ISSN 2321-807X

Comparison of three types of physical aspects of a carbonated hydroxyapatite biomaterial: Study implantaion in vivo in rats of "Wistar" strain and physiological & physicochemical explorations

Salha Boulila[a,b], Kais Mnafgui[a], Hassane Oudadesse[b], Hafed El Feki[c]*, Abdelfattah El Feki[a]

[a] Laboratory of Animal Ecophysiology, Faculty of Sciences of Sfax, University of Sfax, P.O. Box 95, Sfax 3052, Tunisia;
E-mail : rima_lina2008@yahoo.fr
E-mail : mnafgui_kais@yahoo.fr
E-mail : Abdelfattah.ElFeki@fss.rnu.tn
[b] Université of Rennes 1, UMR CNRS 6226, Campus de Beaulieu, 35042 Rennes, France
E-mail : hassane.oudadesse@univ-rennes1.fr
[c] Laboratory of Sciences Material and environment, Faculty of Sciences of Sfax, B.P. 1171, 3000 Sfax, Tunisia;
E-mail : hafed.elfeki@yahoo.fr
*Corresponding author: E-mail address: hafed.elfeki@yahoo.fr

ABSTRACT

Currently, research on biomaterials must meet and demonstrate a set of therapeutic competence to level many health problems. The objective of our work is to normalize the technique of implantation of the biomaterial (carbonated hydroxyapatite: HAC). Three modes subcutaneous implantation was carried out. This technique consists to select the most tolerated by the body without toxicity. Thus, we have applied our biomaterial (HAC) in pellet form under pressure, under pressure sintering pellets and capsules for two weeks. Our results showed that the capsule did not disturb and mainted the equilibrium and balance or ferric ion phosphate balance, prevent against the toxicity of hepato-renal system by comparison with the pellets. These results demonstrated the tolerance, the biocompatibility and the integrity of apatite administered in capsule.

Indexing terms/Keywords

Biocompatibility; Carbonated hydroxyapatite; Oxidative stress; Subcutaneous implantation.

Academic Discipline And Sub-Disciplines

Biomaterials

SUBJECT CLASSIFICATION

Biology Subject Classification

TYPE (METHOD/APPROACH)

Experimental study-implantation.

Council for Innovative Research
Peer Review Research Publishing System
Journal: Journal of Advances in Chemistry
Vol. 8, No. 2
editor@cirjac.com
www.jac.cirworld.com, member.cirworld.com

ISSN 2321-807X

1. Introduction

Biomaterials cover many domains of applications such as ophthalmology, orthopedic surgery, cardiovascular, dentistry, urology, endocrinology, plastic surgery ... etc). Materials science oriented multidisciplinary biomedical and requires close collaborations between experts in various fields: chemist, biologists and Surgeons.

Among bioceramics, Hydroxyapatite (Ca10 (PO4)6 (OH)2) is known for its physical and chemical properties very similar to those of calcified tissues (bone, dentine ....). It can be produced from coral [1-2], the shell [2-3], eggshell [2,4-6] and also from body fluids [2,7]. It is non-toxic, non-inflammatory, non-immunological and bioactive [8-9]. As well, the Hydroxyapatite (HA) is a good candidate for bone substitution because of its chemical and structural similarity with bone mineral [10-11]. In addition, this biomaterial through a crystalline phase of calcium phosphate minerals found naturally in bone, has shown tremendous promise as a graft material [12]. Our biomaterial (carbonated hydroxyapatite) so presents a biocompatibility, bioactivity and bioresorbable properties [13-17].

But, this material has several drawbacks, on the one hand, it is osteoconductive but not osteoinductive, on the other hand, the mechanical behavior of these materials remains very fragile.

In previous work [9], we used a carbonated hydroxyapatite (HAC) biomaterial type. The implantation of HAC "in vivo" was formed in pellet form at the bone of rabbits. The results, obtained by exploration physiological and physico-chemical properties of bone after implantation, showed that the implant is not toxic secondary effects on the body on the one part and permits a good consolidation of the bone without oxidative stress in the other part.

To better see the influence of the purity of the biomaterial HAC on the one hand and the mode of implantation on the other hand on the biological and physicochemical parameters on a model animal (rat strain "Wister"), we studied in the present work a biomaterial type 80% of HAC and 20% β-TCP (β - tricalcium phosphate) as a subcutaneous implant.

The objective of this study is to select the best method to implant our biomaterial in rat, without toxicity and with a good tolerance by the organism.

So, three types of subcutaneous implants were applied. Thus, in this work, the three methods to apply our biomaterial (Hydroxyapatite) pellets under pressure, under pressure sintering pellets and capsules implanted in rats strain "Wistar" were used. Our study is based therefore on the physiological effects (oxidative stress, hepatorenal toxicity, balance iron and phosphocalcic) and the physico-chemical characterization through diagrams analysis (X-ray diffraction and infrared spectroscopy) of these three types of implants.

2. Material and Methods

2.1. Synthesis of the biomaterial

The powder of the carbonated hydroxyapatite (HAC) was prepared by the method of double decomposition (wet), which consists in adding a solution of calcium nitrate to a solution of ammonium phosphate in well-defined operating conditions. The product obtained after filtration of the solution, is subjected to calcination at 400 ° C. The powder thus obtained was transformed into implant in three physical forms:

- Pellet formed under pressure (PP): 300 mg powder of HAC was milled during 5 minutes and then compacted under a pressure of 8 bars during 15 minutes.

- Pellet formed under sintering and pressure (PPS): 300 mg powder of HAC was ground during 5 minutes and then compacted under a pressure of 8 bars during 15 minutes and then was put in the oven with a program for 6 hours in 1000° C.

- Capsule (Cap): 300 mg powder of HAC was stored into capsule which was purchased from local pharmacy.

2.2. Animals and treatments

The assays of the present study were conducted on adult male Wistar rats, weighting 240 ±10 g, which were obtained from the local Central Pharmacy, Tunisia. All rats were kept in an environmentally controlled breeding room (temperature: 20 ± 2°C; humidity: 60 ± 5%; 12h dark/light cycle). They had standard diets and free access to tap water. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics. Animals were anesthetized subcutaneously by injection of chlorhydrate lidocaine (10 mg/Kg body weight). After surgical incision, different physical forms of HAC (300 mg each sample) were implanted in rats. The experimental rats were divided into 4 groups of eight animals each as follows:

Group 1: control rats, considered as referent non-implanted rats. (C)

Group 2: operated rats implanted with HAC as PP form during 2 weeks. (PP)

Group 3: operated rats implanted with HAC as PPS form during 2 weeks. (PPS)

Group 4: operated rats implanted with HAC as capsule form during 2 weeks. (Cap)

The animals were supervised daily to detect the presence of the implant. Two weeks later, the rats were sacrificed by decapitation, and their trunk blood was collected. The serum was prepared by centrifugation (1500×g, 15min, 4°C), frozen

ISSN 2321-807X

and stored at -20°C until analysis. The kidney and liver were removed and cleaned of fat. Samples of femoral bone were collected from each group of animal for further physicochemical analysis. All samples were stored at -80°C until used.

2.3. Oxidative stress measurements

The lipid peroxidation in the liver and kidneys of control and all treated groups of animals was measured by the quantification of thiobarbituric acid-reactive substances (TBARS) [18] and Conjugated Dienes (CD) [19]. The activity of superoxide dismutase (SOD) was assayed by the spectrophotometric method [20]. The glutathione peroxidase (GPx) activity was measured by the method described by Pagila and Valentine [21]. The catalase (Cat) was assayed colorimetrically at 240 nm and expressed as moles of H2O2 consumed per minute per milligram of protein [22]. The level of total protein was determined by the method of Lowry et al. using bovine serum albumin as the standard at 660 nm [23].

2.4.Biochemical analysis

The serum electrolytes, calcium, phosphorus and iron concentrations were determined by an automatic ion analyzer (IA 300, Japan). Serum levels of Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT) activities and creatinine rate were measured in frozen aliquots of serum by standardized enzymatic procedures using commercial kits from (Biolabo, France) on an automatic biochemistry analyzer (Vitalab Flexor E, USA) at the clinic pathological laboratory of Sidi Bouzid Hospital.

2.5.Physicochemical analysis

The samples of powder and bone were characterized by X Ray-Diffraction (XRD) and Infrared Absorption Spectrometry (IR). The IR spectra in the range 4000-400 cm-1 were recorded as KBr pellets with a JASKO FT/IR 420 spectrometer.

The XRD pattern of powder and bone simples was measured on a Seifert XRD 3000 TT diffractometer controlled by an IBM PC microcomputer. Measurements were made using a step-scanning technique at fixed time intervals, the calculation of the crystallographic parameters and the mesh volume apatitic of powder and bone were realized using the program CELREF.

2.6. Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical significance was assessed by the Fisher test. *P < 0.05 was considered statistically significant.

3.Results

3.1. Physiological properties

3.1.1. Effect of HAC implantation on TBARS and CD in liver and kidney of experimental animal

As shown in Fig. 1 and 2, a significant increase (44% and 29%) of TBARS and (23% and 40%) of CD levels was observed in liver of PP and PPS rats respectively in comparison with the control. Remembered that these markers (CD and TBARS) are indicators of lipid peroxidation. This was probably related to generation of free radicals which caused the oxidative damage to the tissues. At the same time an increase in TBARS and CD levels in the kidney respectively of PP (by about 21% and 25%) and PPS (by about 22% and 28%) compared to the controls was obtained (Fig.1 and 2). Whereas hepatic and renal, capsule apatite induces a significant decrease of TBARS and CD compared to treated PP and PPS. We can therefore conclude that the apatite administered in capsule is more tolerated by organism and has no hepato-renal stress.

