26.86±2.91 <sup>Ac</sup> <sub>Pr</sub>
III	27.59±1.15 <sup>b</sup> <sub>q</sub>	37.28±4.8 <sup>Aa</sup> <sub>Pp</sub>	27.99±3.12 <sup>Ab</sup> <sub>Pq</sub>	17.99±2.92 <sup>Cc</sup> <sub>Rr</sub>

\*The values with different superscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at  $p \leq 0.05$ .

\*The values with different subscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at  $p \leq 0.01$ .



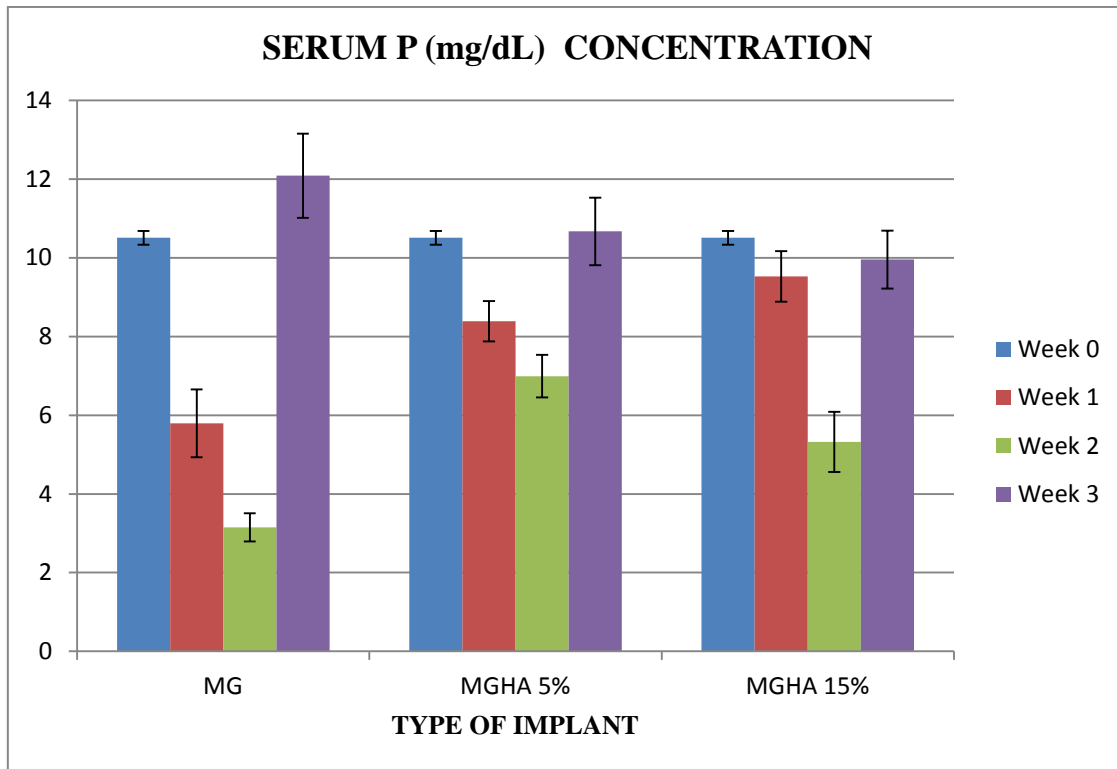
**Figure 12: Changes in serum Ca levels of birds implanted with plain Mg, 5% Mg apatite and 15% Mg-apatite implants after 0<sup>th</sup>, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> postoperative weeks (Mean ± SE)**

**Table 4.9: Serum P level (mg/dL) obtained from birds of different groups (Mean ± SE)**

Group	Postoperative weeks			
	0	1	2	3
I	10.5±0.17 <sup>b</sup> <sub>q</sub>	5.79±0.86 <sup>Cc</sup> <sub>Rr</sub>	3.15±0.35 <sup>Cd</sup> <sub>Rs</sub>	12.08±1.07 <sup>Aa</sup> <sub>Pp</sub>
II	10.5±0.17 <sup>a</sup> <sub>p</sub>	8.38±0.50 <sup>Bb</sup> <sub>Qq</sub>	6.99±0.54 <sup>Ac</sup> <sub>Pr</sub>	10.67±0.85 <sup>Ba</sup> <sub>Qp</sub>
III	10.5±0.17 <sup>a</sup> <sub>p</sub>	9.53±0.64 <sup>Ab</sup> <sub>Pq</sub>	5.32±0.76 <sup>Bc</sup> <sub>Qr</sub>	9.95±0.73 <sup>Cb</sup> <sub>Rq</sub>

\*The values with different superscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at p≤0.05.

\*The values with different subscripts, with capital letters and with small letters differ significantly, between the group and within the group, respectively, at p≤0.01.



**Figure 13: Changes in serum P levels of birds implanted with plain Mg, 5% Mg-apatite and 15% Mg apatite implants after 0<sup>th</sup>, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> postoperative weeks (Mean ± SE)**

