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Determination of the Levels of Some Metal Ions in the Blood, Liver and Kidney of Wistar-Albino Rat after Surgical Implantation of Stainless Steel Arch Bar

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Abstract

The study was carried out to determine the accumulated levels of the anticipated major corrosion products of stainless steel (SS) arch bar used for maxillomandibular fixation and the affinity of the corrosion products of SS arch bar for some organs, when it is implanted into the mandible of Wistar albino rat (*Rattus norvegicus*) for six weeks. Ten experimental rats with implantation and ten control rats were each sacrificed at the end of the six weeks by a 1.5 mL lethal dose of chloroform. The concentrations of Co, Cr, Fe, Mn and Ni in the blood and oven-dried kidney and liver of the two groups were determined using atomic absorption spectrophotometry. The highest mean concentration of Fe ions was accumulated in blood (0.5423 ± 0.0150 mg/kg) and least in the liver (0.4675 ± 0.0060 mg/kg). The accumulated levels of Fe ions in the blood of the experimental and control rat (0.3897 ± 0.0703 mg/kg) are statistically different at $P < 0.05$. The concentration of Ni ions in the experimental rats was statistically elevated compared to the control. The total metal ions accumulated in the blood and kidney followed the ranking Ni > Fe > Co > Mn, this ranking showed a different trend from that accumulated by the liver Fe > Ni > Mn > Co; though the percentage composition of these metals in the arch bar has the ranking Fe > Ni > Cr > Mn > Co. Ni showed a high affinity for kidney and blood of the rat, while Fe showed affinity for all the organs studied**.** Ni ions released from SS arch bar was statistically elevated in kidney and Fe ions in blood of the experimental animal model. The study affirms that SS arch wire like other medical implants corrode when bathe by bio-fluids with small amounts of metal ions being accumulated by surrounding organs.

Key words: Arch bar; Corrosion product; Organ; Affinity; Albino rat

Introduction

The most common metals or alloys in orthopaedics and dentistry are cobalt-chromium-nickel, titanium-aluminum-vanadium, commercially pure titanium and 316L stainless steel (Okazaki

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and Gotoh, 2005). Metallic implants represent a pool of trace elements in the body which by corrosion are gradually leached out and may disturb the delicate balance of trace elements in the surrounding tissue as well as, possibly, the entire organism (Michel, 1987; Tüken, 2006). The metal ions released into the surrounding tissue may be concentrated locally or distributed systemically.

Trace element analyses of specimens from hosts of implants have shown that metal ions can be localized in blood or serum (Koegel and Menchim, 1984; Faccioni *et al.,* 2003), urine and other organs (Lugowski *et al.*, 1991; Omoniyi *et al.,* 2009), as well as in bone tissue adjacent to implants (Salehi *et al.*, 2013; Wetterhahn *et al.*, 1992). Furthermore, many authors have reported increased concentrations of local and systemic trace metals in association with metal implants (Morais *et al.*, 2009; Betts *et al.,* 1992; Urban *et al.,* 2000; Olmedo *et al.,* 2003*;* Case *et al.,* 1994; Dorr *et al.,* 1990; Jacobs *et al.,* 1996; Michel *et al.,* 1991).

The medical grade stainless steel (316L or 317L) contains strongly sensitizing metals, including 13–15% of nickel, 17–19% of chromium and about 2% of molybdenum (Ramsden, 2007; Navarro, 2008). Arch bar is a stainless steel (SS) prosthesis mostly used for treatment of mandible fractures as an aid in intermaxillary fixation procedures (Carl-Peter and Michael, 2010; Andrew and Chandrasekaran, 2004). Lori *et al.* (2009) reported the release of cobalt, iron, manganese, nickel and chromium from arch bar when immersed in pseudo- and actual-biofluids. Arch bars ligated to jaw models and immersed in 0.9% saline solution for 3, 10 and 28 days released Ni, Cr, Fe, Cu, Zn and Cd at levels that were 140 - 600 times higher than those released from the solid arch bar (Torgersen and Gjerdet, 1992).