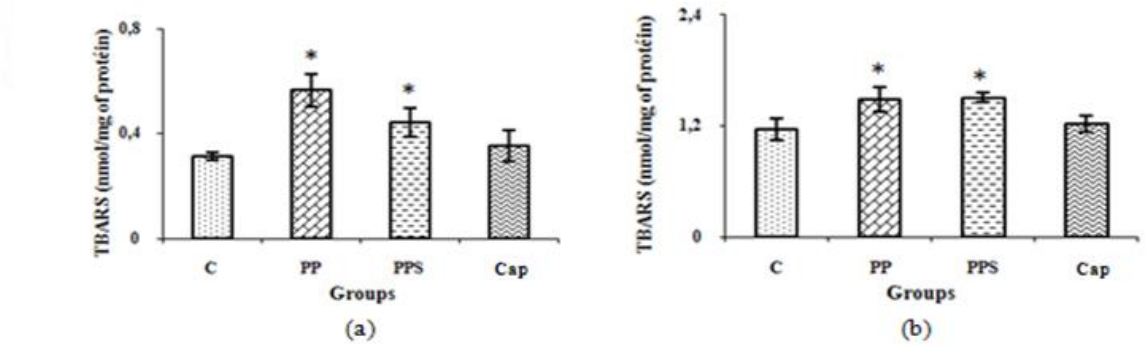

Fig 1: Rate of TBARS hepatic (a) and kidney (b) (nmol / mg of protein) in control rats and implanted
Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

ISSN 2321-807X

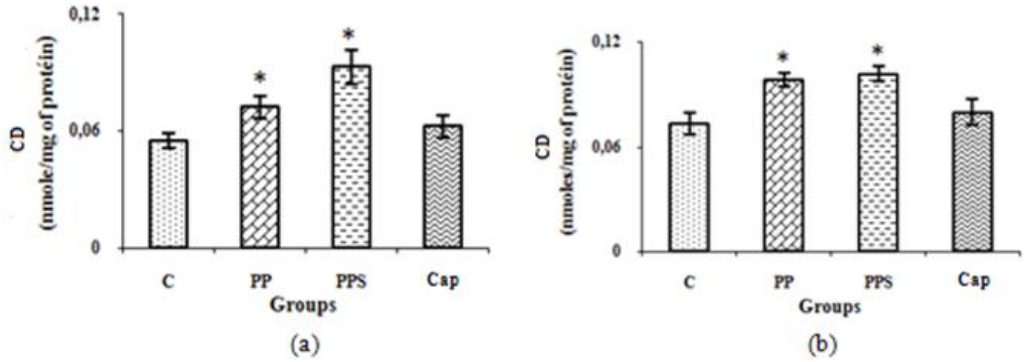

Fig 2: Rate of CD liver (a) and kidney (b) (nmol / mg of protein) in control rats and implanted
Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

3.1.2. Effect of HAC implantation on antioxidant enzymes (SOD, Cat and GPx) in liver and kidney of experimental animal

Activities of SOD, Cat and GPx, were found to be much reduced in liver and kidney of PP and PPS rats as compared to control values (Fig. 3-5). These changes could worsen the oxidative stress by allowing an accumulation of free radicals. In rats implanted capsule, there was no significant change in the activity of these enzymes, as compared to controls (Fig. 3-5). While rats implanted with apatite capsule showed improved antioxidant activity of this enzyme in comparison to groups of rats treated with pellets (PP and PPS).

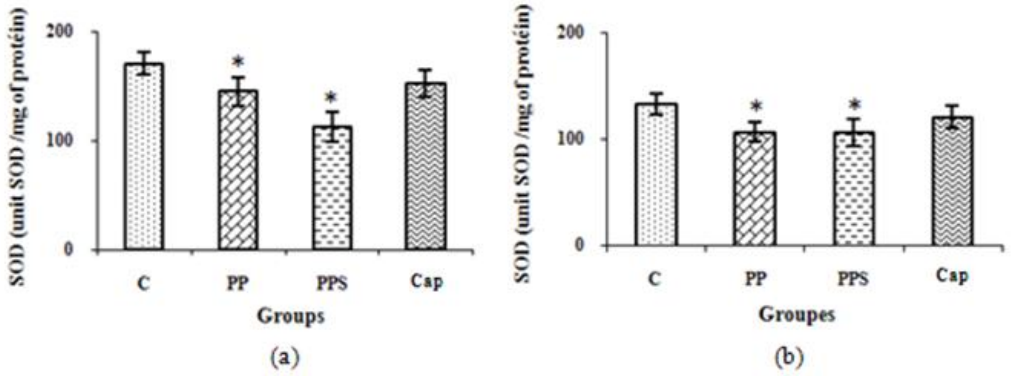

Fig 3: Rate of hepatic (a) and kidney (b) SOD activity (SOD unit / mg protein) in control rats and implanted
Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

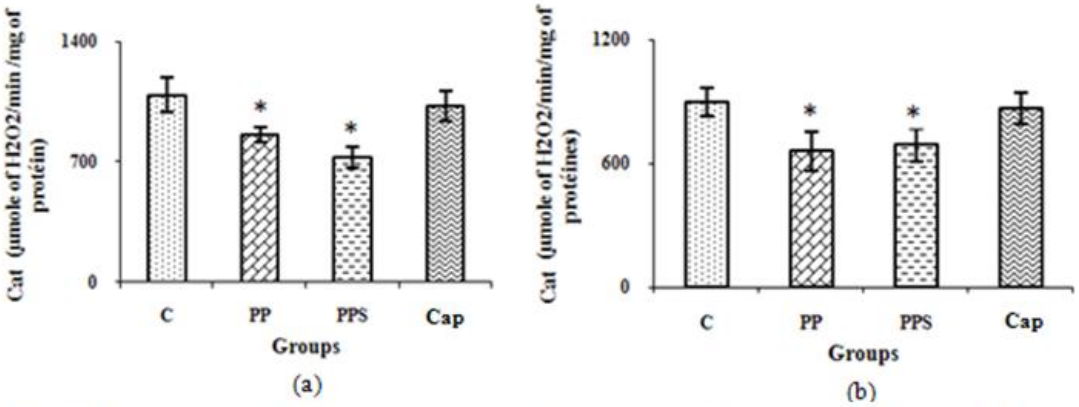

Fig 4: Rate of Liver (a) and kidney (b) Cat activity (micromoles of H_2O_2/min/mg protein) in control rats and implanted
Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

ISSN 2321-807X

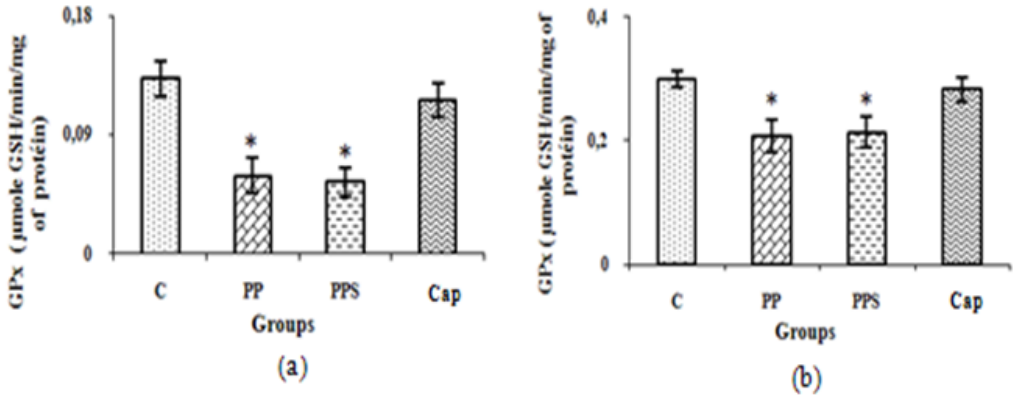

Fig 5: Rate of hepatic (a) and kidney (b) GPx activity (Unit GSH / min / mg protein) in control rats and implanted Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

3.1.3. Effect of HAC implantation on the ionic and serum phosphocalcic balance

Our study (Table 1) shows that serum electrolytes (Na+, K+ and Cl-) and iron show no significant change (P > 0.05) in the group of rats implanted with the capsule in comparison with the control group. Conversely, groups of rats (PP) and (PPS) showed a significant variation (P < 0.05) in serum Na+ and iron compared with the control group. For serum calcium, the group implanted with the capsule (Cap) shows stability compared to the control, by cons, the groups implanted with the pellets (PP and PPS) show a significant decrease. It seems that the capsule is better tolerated by the body, it does not disturb the equilibrium ferric ion and phosphocalcic.

Table 1. Biochemical variation in different lots of rats

Groups / Parameters		C	PP	PPS	Cap
THE ELECTROLYTES (meq/l)	Na^+	134,73 ± 0,64	135,60 ± 0,49 (*)	132,37 ± 1,76 (*)	135,32 ± 1,32
	K^+	5,30 ± 0,31	5,49 ± 0,31	5,16 ± 0,53	5,28 ± 0,42
	Cl^-	105,86 ± 0,72	106,86 ± 1,15	104,85 ± 1,73	107,97 ± 1,07
IRON (µmol/l)		12,33 ± 2,08	10.00 ± 0,81 (*)	10.00 ± 0,81 (*)	12.00 ± 0,81
CALCIUM (mmol/l)		2,90 ± 0,03	2,76 ± 0,05 (*)	2,83 ± 0,04 (*)	2,86 ± 0,04
PHOSPHORUS (mmol/l)		2,69 ± 0,23	2,57 ± 0,12	2,66 ± 0,20	2,65 ± 0,37

Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

3.1.4. Effect of HAC implantation on serum parameters of hepatic and renal toxicity

This study showed a significant change for the level of serum creatinine in the groups implanted with pellets (PP and PPS) compared to controls. But this level has no significant change for groups implanted with capsule (Cap) and this compared to controls. Apatite administered in capsule does not disrupt therefore renal activity.

ISSN 2321-807X

On serum indices of liver toxicity, there is an increase in AST and GGT serum for the treatment groups (PP and PPS) compared to the control group. In contrast, rats treated with the capsule showed a reduction in the level of these two indices compared to treated groups (PP) and (PPS).