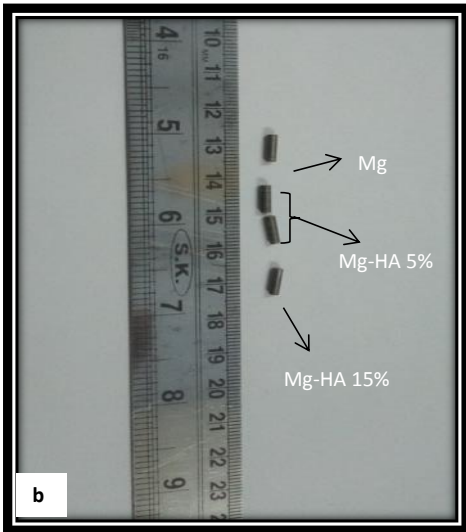
#### 4.3.5. HISTOLOGICAL EVALUATION

Histological study did not reveal any discernible adverse tissue reactions in bone specimen of the birds. There was no obvious architectural change was found in bone matrix and cells on histopathological slides (Fig.25 and 26). New bone formation was observed at the periosteal and endosteal location near the implant site, while no bone mass was induced in the surrounding soft tissue as per the bone histopathological studies. These may be due to higher osteoblastic activity at the site. Soft tissue gas formation that occurred and delineated in radiographs (Fig.23) was almost entirely resorbed (within 5 days) but could not be detected in periosteal site in histological study, this may be due to absorption of air through the respiratory system, at the initial insult. New bone formation over the pilot-hole and increased cortical thickness over the implant site might be an indicative of close contact of implant between bone and trabeculae, during the first four week of implantation. At 24 weeks the trabeculae was regenerated to a nearly normal extent. These findings were in accordance with the findings of the study by Li *et al.*, 2008.

Kraus *et al.* (2012) also reported new bone formation and bone resorption at the same time without inflammation reactions, complete implant degradation and decreased gas evolution, the positive influence of magnesium and resulting rapid full regeneration of the cortical bone defects in 32 male dawley rats. They also concluded that the size of the implant used as intramedullary nail in pediatric orthopedics would be significantly larger than in the current case, a similar scenario would result. This is because higher amounts of evolved gas would go hand-in-hand with more tissue available for gas resorption.

**Figure 14:** Representative photograph showing physical and radiographic characteristics of test implants

- (a) Radiographic image showing similar radio-opacity of implants (1-Mg plain implant, 2-MgHA 5 % implant and 3-MgHA 15 % implant)
- (b) Photograph of implants for showing gross appearance
- (c) Plain radiograph of medio-lateral view of test humerus bone (right) before implanting the test materials, depicting (i) normal pneumatization of intramedullary cavity, (ii) long thick fringelike feathers and petagium can also be observable



**Figure 14**

## Intramedullary insertion of test implant

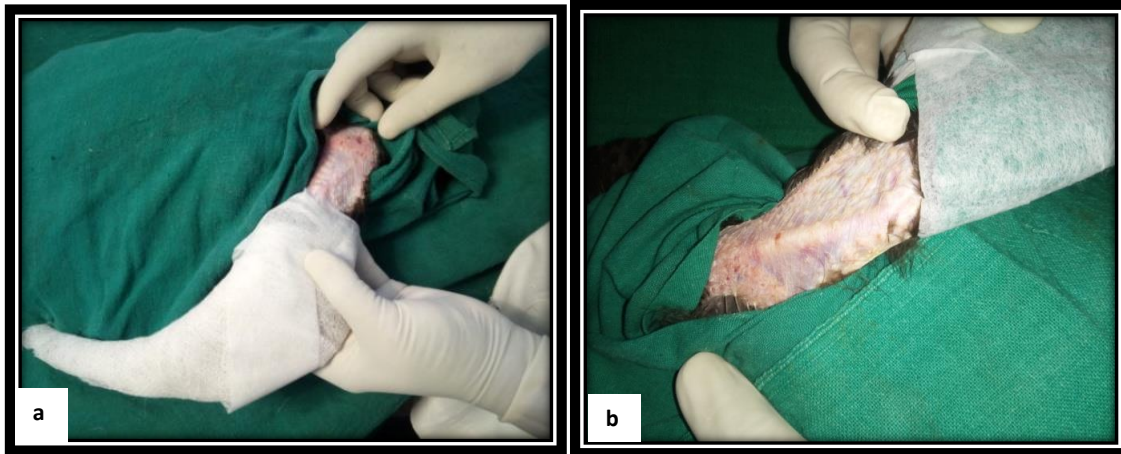


Figure 15: Positioning of bird and aseptic preparation of surgical area i.e. Antero-dorsal aspect of right humerus.

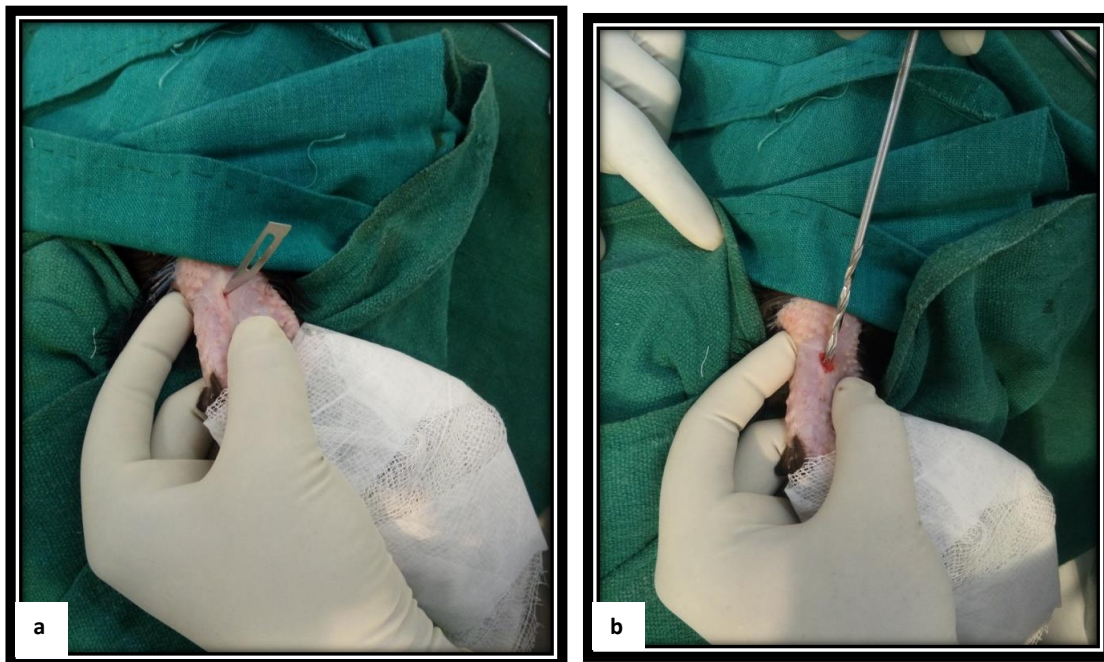


Figure 16: Photographs depicting drilling of pilot hole and reaming of trabeculae for proper intramedullary placement of implant into distal third of humerus by using (i) #11 BP Blade (ii) Drill-bit size 2.4 mm and (iii) K-wire of 1.5 mm diameter.