Counts *et al.* (2002) reported nickel sensitivity of the oral mucosa during the use of a transpalatal arch appliance (TPA) in patients. Using inductive coupled plasma emission spectroscopy, Rai *et al.* (2011) determined the amounts of Ni and Cr in the serum of patients implanted with orthodontic arch wire following a short term implantation; a similar

investigation was conducted on the blood of patients and the level of nickel determined (Bishara, 1993). Notwithstanding, studies have failed to confirm that patients hosting implants containing Ni are at a great risk of developing pathologic entities linked to Ni (Sutow, 2004; Eliades and Athanasiou, 2002); but a slightly higher cases of tumors have been reported (Tariq *et al.*, 1995).

Heavy metal toxicity represents an uncommon, yet clinically significant, medical condition. If unrecognised or inappropriately treated, heavy metal toxicity can result in significant morbidity and mortality. The most common heavy metals implicated in acute and/or chronic conditions include lead, arsenic and mercury (Singh and Junnarkar, 1991; Henryk, 2014; Adal and Wiener, 2013).

The issues associated with metal-ion release are: the amount of metal released from the implant, the site to which the metal is transported and the quantity that is transported, the chemical form of the released metal and the pathophysiological consequences of such metal release (Woodman *et al.*, 1984; Okazaki *et al.*, 2004).

Metal ions have specificity to accumulate in organs, for example, in rabbits Ni shows a high affinity for the kidneys, whereas Mo is selectively accumulated in the spleen (Jacobs *et al.*, 1995; Henryk, 2014). Therefore, the assertion that Ni levels in the blood of orthodontic patients are not different from those in untreated individuals cannot rule out the possibility that Ni has been accumulated in an organ (Jacobs *et al.*, 1995).

This study is aimed to assess the post-operative levels of accumulated metal ions in the blood, liver and kidney of *Rattus norvegicus* (albino rat) and the affinity pattern of the metal ions of SS arch bar for absorption by these organs, by using atomic absorption spectroscopy, six

weeks after implanting SS bar in the mandible of albino rat. The study will help to better elucidate the metal biocompatibility of SS arch bar used for maxillomandibular fixation.

Materials and Methods

Maxillomandibular implantation of stainless steel arch bar

Twenty three female albino rats of Wistar strain (178.0 ± 3.5 g body weight) of age eight weeks were used for the study. The rats obtained from the animal house of the Faculty of Pharmacy, Ahmadu Bello University, Zaria in July 2008 were fed commercial rat pellets (Bendel Feeds and Flour Mill, Ewu, Nigeria) and tap water *ad lib.* Ten rats marked E1 to E10 (experimental group) and the control group marked C1 to C10 were caged separately. Three rats marked A1 to A3 were used for anaesthesia experimentation, in order to determine the quantity of the pre-anaesthetic (to weaken) and the anaesthetic (to knock off) required for minimum recovery time of the animal after implantation.

Injection of 0.2 mL chlorpromazine (CLP), and 0.3 mL Ketamine (KTM) 15 min after, was administered to rat A1; 0.2 mL CLP and 0.2 mL KTM 15 min after, to rat A2; and 0.2 mL CLP and 0.1 mL KTM 15 min after, to rat A3.

Into each of the experimental rats anaesthetised with 0.2 mL CLP and 0.1 mL KTM; 0.6 ± 0.05 cm length of SS arch bar of average weight 75.05 ± 0.90 mg in the form 'as-received' from the manufacturer (Unitek, Monrovia, California, USA) was implanted in the lower jaw following the procedures: The skin incision was made slightly lateral to the ventral midline of the mandible from the level of the diastema to the level of the molar teeth. The flat and very thin platysma muscle was incised with the subcutaneous fascia and was then retracted with the fascia and skin. Then, dorsal retraction of the platysma and skin, exposed the body of the mandible (Plate 1). The arch bar was then implanted in-between the mandible using circlage wire to anchor it. The platysma and subcutaneous fascia were closed in one layer using 2/0 nylon as depicted by Plate 2. The positioning of the arch bar between the mandible allowed its interaction with the saliva and blood; for experimental purpose, this

would signal the influence of these bio-fluids on the leaching of metal ions from arch bar, and the levels accumulated in the organs studied.