The PAL serum significantly increased the range of 19.22% and 20.31% respectively for the treated groups (PP and PPS) compared to control rats, conversely, these rates decreased remarkably in the group implanted with the capsule, and this in comparison groups (PP) and (PPS) which explains the protective effect of apatite implanted capsule form against hepatotoxicity (Table 2).

Table 2: Change in serum parameters of hepatic and renal toxicity in different lots of rats

Groups / Parameters	C	PP	PPS	Cap
CREATININE (µmol/l)	35,43 ± 1,35	39,42 ± 2.87 (*)	32,73 ± 0,35 (*)	35,83 ± 1,24
AST (UI/L)	55 .00 ± 2,82	59.00 ± 1,73	66,50 ± 9,84	55,66 ± 4,61
GGT (UI/L)	2,33 ± 0,57	3,75 ± 0,95 (*)	3,50 ± 0,57 (*)	2,75 ± 0,43 (*)
PAL (UI/L)	652,66 ±11,59	808.00 ± 77,09 (*)	819.00 ± 45,25 (*)	576,50± 37,47 (*)

Data represent mean ± standard deviation to the mean value of controls. * Significant as compared with control rats.

3.2. Physico-chemical properties

The purpose of a physico-chemical characterization of our apatite biomaterial and cohabitation at the implantation site often we use two analytical techniques. The best known are infrared spectroscopy and X-ray diffraction. Indeed, these methods are used to determine of the one part, the nature of certain exchange ions from the body with the network apatite. On the other part, the detection of the integration of this implant (HAC).

3.2.1. Study of hydroxyapatite before and after implantation

3.2.1.1. X-ray diffraction

Obtained results by X-ray diffraction (Fig. 6-9) of not implanted biomaterial used show the presence of two phases: apatite phase and β-TCP phase. We tried to follow the evolution of only apatite phase during implantation. The strongest lines are indexed in the hexagonal system with space group P63 / m. From the 2θ values of lines and their Miller indices hkl, the program CELREF was used. The crystallographic parameters a, b, c and cell volume apatite before implantation were calculated. After implantation of the biomaterial that exists in three physical forms (PP, PPS and Cap), we observed the movement of certain lines such as the strongest lines of Miller indices hkl: 200, 002, 211, 300 and 222. To better see these displacements, we use the calculation of crystallographic parameters (a, b and c) and the cell volume (Table III). We find in general significant variations in these parameters, except that the implants are in capsule form (Cap) and pellet (PPS) were the most changed from one form of biomaterial (PP). We thus demonstrate the high reactivity of the HA as capsule (Cap) and pellet (PPS) with the organization. To distinguish between these two types of biomaterials we use infrared technical.

ISSN 2321-807X

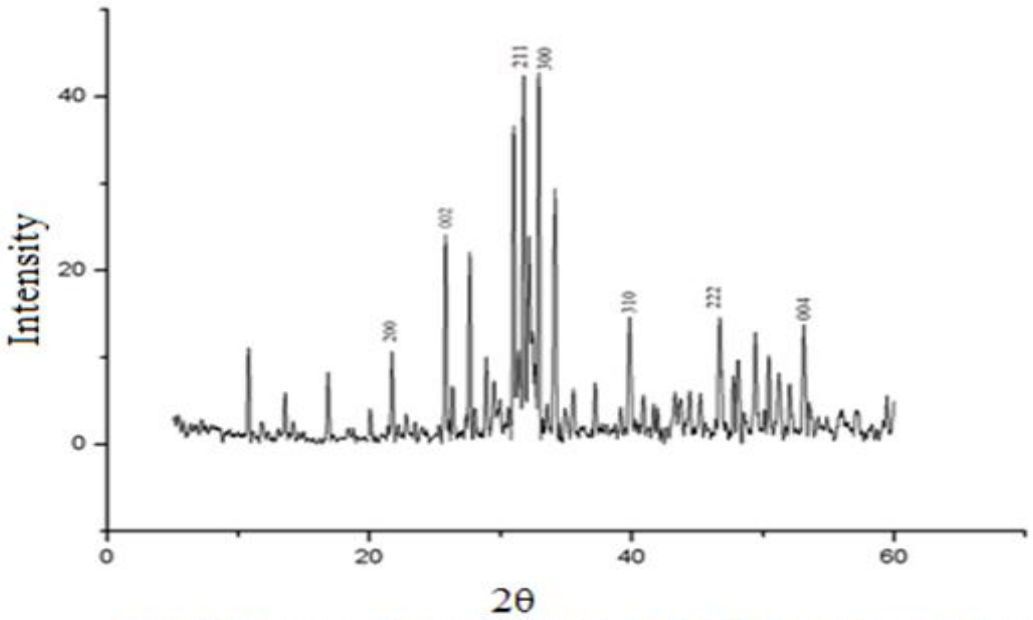

Fig. 6: Diagram of X-ray diffraction of the HA before implantation

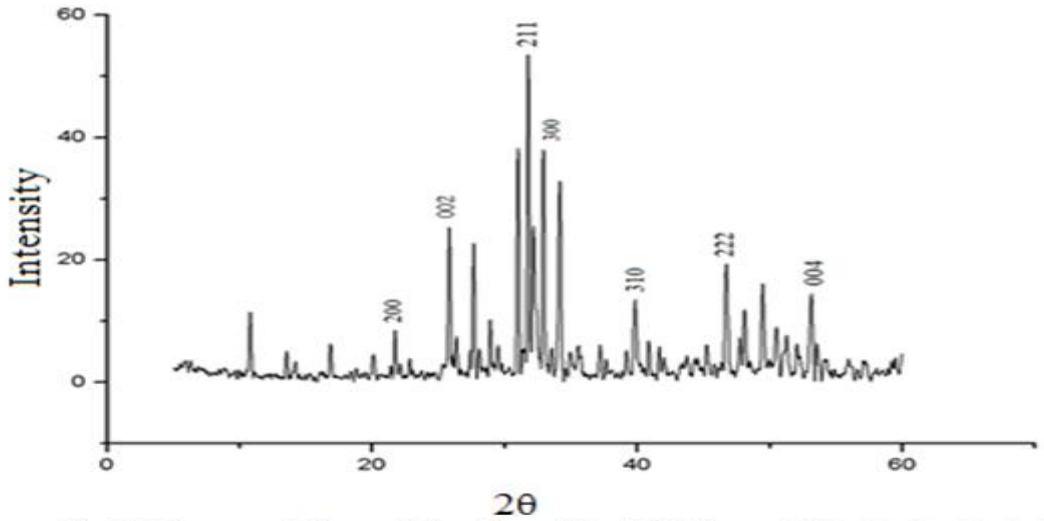

Fig 7: Diagram of X-ray diffraction of the HAC form of PP after implantation

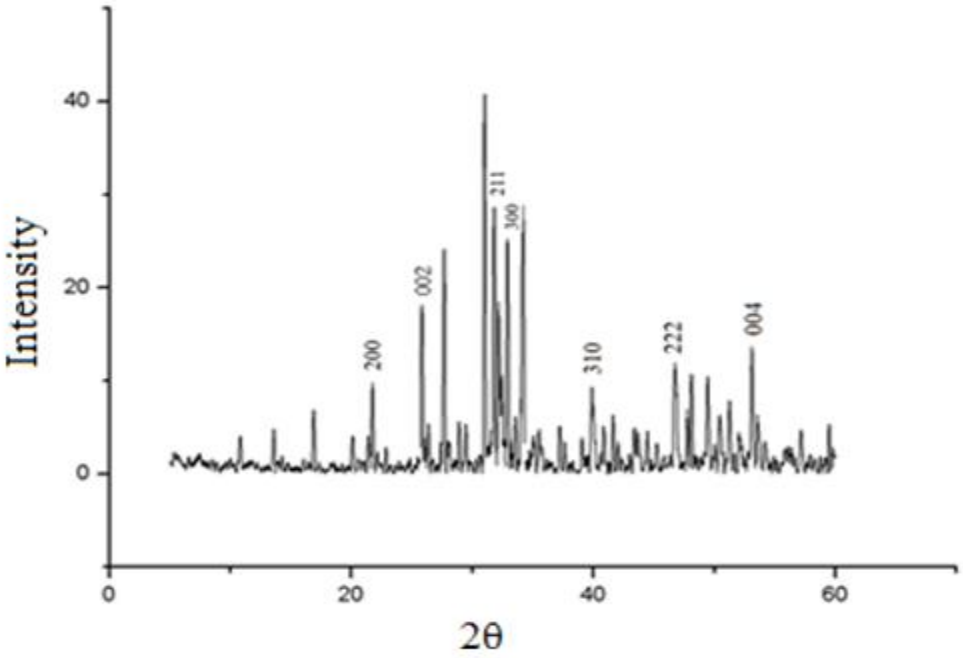

Fig 8: Diagram of X-ray diffraction of the HAC form of PPS after implantation

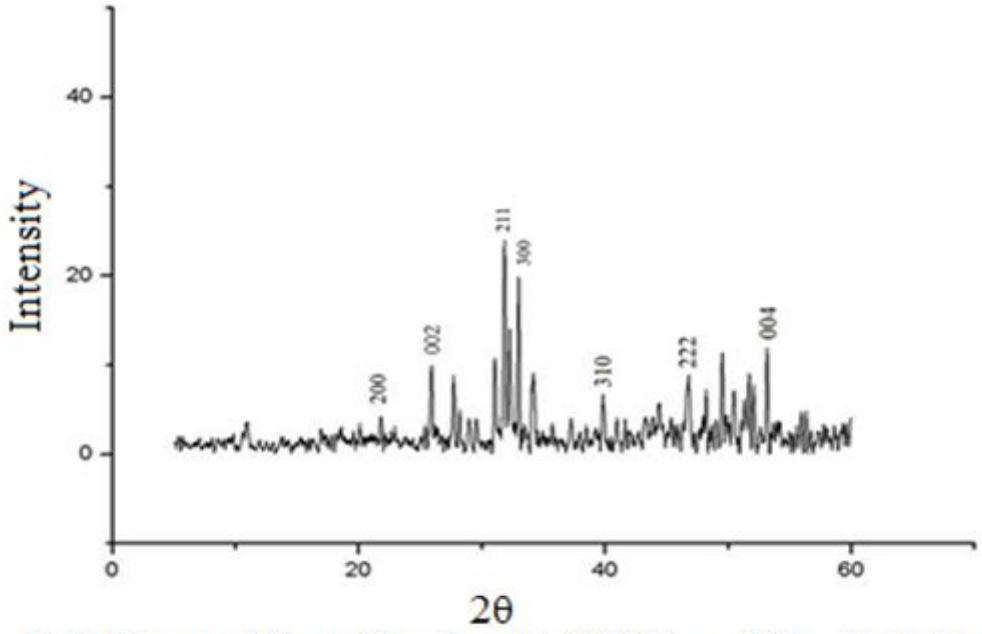

Fig 9: Diagram of X-ray diffraction of the HAC form of Cap after implantation

3.2.1.2. Infrared spectroscopy of compound

Infrared spectroscopic results, for samples of the non-implanted HAC and those located in different forms: pellet pressure (PP), pellet pressure and sintering (PPS) and capsule (Cap) are illustrated in Figs .10-13.