## Postoperative complications

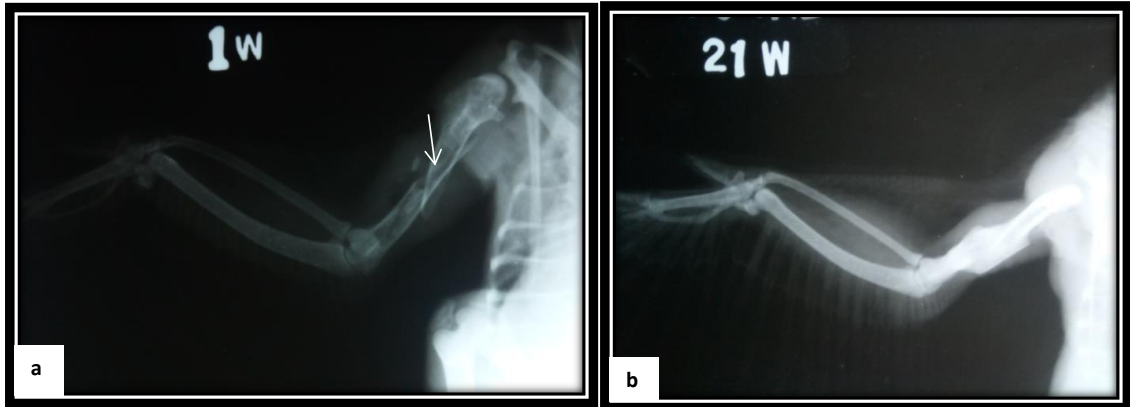


Figure 17: Postoperative complication in a bird, showing development of split fracture as a consequence of drilling of hole and reaming during the operation (a); and subsequent fracture healing by callus formation is discernible (b); the escape of generated hydrogen gas into soft tissue as black shadows (indicated in arrow) can be observed.

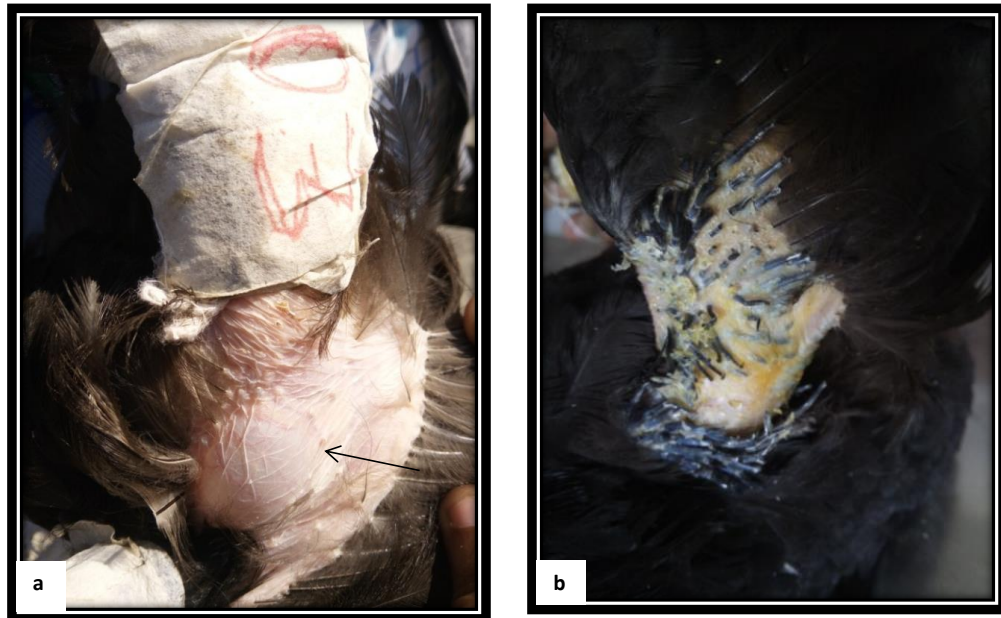


Figure 18: Photographs showing subcutaneous emphysema formation as a result of escape of formed hydrogen gas via pilot hole (a) and their subsequent clearing over next couple of days, (b) showing postoperative seventh day recovery with no appreciable inflammation, swelling with normal developed feathers over incision site.