The surgical implantation was carried out at the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria.

Collection of biological samples pre- and post-operative

The experimental animals were bled at the tails 24 hours before the implantation, and 0.5 mL of blood samples collected with a 2 mL syringe. At the end of six weeks of implantation, the experimental and control animals were sacrificed by a 1.5 mL lethal dose of chloroform. The kidney and liver obtained from the two rat groups were oven-dried at 55^{o}C for 48 h, in order to obtain constant weight. Two millilitres of blood samples was collected from each animal group, post-operative. The six weeks implantation period simulates the healing period after maxillomandibular surgery.

The experiment was conducted in accordance with international ethics on animal use and care. Also, all the animals received humane care according to the European Convention on Animal Care.

Digestion of the biological samples

Samples of the dried organs (1.0 g) were each pulverized with an agate mortar and pestle, following which 3 mL of concentrated HNO_3 and 10 mL of concentrated HCl solution was added on a hot plate at 90^{o}C. The digest was then filtered through a Whatman no. 1 filter paper and made up to 25 mL mark in a volumetric flask with doubly distilled water.

Each of the 0.5 mL blood samples was digested with 0.15 mL of concentrated HNO_3 at 80^{o}C for 8 h in a hot air oven; drops of water were added to prevent drying. On the other hand, elemental composition of the arch bar was conducted by the dissolution of 0.20 g of the metallic sample in a beaker containing 3 mL of concentrated HNO_3 and 10 mL of concentrated HCl solution on a hot plate adjusted to 90^{o}C. The solutions were each diluted to 25 mL in a volumetric flask.

Methodology of utilising the atomic absorption spectrophotometer

The amounts of Co, Cr, Mn and Fe in the diluted solutions were determined using atomic absorption spectrophotometer (Varian AA240FS) at the Multi-user Laboratory, Ahmadu Bello University, Zaria, Nigeria. Each of the samples was aspirated into a flame and the sample element converted to vapour.

For each metal a beam of electromagnetic radiation from a specific hollow cathode lamp (Co- 240.7 nm; Cr- 357.9 nm; Fe- 248.3 nm; Mn- 279.5 nm; Ni- 232.0 nm) passed through the vapourised sample. Each metal absorbed radiation that is directly proportional to the concentration of the atomic vapour in the flame (analyte in the sample); the absorbance is measured and converted to concentration using the calibration curve. All measurements were taken in triplicate, the quality assurance for the analyses was conducted through the spiking method, and mean % recovery for the analyses determined.

Statistical Analysis

Duncan's Post-hoc multiple range test (DMRT) was used to assess the statistical difference in the mean values of the various metal ions accumulated by each of the organs, and to evaluate the difference between the amount of a metal ion accumulated by an organ in the experimental compared to the control group (two-way ANOVA, $P < 0.05$).

Plate1: Maxillomandibular fixation of stainless steel arch bar into an albino rat