The product used, according to the infrared spectrum, is slightly carbonated hydroxyapatite of the majority of type B and the rest is Type A. This is testified by the presence of four vibration modes of the phosphate group observed to: 1088.23, 983.24, 603.06 and 473.28 cm-1. Thus, two modes of vibration that bundling CO32- observed at 1447.97 and 858.08 cm-1. Finally, we note the presence of two modes us and ul related to OH- group and are observed respectively at 631.67 and 3570.53 cm-1. It should be noted the presence of even the IR spectrum two bands observed at 724.78 and 3214.05 cm-1 which are attributed from the literature [14] respectively us vibration modes of OH- and ul adjacent groups carbonates CO3 2-. Hence, the existence of a small quantity of carbonate type A is confirmed. We note also the presence of some shoulders observed near the modes of vibration of the phosphate groups, at 1072.39, 962.26, 586.98 and 562.22 cm-1, which is probably attributed to the vibration modes of the PO4 3- groups to a second phase which corresponds to the non apatitic β-TCP.

ISSN 2321-807X

The spectra of the apatite located in different physical forms (PP, PPS and Cap) show clearly the presence of different modes of vibration observed at hydroxyapatite implantation but before we can high light displacement of these bands and the disappearance of others. We also note the disappearance of the bands corresponding mainly to β-TCP because it is more absorbable than apatite phase. Indeed, these spectra mount the capsule undergoes further change and decline of the bands in the case of pellets. The form of the implant so the more resorbed and more bioactive form is the capsule.