## Diagnostic techniques for implant visualization

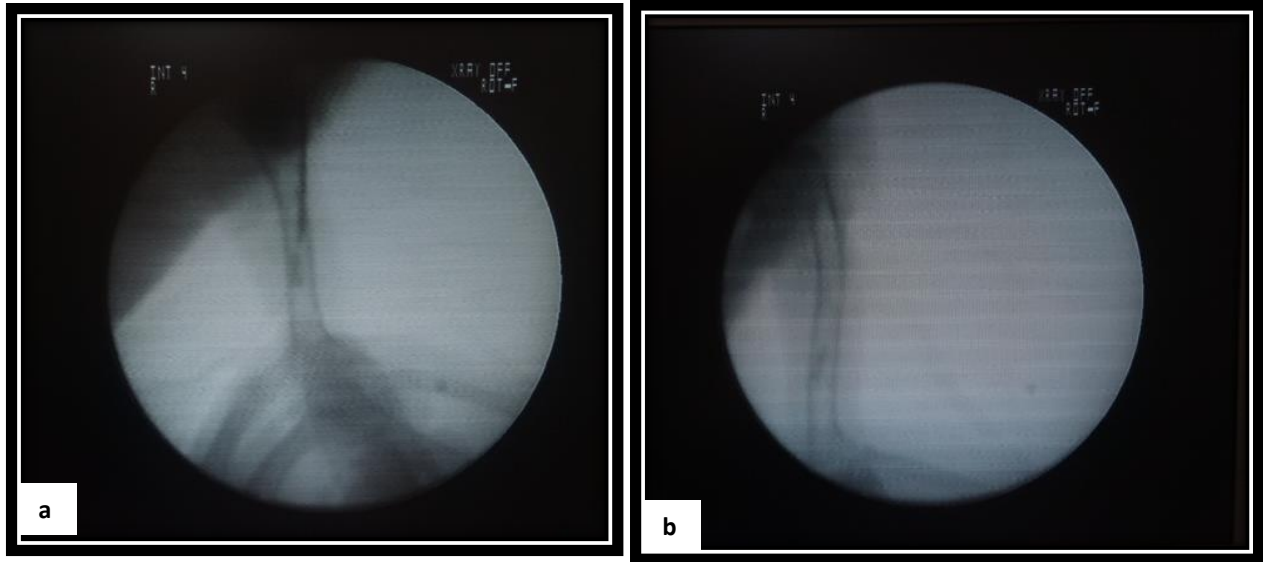


Figure 19: Photographs showing C-ARM guided intramedullary insertion and assessment of proper positioning of test implants over distal third space of humerus by using K-wire.

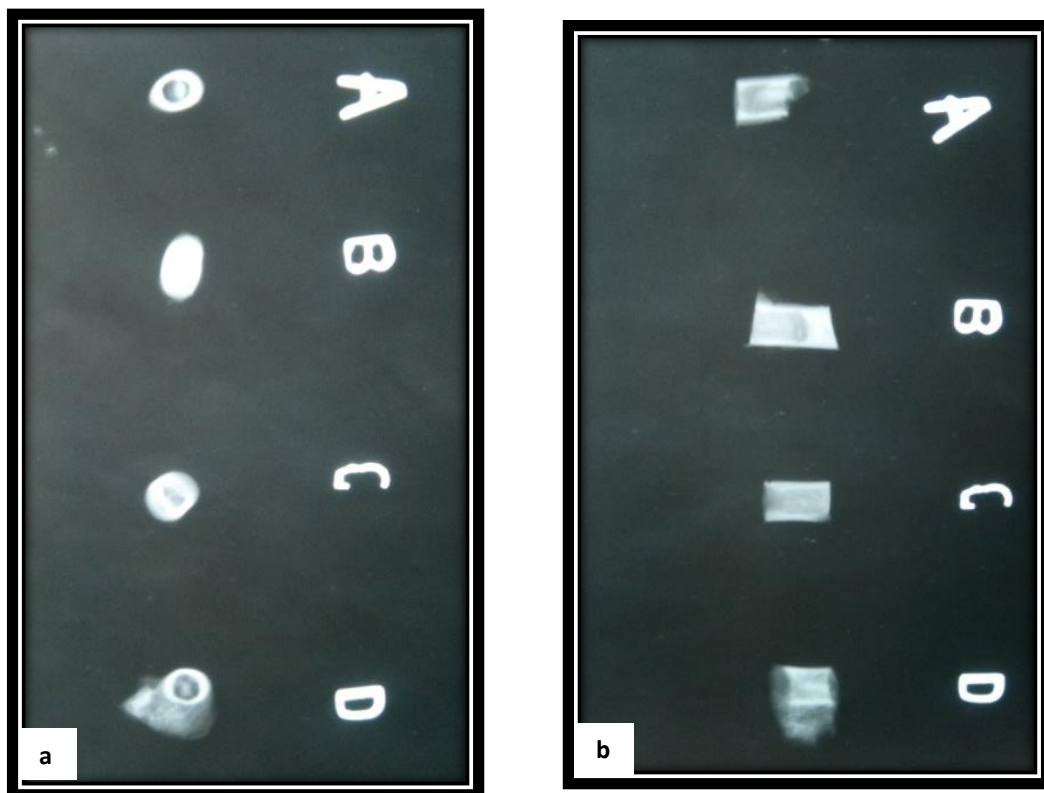


Figure 20: Photographs showing radiographic views of segmental bones for locating implant for histological sectioning (A - Mg plain, B - Mg 5% HA, C - Mg 5% HA 16<sup>th</sup> week and D - Mg plain), (a) Cross section of bone segments, (b) Longitudinal positioning of bone segments.

**Figure 21:** Representative photograph of medio-lateral view radiograph depicting sequestration (arrow) of the implant material on lower third of the intramedullary space with obliteration of medullary cavity by endosteal bone reaction.

**Figure 22:** Representative medio-lateral radiograph showing endosteal bone thickening (arrow) with obliteration of intramedullary cavity at later half of implantation with established channel (Magnified using lens).

**Figure 23:** Representative medio-lateral radiograph showing the escape of gas via insertion port into the soft tissue (flower bracket) on first postoperative week.

**Figure 24:** Representative leading edge view of test bone (group I) showing increased radio-density at 24<sup>th</sup> postoperative week (flower bracket) indicative of bone neoformation and remodeling.