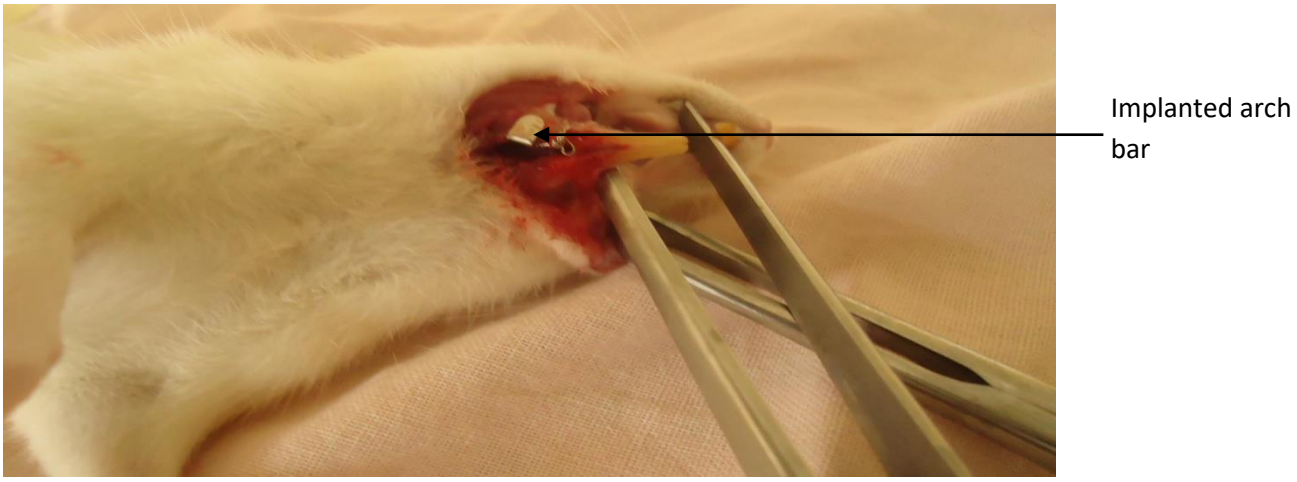
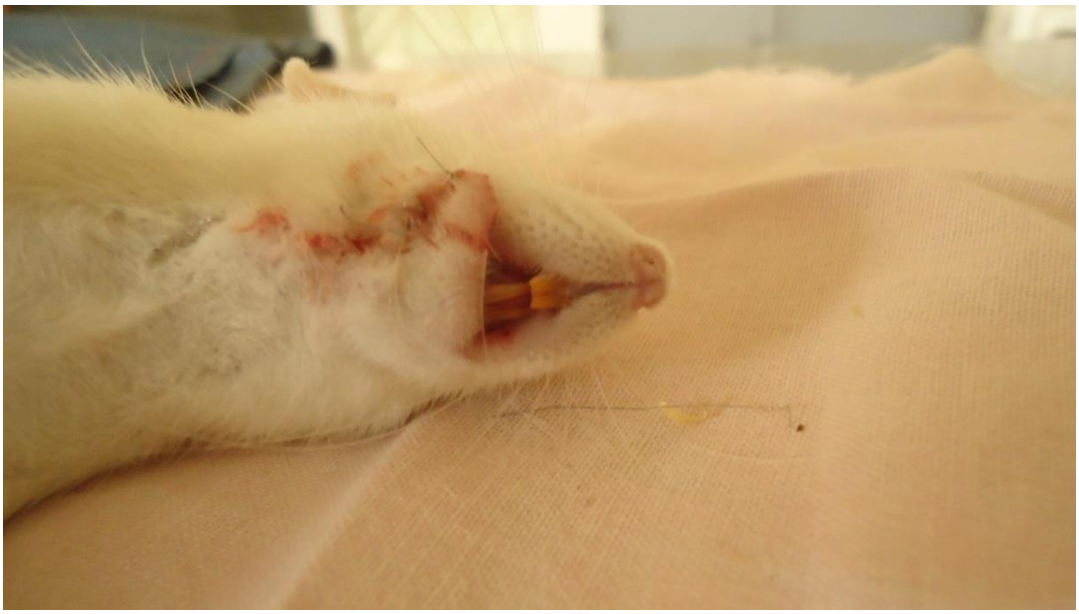


Plate 2: Closure of the platysma and subcutaneous fascia using 2/0 nylon



Result and Discussion

The spiking procedure indicated that the mean percentage recovery for the metals ranged from 81.9 ± 0.15 to 94.9 ± 0.40.

The result indicated that the arch bar used has the following elemental composition: 60.05 % Fe, 18.35 % Cr, 18.62 % Ni, 2.94 % Mn and 0.03 % Co.

Metal ions accumulated by the organs

The minimum doses of chlorpromazine (CLP) and Ketamine (KTM) needed for recovery of the rats after the implantation of SS arch bar, was by the administration of 0.2 mL of CLP and 0.1 mL of KTM, these doses led to the recovery of the rats from anaesthesia after 7 h. As presented in Figure 1 - 3, the Cr ion was not detected by the atomic absorption spectrophotometer in the other organs, except the kidney of the experimental rats, which recorded a mean value of 0.0119 ± 0.0010 mg/kg (Figure 1). This can be accounted for by the fact that a small amount (about 0.4 - 2.1%) of ingested Cr pass through the intestine and enter the bloodstream (Merrit and Brown, 1996; O'flaherty *et al.*, 2000).