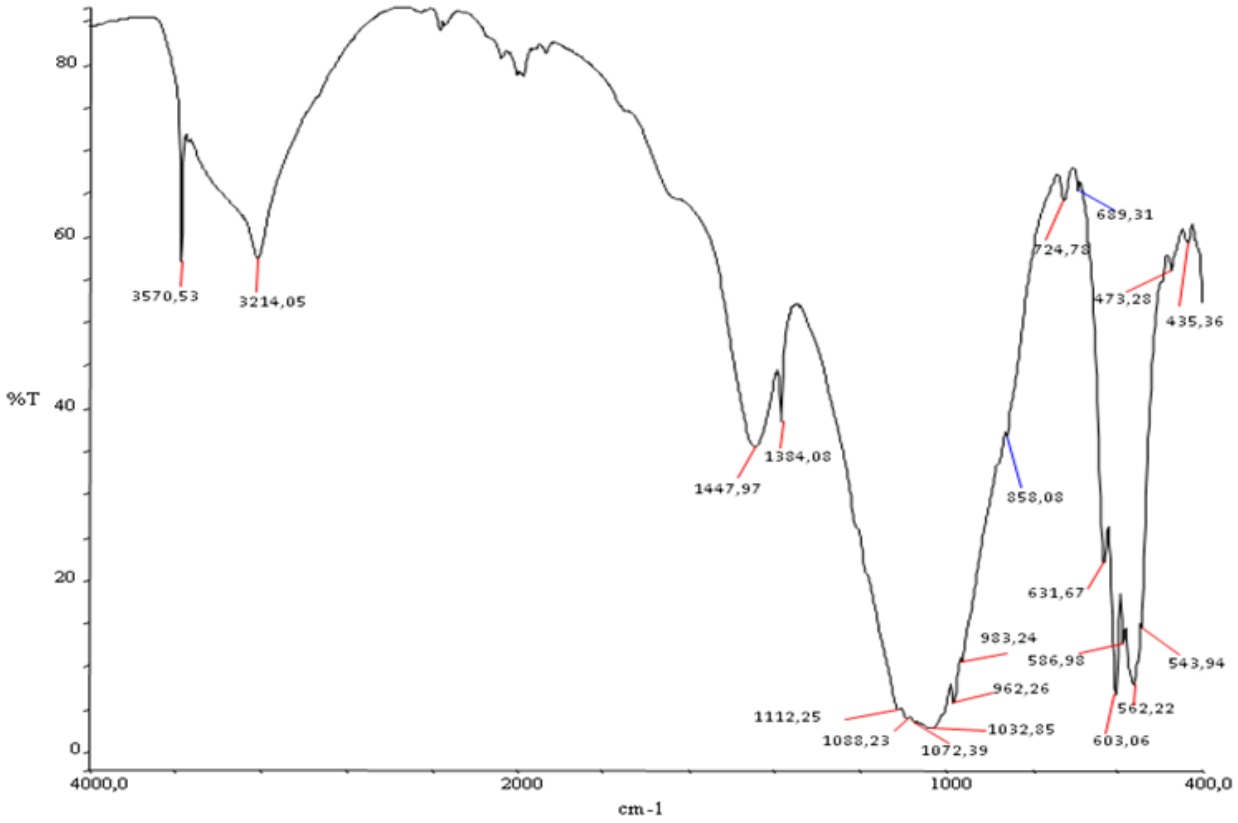

Fig 10: IR spectrum of HAC before implantation

ISSN 2321-807X

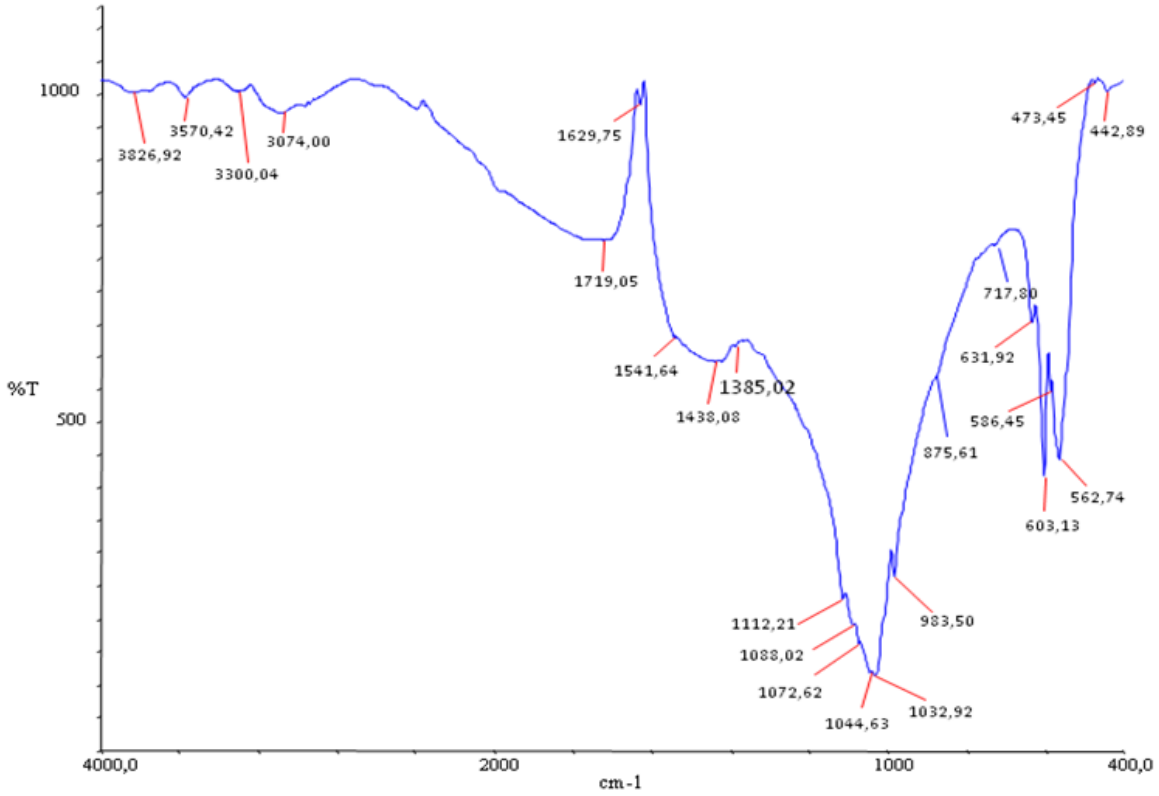

Fig 11: IR spectrum of HAC pellet form under pressure (PP) after implantation

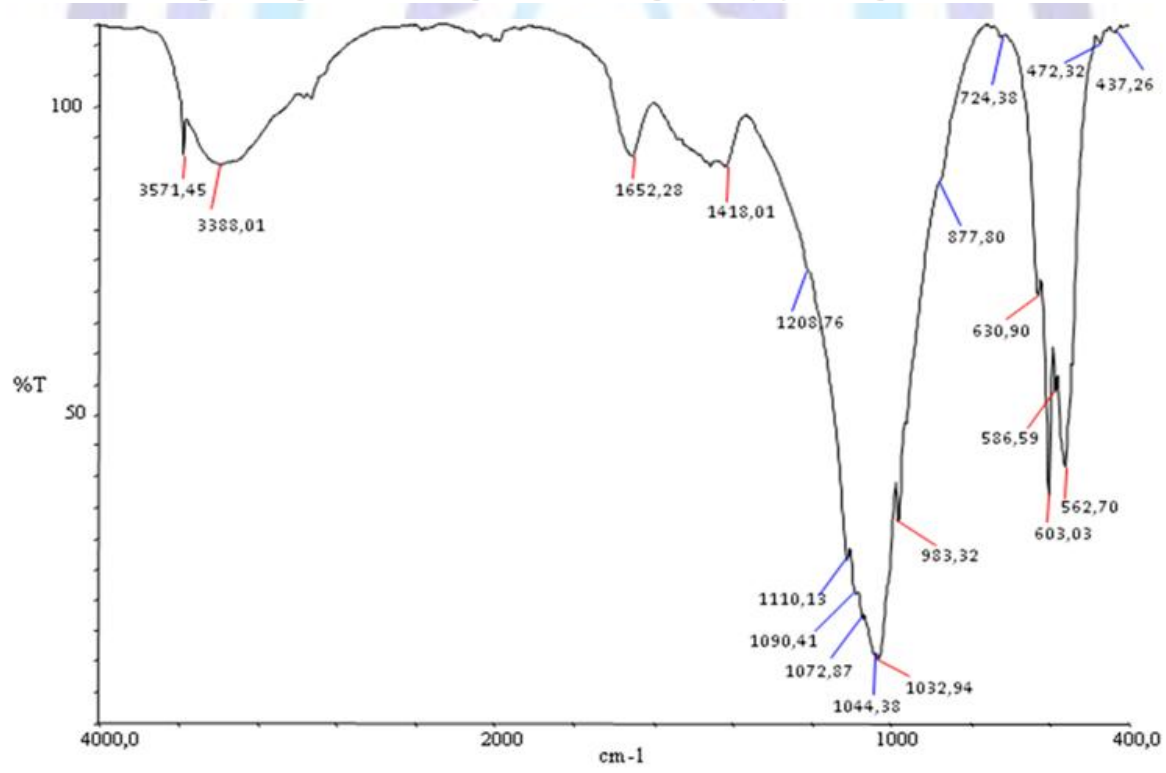

Fig 12: IR spectrum of HAC as a pellet and sintering under pressure (PPS) after implantation

ISSN 2321-807X

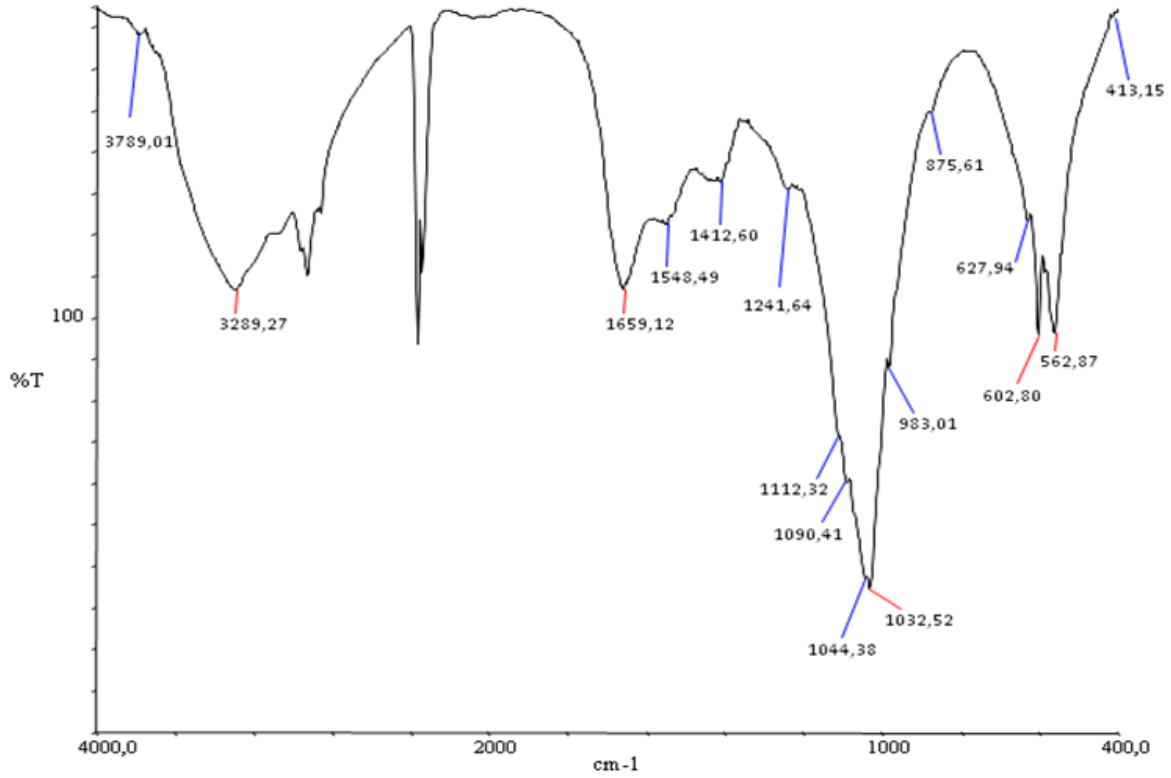

Fig 13: IR spectrum of HAC in capsule form (Cap) after implantation

3.2.2. Study of bone before and after implantation

3.2.2.1. The X-ray diffraction

The analysis of X-ray diagrams, performed on bone tranches not calcined in the case of different implants (C, PP, PPS and Cap) shows the presence of the lines large overlapping reflecting the presence of organic matter. The purpose of the one part is to improve the crystallization of the sample and on the other part the power of the resolution of the spectrum, we have calcined our bone samples at 400○C. The first results led to the elimination of the organic phase but the crystallization was not too improved. To do this we calcined bones at 600○C, the diffractograms presented in figures 14-17 show much finer lines. In addition, they showed the presence of a single phase pure apatite. We note that all the samples control and implanted have practically the same types of lines (Miller index) except that their positions 2θ and their intensities different from sample to another. This encouraged us to calculate the cell parameters and the cell volume apatite using the program "CELREF" (Table 4). We note that these parameters and the cell volume of apatite samples implanted rats vary in comparison to those of control rat bone. These variations observed before and after implantation show that there is good interaction between the implant and the bone but that is manifested by an ion exchange important bone to the implant. In addition, we note that the capsule is the implant showed more bioreactivity than in the case of pellets.

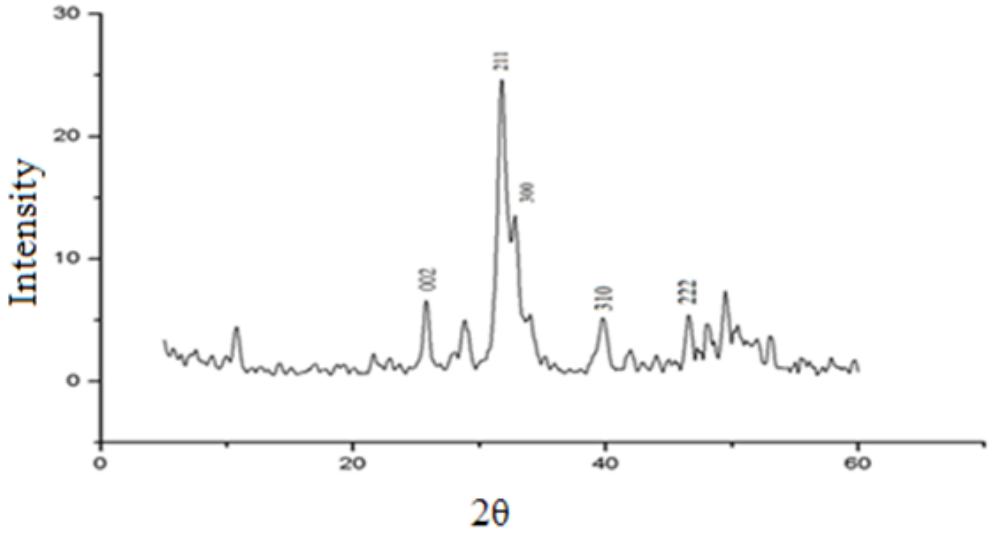

Fig 14: Diagram of X-ray diffraction of a sample bone of a rat not implanted (C) calcined at 600°C

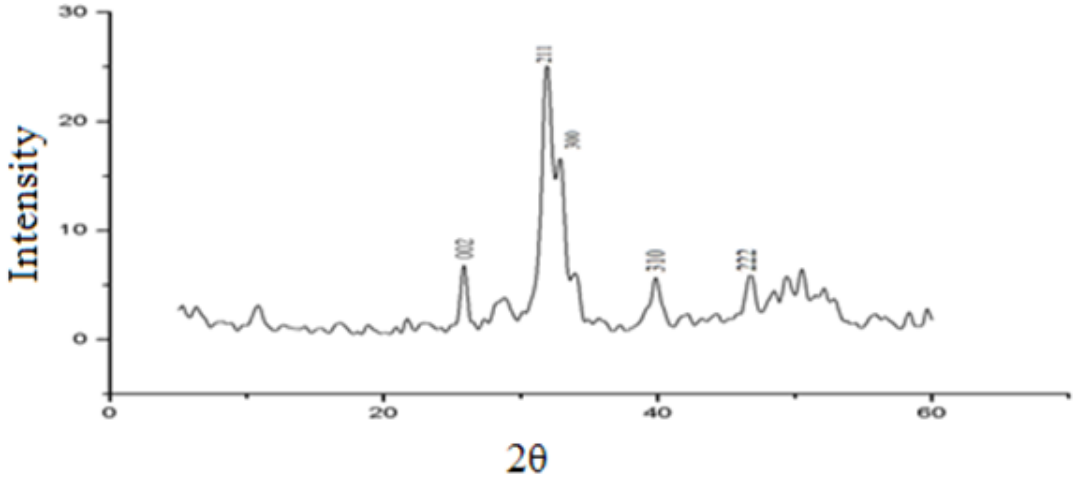

Fig 15: Diagram of X-ray diffraction of a sample bone of a rat implanted PP calcined at 600°C