Radiographs of special features



Figure 21



Figure 22



Figure 23



Figure 24

Histopathological photographs with H&E stain

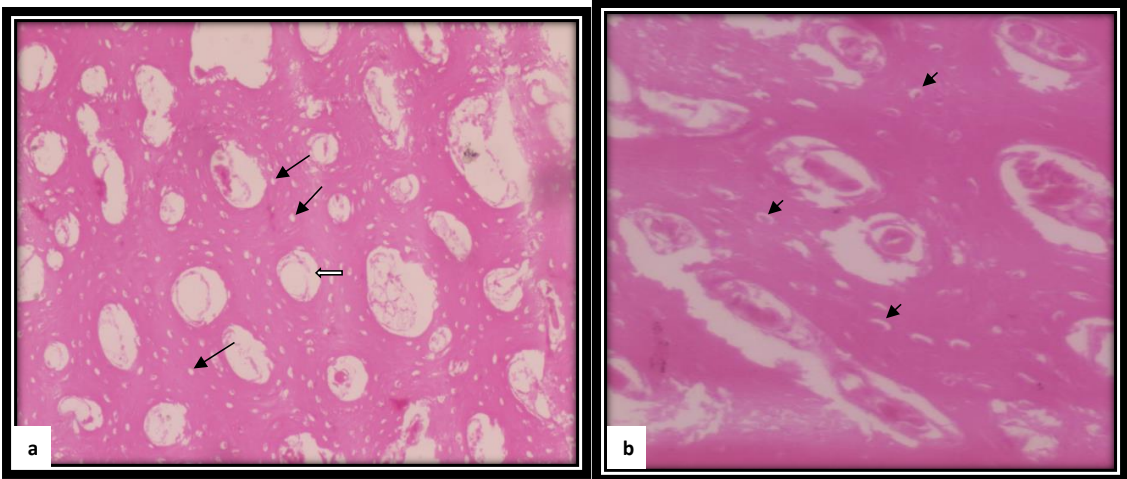


Figure 25: Representative histological photographs of hematoxylin and eosin stained sections of bone samples (small arrows: osteoblasts, long arrows: osteocytes in lacunae, white arrow: Haversian canal)

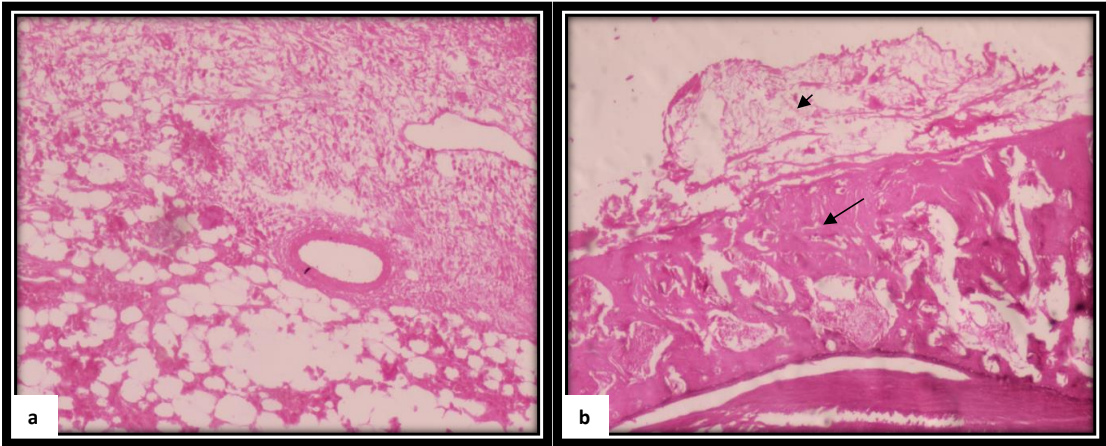


Figure 26: Representative histological photographs of hematoxylin and eosin stained sections of bone samples (small arrow: periosteum, long arrows: cortical bone)



*Summary  
and  
Conclusions*



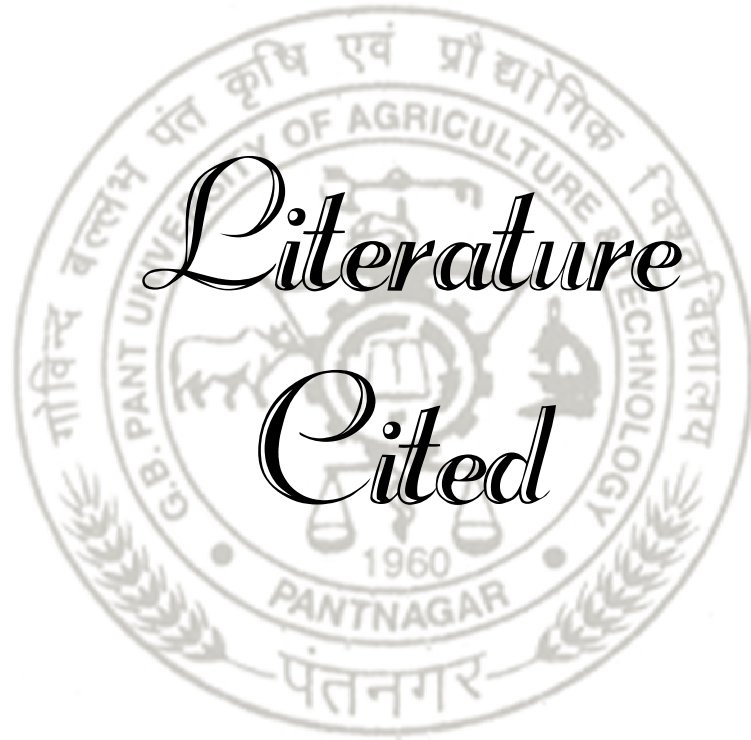
The study was conducted in eighteen healthy adult Uttara-fowl birds that were subjected to intramedullary insertion of Magnesium based (three types) orthopaedic bone implants with the objective of studying their biocompatibility and biodegradability for the period of 180 days. These birds were divided into three groups with six birds in each group. Birds of group-I were inserted with plain Magnesium intramedullary implants, birds of group-II were inserted with five per cent weight Magnesium-apatite intramedullary implants and birds of group-III with fifteen per cent weight Magnesium-apatite intramedullary implants. The anesthetic regimen with atropine sulphate (at the rate of 0.04 mg/ kg, body weight, SC) premedication followed with intravenous induction of ketamine (at the rate of 35 mg/kg body weight) anaesthesia. Birds were observed clinically for different parameters like dropping of the wing, flight test and postoperative complications. Laboratory investigations included histological evaluation of implant bone interaction and serum Magnesium, Calcium and Phosphorous estimation (three successive postoperative weeks with preoperative one). Medio-lateral (soft tissue technique) and leading edge (Hanging-drop technique) views of radiographs were taken, with radiographic factors of 1.5-2.0 mAs, 45-46 kVp and a constant FFD of 90 cm for evaluation of radiological degradation of implant materials on, immediate postoperative, 1<sup>st</sup> week, 2<sup>nd</sup> week, 3<sup>rd</sup> week, 4<sup>th</sup> week, 6<sup>th</sup> week, 8<sup>th</sup> week, 10<sup>th</sup> week, 12<sup>th</sup> week, 15<sup>th</sup> week, 18<sup>th</sup> week, 21<sup>st</sup> week and 24<sup>th</sup> week. Initiation of biodegradation was discernible in radiographs of Group-I on 2<sup>nd</sup> week (13<sup>th</sup> day) and complete radiological degradation was observed on 19-20<sup>th</sup> week; whereas in Group-II initiation of biodegradation was discernible on 3<sup>rd</sup> week (17<sup>th</sup> day) and complete radiological degradation was observed on 20<sup>th</sup> week; and in Group-III initiation of biodegradation was discernible on 1<sup>st</sup> week (6<sup>th</sup> day) and complete radiological degradation was observed on 21<sup>st</sup> week.