As presented in Figure 2 and 3, for the experimental group, the highest mean level of Co ions was in blood (0.0387 ± 0.0090 mg/kg) and the least in liver (0.0037 ± 0.0030 mg/kg). There was a significant decrease in the amount of Co ions measured in liver compared to the other organs considered. The experimental group had mean level of Co ions in blood being about one-and-half fold that in the control (0.0211 ± 0.0043 mg/kg), there was significant elevation in the level of Co ions in the blood of the experimental group compared to the control. The highest mean concentration of Fe ions accumulated was in blood (0.5423 ± 0.0150 mg/kg) and the least in liver (0.4675 ± 0.0060 mg/kg); there was no statistical difference in the levels of Fe ions accumulated in the three organs investigated.

Following from Figure 3, DMRT indicated that the accumulated levels of Fe ions in the blood of the experimental and control (0.3897 ± 0.0703 mg/kg) are statistically different at $P < 0.05$. For Mn ion, the highest mean concentration was in kidney (0.0310 ± 0. 0.0009 mg/kg) and the least was in liver (0.0171 ± 0.0010 mg/kg), the mean concentration of Mn ions in kidney and blood was found to be significantly elevated compared to the levels accumulated in liver ($P < 0.05$). However, the mean level of Mn ions in the kidney of the control group was higher than the mean value in the

experimental counterpart; this could be connected to the physiological metabolism of manganese in the animal model.

The accumulated amount of Ni ions was highest in blood (0.8764 ± 0.0070 mg/kg) and closely followed by kidney (0.8280 ± 0.0090 mg/kg), the least amount was found in liver (0.0821 ± 0.004 mg/kg). The ranking of the total amount of metal ions accumulated by the organs investigated in the study are in the order: Ni > Fe > Mn > Co > Cr.

Duncan's grouping (two-way) that considered the reservoir organs and types of metal ions as discriminating variables, indicated that, the amount of mean Ni ions accumulated in kidney of the experimental rats was significant elevated compared to the other metal ions investigated.

This report showed that, the total concentrations of Fe and Ni ions accumulated in the organs and blood are significantly different from the concentrations of the other metals investigated (DMRT).

The findings show that about 60% of the total metal ions accumulated in blood was Ni, Fe constituted about 25% and Co about 1.5% in the blood; these are close in values to that in kidney which are, 58% for Ni, Fe 36% and Co 2%. In the liver, 14% of the total metal ions accumulated was Ni, Fe constituted about 80% while Co was about 0.7%. The total metal ions accumulated in the blood and kidney follow the ranking Ni > Fe > Co > Mn, this ranking showed a different trend from that accumulated by the liver, Fe > Ni > Mn > Co; the percentage composition of these metals in the arch bar is in the ranking Fe > Ni > Cr > Mn > Co; though the extremely low levels of accumulated Cr ions in all the organs could not be immediately understood, but might be attributed to physiological processes responsible for the depuration of Cr ions by the animal.

Following the implantation of SS arch in the rats, the study indicated that the amount of metal ions released from the arch bar led to statistical increase in the level of Ni ions in the kidney and Fe ions in blood of the experimental animal model.

The metallic composition of the arch bar being presented above did not correlate with the accumulated levels of the various corrosion products in blood, liver and kidney. This agrees with the

assertion of Williams and Roaf (1973), Kong *et al.* (2002) and that by Katharine and Stanley (1985) that tissues retrieved from the site of implants revealed that metal is present, but analysis of the amount of corrosion of the implant and amount of metal in the tissue does not reveal a strong correlation; also the composition of the alloy and the composition of the metal in the tissue often does not always correlate, like the report of Morais *et al.* 2009.

However, the report of Smith and Black (1985) differs, when Type 316L stainless steel were implanted into laboratory rabbits, it was indicated that levels of circulating iron and chromium in the blood-transport compartment reflect the implanted surface area of Type 316L stainless steel. A similar positive correlation was found between implanted Type 316L stainless steel surface area and liver (reticuloendothelial) accumulations of iron and chromium. The kidney demonstrated no constituent element accumulations (Smith and Black, 1985).