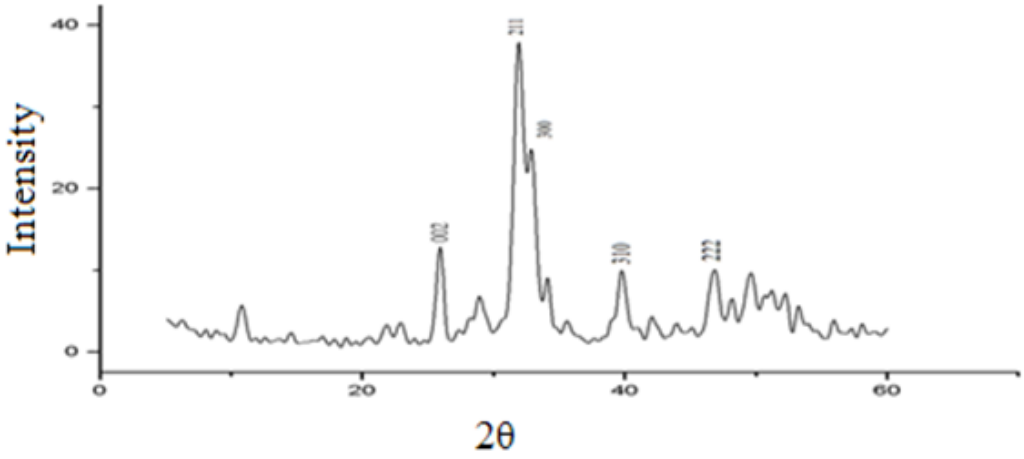

Fig 16: Diagram of X-ray diffraction of a sample bone of a rat implanted PPS calcined at 600°C

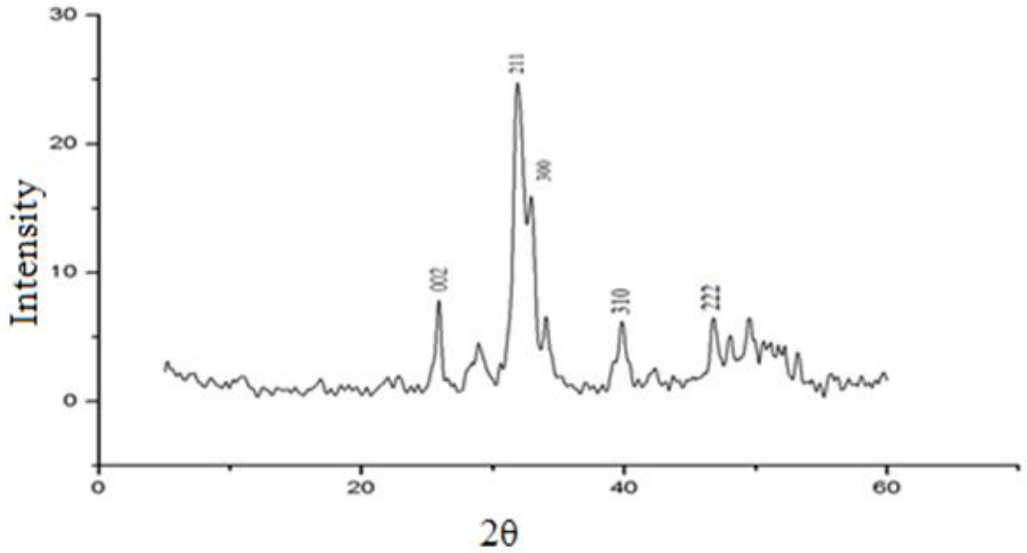

Fig 17: Diagram of X-ray diffraction of a sample bone of a rat implanted by Cap calcined at 600°C

3.2.2.2.The Infrared spectroscopy

The study by infrared spectroscopy shows that the spectra of bone samples calcined up to 600○C have an apatite structure. Indeed, these spectra show also the existence, in addition to vibration bands related groups PO4 3 -, vibration modes bundling CO3 2 - observed at 1462.99, 1417.36 and 872.18 cm-1. In addition, we note the presence of two modes us and ul related to OH- group and are observed respectively at 630.13 and 3569.23 cm-1. It should be noted the presence of even the IR spectrum of a single band observed at 3439.38 cm-1 is attributed to the vibration mode ul OH- ions adjacent the carbonate group CO3 2 -. Hence, the existence of a small quantity of carbonate type A. We also note that spectra of figures 18-21 of the bones of rats implanted groups showed the same except that their different modes of vibration. This proves that the mechanisms of the exchanges between the biomaterial used and the bone is totally normal and natural.

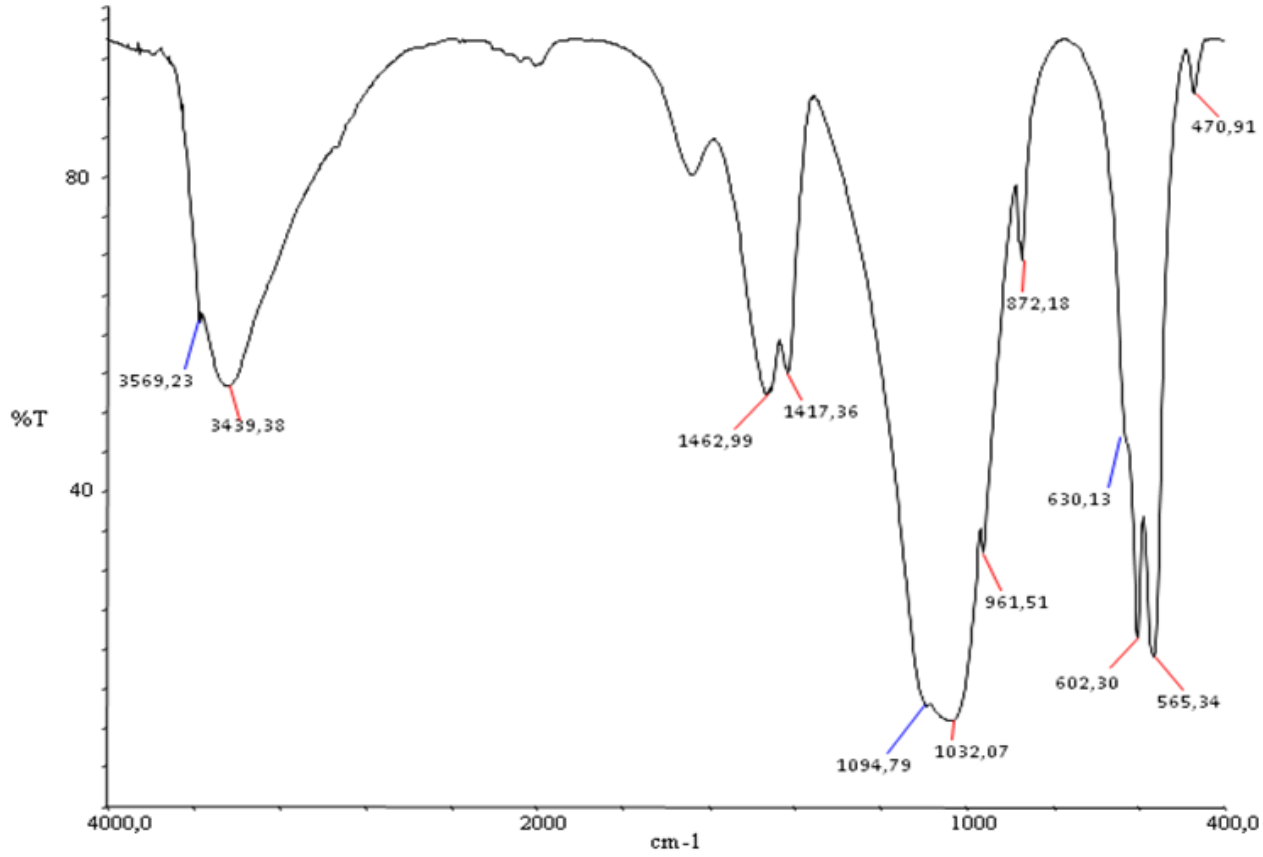

Fig18: IR spectrum of rat bone in a non-implanted (C)