Some of the birds showed the postoperative subcutaneous emphysema increased up to fourth postoperative days (first week) after which it gradually returned to the normal. Some of the birds of every group showed wing dropping for first postoperative week, but then returned to normal and attained normal flight within first two

postoperative weeks. Serum Mg and Ca gives statistical significance conclusions about their concentrations in various postoperative weeks with respect to preoperative one, whereas serum P level could able to give significant difference even among different groups, which coincides with the cortical thinning and cortical thickening of test bone, over implant material and at implant extremities, respectively, due to contribution of minerals for osteosynthesis.

Following conclusions can be made on the basis of results of this study;

1. All the test varieties of Mg-based radio-opaque implants in this study maintain their radiographical integrity, in the intramedullary space for about 2 weeks and thereafter start degrading on subsequent days. The implants that were employed in birds of group II (Mg-based five per cent weight HA) showed longer duration for biodegradation and plain Mg- spacer was faster in the course of degradability (group-I) and 15 per cent HA mixed spacer (group-III) remained intermediate in that response.
2. Plain Mg implants generates enhanced bone reaction and cortical thickening around the intramedullary implantation site and that made good evidence of osteoactivity and osteoinductivity property of Mg.
3. These all biodegradable test implants can be used as replacers of stainless steel intramedullary pinning technique for simple fracture fixation in birds, though Mg with five per cent HA being preferred for those fractures which need, relatively, longer duration for osteosynthesis.



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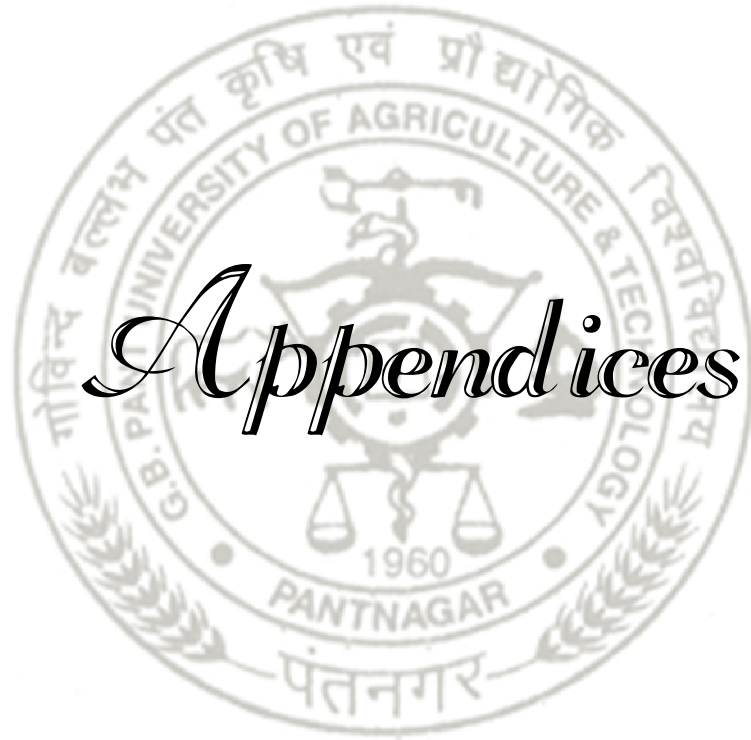
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- Registered Uttara-fowl bird on October, 05, 2018.
- Injured birds in kite (Uttarayana) festival on January, 18, 2018.



## Appendix-I

**4,026 birds injured, 214 die during treatment**

**Chinese Manja Killed Most, Say Volunteers**

TIMES NEWS NETWORK

**Ahmedabad:** In the two days of Uttarayan festivities, the forest department rescued and treated 4,026 birds, of which 214 died during treatment. The city reported 1,935 bird injuries, of which 139 birds died.

Several NGOs were also involved in bird rescues in the state. Principal chief conservator of forest (wildlife) G K Sinha said, "This year 4,026 birds have been rescued and the number was less than in 2017. Last year, there were 8,300 rescues." Sinha said, "This year, Karuna Abhiyan has reached all talukas and reports from smaller towns or cities have been received. Moreover, around 20 lakh schoolchildren took pledges to save birds. We take this as a credit, as despite extensive coverage, the number of birds treated is less than last year."