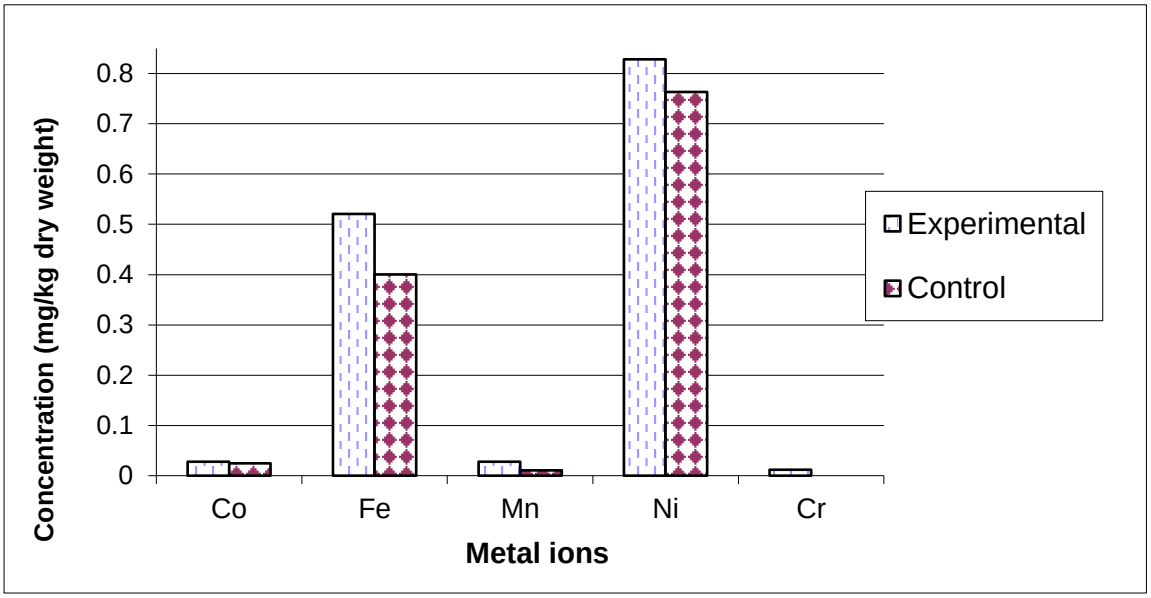


Fig 1: Concentration of Metal Ions in Kidney of Albino Rat (mg/kg Dry Weight)

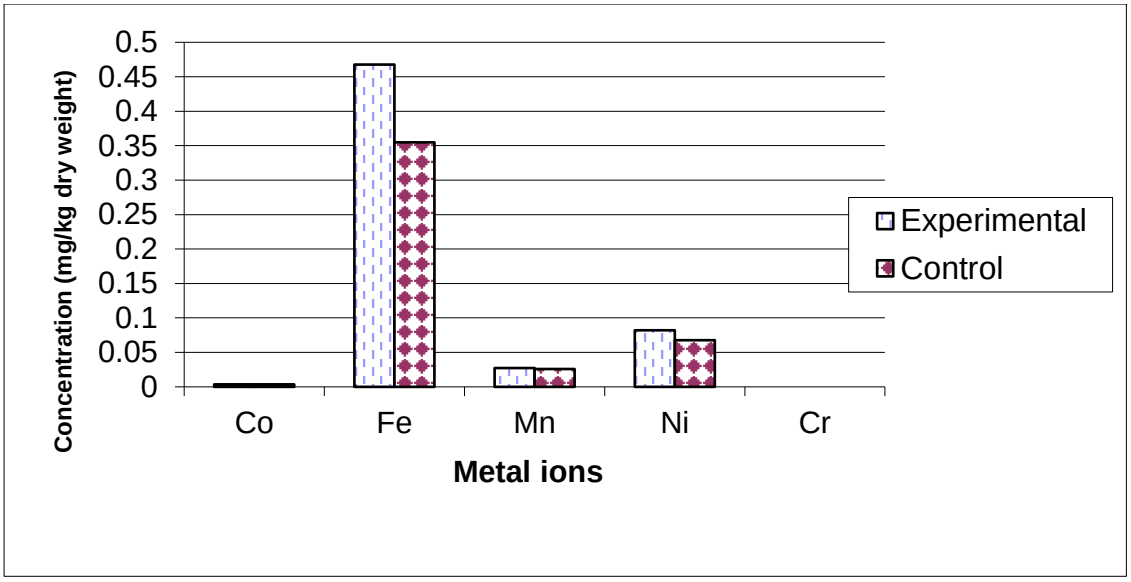


Fig 2: Concentration of Metal Ions in Liver of Albino Rat (mg/kg Dry Weight)