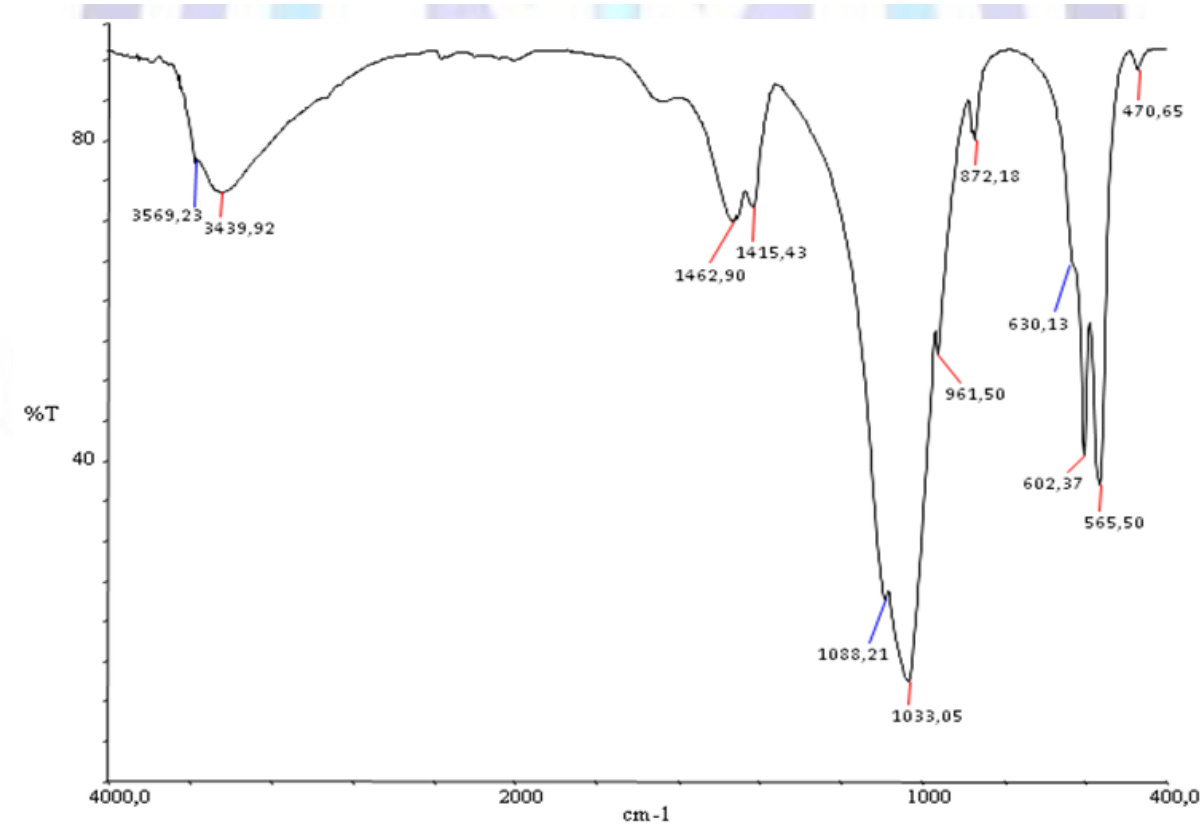

Fig 19: IR spectrum of bone in a rat implanted by PP

ISSN 2321-807X

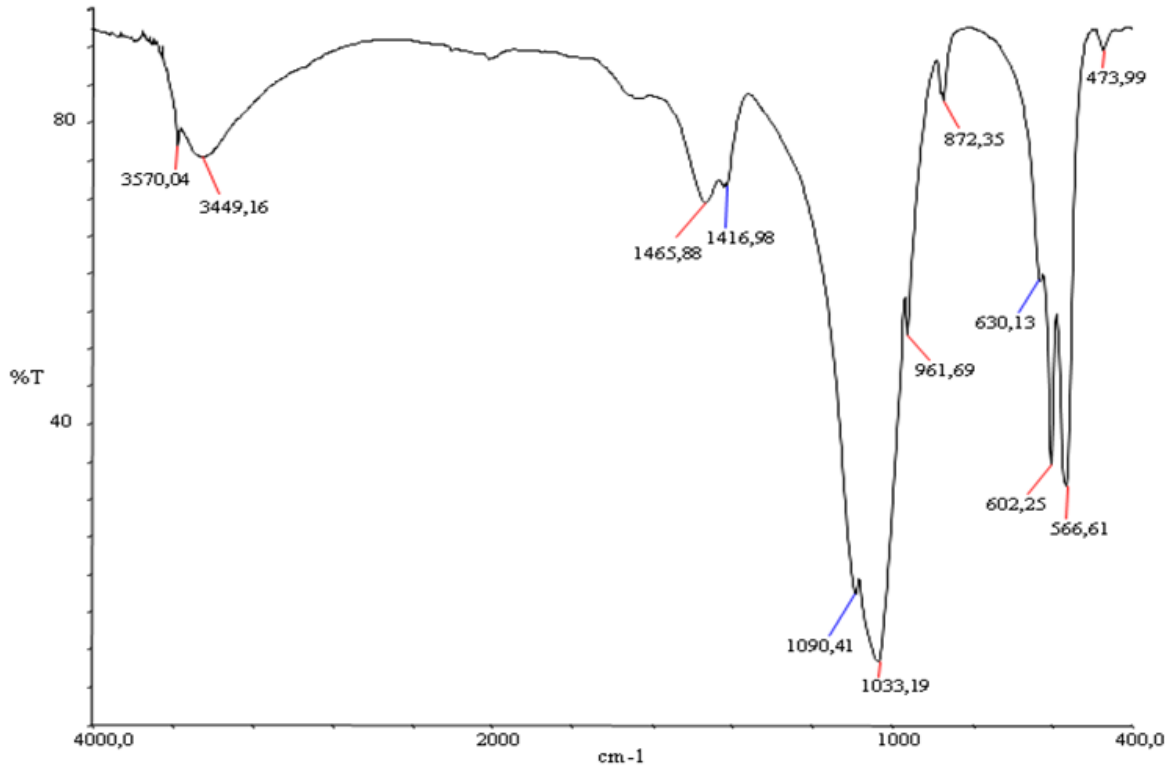

Fig 20: IR spectrum of bone in a rat implanted by PPS

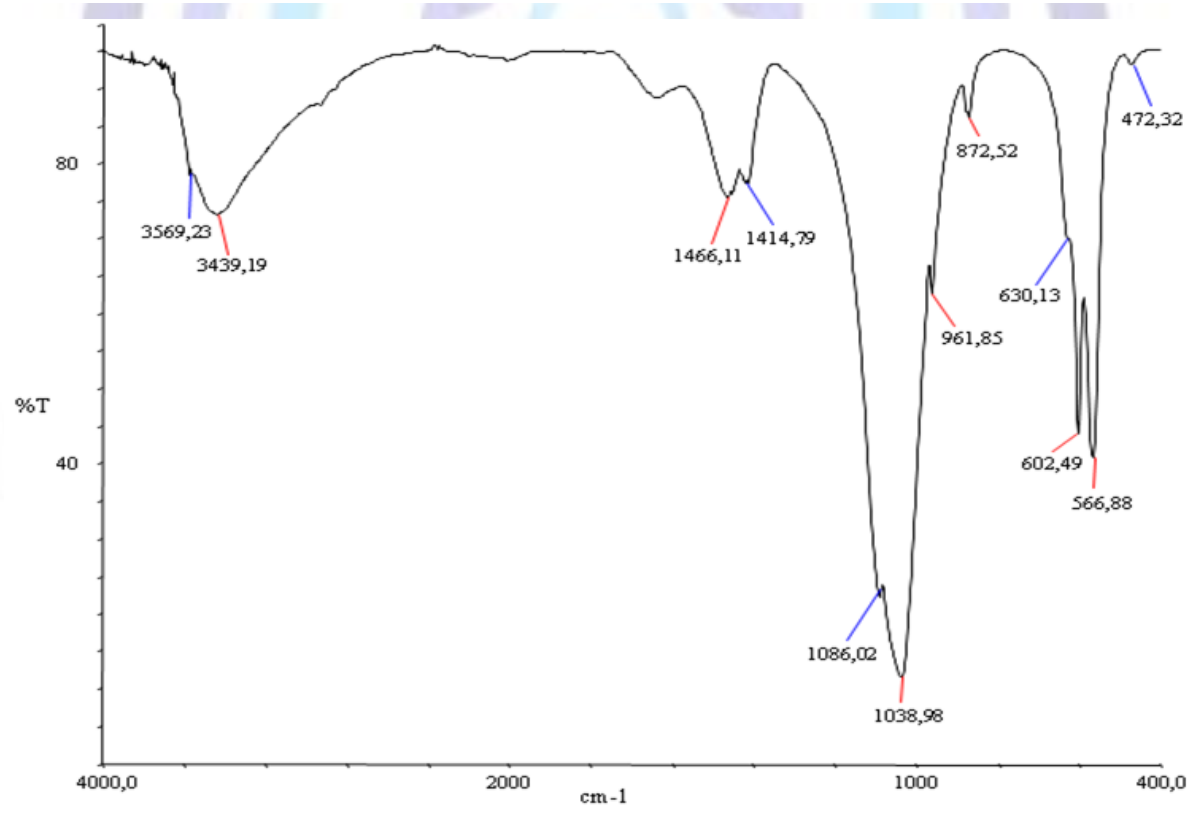

Fig 21: IR spectrum of bone in a rat implanted by Cap

4. Discussion

The vast majority of epidemiological studies indicate that there is a strong association between alteration of the antioxidant defenses and increased markers of oxidation [24]. Indeed, the conduct of various physiological mechanisms are still not generators of a state of oxidative stress that when the production of free radicals is important. Our results on the oxidative stress show that the stress is not appeared in implanted rats by a capsule apatite and without any significant change in the concentration of hepatic and renal of markers of lipidic peroxidation (TBARS and CD) and activities of SOD, GPx and Cat. The fact, we conclude that our implantation technique by the capsule (Cap) has no deleterious effect on the body by inducing lipid peroxidation processes in acid polyunsaturated fatty acids of membrane lipoproteins. It shows thus that although there free radical production at the beginning of the treatment period or postoperative period, the rats of group Cap, by their antioxidant reserves, were able to fight and finally return their status homeostatsique. We have shown in previous work [25] that the HA does not induce an imbalance of the oxidative balance. Our study shows that the HAC pellet form PP and PPS implanted subcutaneous ventral has stressful effects to the body. In addition, previous work [9], showed that the use of HA in the form of pellet, such as bone grafting, may increase the stability of the implant, lead to accelerate bone regeneration and facilitate the healing process without problems immunogenic. However, the efficacy of biomaterial depends on one hand of its physico-chemical and biological and on second hand its mechanical environment where it is located.

Our results showed that the capsule is better tolerated by the body because it does not disturb the equilibrium ferric ion and calcium phosphate because the HAC powder in the capsule is more bioresorbable than the pellets due to its stability exchange ionic.

The bioresorbable phenomenon is dependent on the physico-chemical characteristics of materials. This is important: It will determine the speed when the material will be degraded “in vivo” by the phenomena of spontaneous dissolution. The dissolution speed of ceramics can be modified by various factors such as crystallite size and porosity of the material [26]. In addition, our study is in agreement with the earlier work of Mallek et al. 2005 which showed that the HA is among phophocalcic biomaterials that have no negative impact on the phosphocalcic homeostasis and on the mineral composition.