S J Pandit, deputy conservator of forests, said that on Sunday there were 643 bird injuries, while on Monday the number was 1,292. He said that on Uttarayan day, there was virtually no wind and hence casualties were low. Officials said migratory birds were also among those injured. A flamingo and a painted stork were brought to Jivdaya from near Thol after they were injured because of manja. An Egyptian vulture was also among the birds rescued.

Kartik Shastri of Jivdaya said, "This year the number of birds received by Jivdaya trust was 249 on Sunday and 400 on Monday. Last year, on January 15, the number of birds injured was 372."

Shastri said that despite the ban on 'Chinese manja' volunteers found many birds injured by the banned thread. He said most bird fatalities were from Chinese manja.

Several NGOs were involved in bird rescues in the state.

Vrushank Patel of Gopal Tinagar said "We rescued around 15% of birds were Chinese manja."



### Paper cuttings depicting injured birds during kite festival

## राज्य की पहली कुक्कुट नस्ल उत्तरा

अमर उजाला ब्यूरो

पंतनगर। पंतनगर कृषि विवि की ओर से विकसित मुर्गी की उत्तरा फाउल प्रजाति अब उत्तरा नस्ल के रूप में जानी जाएगी। भारतीय कृषि अनुसंधान परिषद दिल्ली में बीते दिनों हुई बैठक में यह निर्णय लिया गया है। पशु विज्ञान महाविद्यालय के अधिष्ठाता डॉ. जीके सिंह ने बताया कि उत्तरा को देश की 19वीं कुक्कुट नस्ल एवं उत्तराखंड की पहली कुक्कुट नस्ल घोषित किया गया है।

महाविद्यालय के पशुधन उत्पादन प्रबंध विभाग के वैज्ञानिक डॉ. शिव कुमार, डॉ. आरके शर्मा, डॉ. डी कुमार, डॉ. अनिल यादव इस नस्ल पर पिछले 10 वर्षों से शोध कर रहे थे। शोध टीम के टीम लीडर डॉ. शिव कुमार ने बताया कि उत्तरा फाउल गहरे काले रंग की है। इसके पैरों पर भी पंख होते हैं। सिर पर पंखों का गुच्छा (क्राउन) होता है।

कुछ स्थानीय क्षेत्रों में इसे डोटियाल, बुलबुल, ताज मुर्गी के नाम से भी जाना जाता है। उत्तरा फाउल कठिन पर्यावरणीय परिस्थितियों, पहाड़ी एवं ठंडे क्षेत्रों में रहने के लिए अनुकूल है। उत्तरा फाउल में अंडों से चूजे निकालने और छोटे चूजों की देखभाल करने के आनुवांशिक लक्षण विद्यमान हैं। शैक्षणिक कुक्कुट फार्म के सह निदेशक डॉ. अनिल यादव ने बताया कि वर्तमान में फार्म पर दो हजार से



पंतनगर में विकसित मुर्गी की उत्तरा फाउल प्रजाति।

ज्यादा उत्तरा नस्ल के पक्षी हैं। शैक्षणिक कुक्कुट फार्म के सहायक निदेशक (स्वास्थ्य) डॉ. राजीव रंजन कुमार ने बताया कि इस प्रजाति के कुक्कुटों में रोग-प्रतिरोधक क्षमता अन्य नस्लों की तुलना में अधिक होती है।

### Paper cuttings of registered bird Uttara-fowl used in the present study as the laboratory animal

## Appendix-II

### TWO WAY ANOVA TABLE FOR SERUM MAGNESIUM CONCENTRATION

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>	
Sample	59.21725325	3	19.73908442	93.50422013	1.369E-22	2.758078	**
Columns	0.024975507	2	0.012487753	0.059154599	0.9426159	3.150411	ns
Interaction	2.078446165	6	0.346407694	1.640936358	0.1516317	2.254053	ns
Within	12.66622045	60	0.211103674				
Total	73.98689537	71					

### TWO WAY ANOVA TABLE FOR SERUM CALCIUM CONCENTRATION

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>	
Sample	1103.360819	3	367.7869397	6.281066608	0.0008872	2.758078	**
Columns	286.0057295	2	143.0028648	2.442203411	0.0955713	3.150411	ns
Interaction	802.2041266	6	133.7006878	2.283340801	0.0473664	2.254053	ns
Within	3513.291254	60	58.55485424				
Total	5704.861929	71					

### TWO WAY ANOVA TABLE FOR SERUM PHOSPHOROUS CONCENTRATION

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>	
Sample	383.4631468	3	127.8210489	51.63544308	1.274E-16	2.758078	**
Columns	20.47962886	2	10.23981443	4.136543704	0.0207516	3.150411	**
Interaction	81.94300814	6	13.65716802	5.517040644	0.0001329	2.254053	**
Within	148.5271062	60	2.475451769				
Total	634.41289	71					

# CURRICULUM VITAE

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## ABSTRACT

Name : Abhishek M S Id. No. : 52737  
Sem. & Year : 1<sup>st</sup>, 2017-18 Degree : M.V.Sc.  
Major : Veterinary Surgery & Radiology  
Minor : Veterinary Gynaecology and Obstetrics  
Department : Veterinary Surgery & Radiology  
Thesis title : “Studies on biocompatibility and biodegradability of magnesium based orthopaedic bone implants in avian model”  
Advisor : Dr. Arup Kumar Das