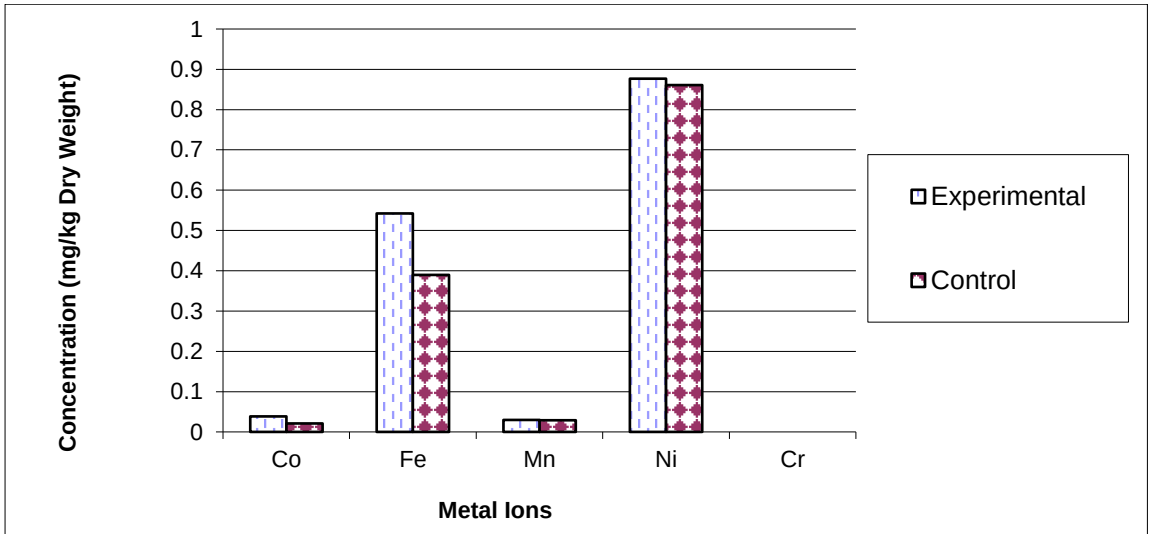


Fig 3: Concentration of Metal Ions in Blood of Albino Rat (mg/kg Dry Weight)

Since clinical studies of metal toxicity in host of metallic implants are mainly from sampling blood and urine specimens. The level of accumulated Ni in blood is indicative of that in kidney; while accumulated Fe in blood is indicative of the levels in liver and kidney of the animal model. Therefore, it can be inferred that, for Wistar albino rat, Ni showed a high affinity for the kidneys and blood; while Fe has affinity for all the organs studied.

Conclusion

Conclusively, the results inform that kidney, blood and liver are some of the reservoirs for the corrosion products of stainless steel arch bar. Since, the levels of accumulation of these anticipated major corrosion products of SS arch bar (Ni and Fe ions) in the distant organs investigated were in low levels compared to the control animal; this suggests that there is no potential physiological adverse consequence on the host when SS arch bar is implanted.

However, cautious considerations should be taken due to elevation in the levels of leached metal ions through this route especially for restorations with consecutively re-used arch bars; as SS arch bar are consecutively re-used for economic reason.

Although, the metal ion levels from most reports in human studies appear to be in the range that can be handled by the body's compensatory mechanisms. Corrosion of metallic implants remains a serious clinical concern, as deleterious corrosion processes have been observed in certain clinical settings. Therefore, research efforts should be directed towards inhibition of the corrosion processes that come to play when metallic implants contact bio-fluids.

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