In addition, the cytotoxic potential of biomaterials is considered one of the principal parameters to evaluate their biocompatibility. Our results showed that the apatite administered in capsule does not disturb liver function or renal because our biomaterial (HAC) is widely used because of its biocompatibility and bioactivity [27] in a medical field. However, the pellets cause liver and kidney alterations due to temperature and compaction because they affect the hardness, density, grain size, mesh size and porosity of our product. In addition, work [28] showed that the density of the HAC is increasing for very high sintering temperatures. In addition, other studies show that in the case of ceramics phosphocalcic microporosity is also inversely correlated with the sintering temperature: as the sintering temperature is low, the microporosity increases [29]. It has been suggested that these micropores were likely to play a role at many levels: the increasing of specific surface area materials, roughness and resorption as well as the creating of microenvironment inside these pores. These various parameters, associated with the reactivity of the material, could have an influence on the bioactivity of the material or on its adsorption capacity [30-31] All these results were confirmed by physico-chemical study (DRX and IR), which has shown that the mode of the implant capsule is better than the pellet form. In fact, according to the results of DRX, the differences of the cell volume between HAC unimplemented one hand and those implanted on the other hand showed that the Cap and the PPS have undergone more modification than in the case of PP. This shows that the physical aspect of the implant plays a very important role in ion exchange with the biological environment (El feki and al. 1991). Moreover, the IR spectra of the apatite implanted show that the capsule is the most bioactive implant, as is testified by the movement of many bands. This is probably due to the physical aspect of the implant which is a non-compacted powder. Because why we have chosen a implant for the treatment of rats is the capsule type. The diffractograms bones of rats implanted with pellets types of PP and PPS show that the volume of the mesh decreases relatively to the control. Remember that those implants PP and PPS have increased, then we can understand the scavenging effect of heavy metals in these types of apatite implants. For cons, the cell volume of apatite implant capsule administered in the same decreased bone implanted in rats of the same group decreased significantly. Which explains that the capsule thereafter passing in urine and not stocked in the kidneys because the biochemical analysis renal function (creatinine) showed no nephrotoxicity. This phenomenon was not observed in the case of groups implanted pellets type PP and PPS, this is probably due to the physical aspect of implants (compacted powders). We can be explained that by the blood circulation, which has not a relatively significant quitter effect of the heavy metals. So, this is not the case of implant apatite powder (capsule), where the departure of heavy metals is easy and will be therefore compensated by an excess migration of other metals to bone from implant. Finally, we can confirm that the capsule is considered a reliable sponge to retain the heavy metals and the impurities habitually located in living bone. These results are in good agreement with those obtained by infrared spectroscopy.

5. Conclusion

the apatite biomaterial capsule has less oxidative stress, does not disturb the balance or ferric ion balance, maintains phosphocalcic balance, and does not cause liver- kidney toxicity. By cons, other physical forms (PP and PPS) of the implant did not present these beneficial effects to the body. Also, the implantation technique with capsule presents more ion exchange in comparison with the groups of rats implanted by the same apatite biomaterial into pellets.

Acknowledgments

This research was supported by the Tunisian Ministry of Higher Education and Scientific Research and the Tunisian Ministry of Public Health.

References

[1] Ripamonti, U., Crooks, J., Khoali, L., Roden, L. 2009. The induction of bone formationby coral-derived calcium carbonate/hydroxyapatite constructs. Biomaterials. 30, 1428–1439.

[2] Gergely, G., Weber, F., Lukacs, I., Toth, A.L., Horvath, Z. E., Mihaly. J., Balazsi, C. 2010 Preparation and characterization of hydroxyapatite from eggshell. Ceramics International. 36, 803–806.

[3] Vecchio, K. S., Zhang, X., Massie, J. B., Wang, M., Kim, C. W. 2007. Conversion of bulk seashells to biocompatible hydroxyapatite for bone implants. Acta Biomaterialia. 3, 910–918.

[4] Rivera, E. M., Araiza, M., Brostow, W., Victor, M., Castano, J. R., DıazEstrada, R., Hernandez, J., Rodrıguez, R. 1999. Synthesis of hydroxyapatite from eggshells. Materials Letters. 41, 128–134.

[5] Leea, S. J., Oh, S. H. 2003. Fabrication of calcium phosphate bioceramics by using eggshell and phosphoric acid. Materials Letters. 57, 4570–4574.

[6] Balazsi, C., Weber, F., Kover, Z., Horvath, E., Nemeth, C. 2007. Preparation of calcium-phosphate bioceramics from natural resources. Journal of the European Ceramic Society. 27, 1601–1606.

[7] Tas, A. C. 2000. Synthesis of biomimetic Ca-hydroxyapatite powders at 373C in synthetic body fluids, Biomaterials 21, 1429–1438.

[8] Picart, C. 2006. Bulletin SFP. Materials transactions. 155, 11-14.

[9] Jebahi, S., Saoudi, M., Badraoui, R., Rebai, T., Oudadesse, H., Ellouz, Z., Keskes, H., El Feki, A., El Feki, H. 2012. Biologic Response to Carbonated Hydrxyapatite Associated with Orthopedic Device: Experimental Study in a Rabbit Model. The Korean Journal of Pathology. 46, 1-7.

[10] Fathi, M. H., Hanifi, A., Mortazavi, V. 2008. Preparation and bioactivity evaluation of bone-likehydroxyapatite nanopowder. journal of materials processing technology. 202, 536-542.

[11] Kim, K., Dean, D., Lu, A., Mikos, A. G., Fisher, J. P. 2011. Early osteogenic signal expression of rat bone marrow stromal cells is influenced by both hydroxyapatite nanoparticle content and initial cell seeding density in biodegradable nanocomposite scaffolds. Acta Biomaterialia. 7, 1249–1264.

[12] Shafiei-Sarvestani, Z., Oryan, A., Bighamc, A. S., Meimandi-Parizi, A. 2012. The effect of hydroxyapatite-hPRP, and coral-hPRP on bone healing in rabbits: Radiological, biomechanical, macroscopic and histopathologic evaluation. International Journal of Surgery. 10, 96-101.

[13] El feki, H., Rey, C., Vignoles, M. 1991. Carbonate Ions in Apatities : Infrared Investigations in the υ4 CO3 Domain. Journal of Calcif Tissue Int. 49, 269-274.

[14] El feki, H., Savariault, J. M., Ben salah, A., Jemal, M. 2000. Sodium and carbonate distribution in substituted calcium hydroxyapatite. Solid State Sciences. 2, 577-586.

[15] Thamaraiselvi, T. V., Rajeswari, S. 2004. Biological Evaluation of Bioceramic Materials - A Review. Trends Biomater. Artif. Organs. 18 (1), 9-17.

[16] Chris Arts, J. J., Verdonschot, N., Schreurs, B. W., Buma, P. 2006. The use of a bioresorbable nano-crystalline hydroxyapatite paste in acetabular bone impaction grafting. Biomaterials. 27, 1110–1118.

[17] Krisanapiboon, A., Buranapanitkit, B., Oungbho, K. 2006. Biocompatability of hydroxyapatite composite as a local drug delivery system. Orthopaedic Surgery. 14(3), 315-8.

[18] Buege, J. A., Auct, S. D. 1984. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

[19] Kurien, B. T., scofield, R. H. 2003. Free radical medited peroxidative damage in systemic lupus erythematosus. Journal of life science. 73, 1655-1666.

[20] Marklund, S., Marklund, G. 1975. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. Eur Journal Biochem. 47, 469–474.

[21] Pagila, D. E., Valentine, W. N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine. 70, 158–169.

[22] Aebi H. 1984. Catalase in vitro. Methods Enzymol. 121-126.

[23] Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with Folin phenol reagent. Journal of Biological Chemistry. 193, 265-275.

[24] Pincemail, J., Lecomite, J., Castiauj, P. 2000. Evaluation of autoantibodes against oxidized LDL and antioxidant status in top soccer and basketball players after 4 months of competition. Free Rad Biol Med. 28, 559-565.

[25] Mallek, K., El feki, H., El feki, A. 2005. Master. Etude des impacts biologiques et physico -chimiques d'un biomatériau apatitique sur la maturation de l'os chez le rat male de souche « wistar ».

[26] Legeros, R. Z. 2002. Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthop Relat Res. 395, 81-98.

[27] Velard, F., Laurent-maquin, D., Braux, J., Guillaume, C., Bouthors, S., Jallot, E., Nedelec, J, M., Belaaouaj, A., Laquerriere, P. 2010. Effect of zinc on hydroxyapatite-mediated activation of human polymorphonuclear neutrophils: potential modulatory role in bone implant-associated acute inflammation. Biomaterials. 31.

[28] Oktar, F. N. 2007. microstructure and mechanical properties of sintered enamel hydroxyapatite. ceramics international. 33, 1309-1314.

[29] Wilson, C. E., Kruyt, M. C., De, Bruijn, J. D., Van Blitterswijk, C. A., Oner, F. C., Verbout, A. J., Dhert, W. J. 2006. A new in vivo screening model for posterior spinal bone formation: comparison of ten calcium phosphate ceramic material treatments. Biomaterials. 27 (3), 302-14.

[30] Habibovic, P., Kruyt, M. C., Juhl, M. V., Clyens, S., Martinetti, R., Dolcini, L, Theilgaard, N., Van Blitterswijk, C. A. 2008. Comparative in vivo study of six hydroxyapatite-based bone graft substitutes. Orthop Res. 26 (10), 1363-70.

[31] Zhu, X. D., Fan, H. S., Xiao, Y. M., Li, D. X., Zhang, H. J., Luxbacher, T., Zhang, X. D. 2008. Effect of surface structure on protein adsorption to biphasic calcium-phosphate ceramics in vitro and in vivo. Acta Biomaterials.