The present study was conducted in 18 adult Uttara-fowls of 8-10 months of either sex, divided into three groups with six birds each, for intramedullary insertion of Mg and Mg-apatite orthopaedic bone spacers with the objective of finding their biodegradability and biocompatibility for the period of 180 days. In first, second and third group, plain Magnesium (Mg), Mg with five per cent Hydroxyapatite (HA) and Mg with 15 per cent HA containing implants, were surgically inserted in intramedullary space of humerus, respectively. The anaesthetic regimen with atropine sulphate premedication and induction of ketamine anaesthesia was pursued. For radiography medio-lateral and leading edge (Hanging-drop positioning technique) views for humerus of the birds were taken on scheduled intervals (immediate postoperative, 1<sup>st</sup> week, 2<sup>nd</sup> week, 3<sup>rd</sup> week, 4<sup>th</sup> week, 6<sup>th</sup> week, 8<sup>th</sup> week, 10<sup>th</sup> week, 12<sup>th</sup> week, 15<sup>th</sup> week, 18<sup>th</sup> week, 21<sup>st</sup> week and 24<sup>th</sup> week) and clinical parameters like heart rate, cloacal temperature, surgical duration, flight test, wing dropping test, histological evaluation and serum magnesium, calcium and phosphorous estimation were assessed to evaluate biodegradability and biocompatibility of implant material. Similar postoperative treatment and care were given to every bird during surgical convalescence. At the end of the study all the birds were euthanized with intravenous injection of thiopentone sodium as recommended by The Animal Welfare Board of India.

Initiation of biodegradation was discernible in radiographs in birds of Group-I on 1<sup>st</sup>-2<sup>nd</sup> week (11<sup>th</sup> day); in Group-II on 3<sup>rd</sup> week (17<sup>th</sup> day); and Group-III on 1<sup>st</sup> week (6<sup>th</sup> day).

In this study the biodegradable magnesium based orthopaedic bone implants were evaluated as the best choice for the avian fracture management. From this study following conclusions can be made (i) all Mg based metallic implants are biocompatible and biodegradable (ii) concentration of HA relates directly in the biodegradation of the Mg-HA matrix implant.

  
(Arup Kumar Das)  
Advisor

  
(Abhishek M S)  
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## सारांश


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प्रवेश वर्ष	: प्रथम, 2017-18	उपाधि	: एम.वी.एस.सी.
विभाग	: पशु शल्य चिकित्सा एवं विकिरण		
मुख्य विषय	: पशु शल्य चिकित्सा एवं विकिरण विज्ञान		
गौण विषय	: पशु मादा रोग एवं प्रसूति विज्ञान		
शोध शीर्षक	: "मैग्नीशियम धातु निर्मित अस्थि प्रतिस्थापक की पक्षी प्रतिरूप में स्थापन के उपरान्त जैव अनुकूलता एवं जैव क्षरण परीक्षण"		
सलाहकार	: डॉ. अरूप कुमार दास		

उक्त शोध कार्य में उत्तरा प्रजातिकी 18 (अट्टारह) कुक्कुटों में प्रयोग किया गया, जिनकी आयु आठ से दस माह की थी। सफल पक्षियों को लिंगविभेद रहित तीन वर्गों में विभाजीत किया गया। प्रत्येक वर्ग के कुक्कुटों में पृथक अस्थि प्रतिस्थापक प्रयोग किया गया। प्रथम समूह में छः (6) पक्षियों में शुद्ध मैग्नीशियम (Mg) धातु निर्मित; द्वितीय समूह में छः (6) पक्षियों में मैग्नीशियम (Mg) एवं 5 प्रतिशत के हाइड्रॉक्सियापटाइट शल्य चिकित्सा द्वारा प्रगंदिका अस्थि भुजा में प्रतिस्थापन किया गया एवं तृतीय वर्ग में छः (6) कुक्कुटों को प्रगंदिका अस्थि भुजा में 15 प्रतिशत हाइड्रॉक्सियापटाइट समनवीत मैग्नीशियम धातु की प्रतिस्थापक शल्य चिकित्सा द्वारा स्थापित किया गया। शल्यचिकित्सा उपरान्त सफल वर्गों के पक्षियों को 180 दिन तक निरीक्षण किया गया। इस दौरान रंजन रश्मि परीक्षण निर्दिष्ट दिनांतर में (शल्यचिकित्सा उपरान्त, प्रथम-, द्वितीय-, तृतीय-, चतुर्थ-, पंचम-, षष्ठम-, अष्टम-, दशम-, द्वादश-, पंचदश-, अष्टदश-, एकविंश- एवं चतुर्विंश- सप्ताह) में सम्पन्न किया गया। सकल नैदानिक एवं लाक्षणिक परीक्षण एवं जैव रसायनिक परीक्षण (मैग्नीशियम, सीरम कैल्शियम एवं सीरम फास्फोरस) सम्पन्न किया गया।

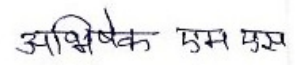
शल्य प्रतिस्थापनोपरान्त सकल पक्षियों को एंटीबायोटिक एवं मरहम पट्टी चिकित्सा प्रदान किया गया। अंत में उत्तकीय परीक्षण हेतु भारतीय पशु कल्याण बोर्ड के निर्देशानुसार क्लेश निवारण मृत्यु प्रदान किया गया, तदुपरान्त उन्हें विधिजनक भूमिगत कर दिया गया।

निरीक्षणनुसार- प्रथम वर्ग के पक्षियों में एकादश दिवस में, द्वितीय वर्ग में सप्तदशदिवस में एवं तृतीय वर्ग में षष्ठम दिवस में अस्थि प्रतिस्थापकों का जैविक क्षय प्रारम्भ हो चुका था।

निष्कर्षतः यह स्पष्ट होता है कि पक्षियों में आस्थभंग हेतु (Mg) मैग्नीशियम एवं हाइड्रॉक्सी एपेटाइट मैग्नीशियम अस्थि प्रतिस्थापक को उत्तम साधन के रूप में माना जा सकता है। किन्तु मैग्नीशियम धातु के जैविक क्षरक हाइड्रॉक्सियापटाइट का संरक्षण एवं उपस्थिति पर निर्भरशील है।



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