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Enhanced Removal of Heavy Metals and SOD-1 Rises by Chronic Nutraceutical Supplementation in Patients with Long-Term Dental Amalgams

José Joaquín Merino[1], José María Parmigiani-Izquierdo[2], María Eugenia Cabaña-Muñoz[3]

[1]Scientific Assessor of CIROM (Murcia)

[2, 3]Clínica CIROM. Centro de Rehabilitación Oral Multidisciplinaria, Murcia, Spain

Short title: Heavy metal detoxification by nutraceuticals in patients with long-term dental amalgams

Abstract: ***Introduction: The accumulations of heavy metal/s in human body is a serious health problem. Aim: we have evaluated whether 80 days of chronic supplementation with several nutraceuticals (selenium, silimarine, L-Glutamine, L-Glutathione, Cordiceps among others) could prevent heavy metal accumulation in patients with long-term amalgams (n=17, average: 5 dental amalgams for 15 years). These selected patients have long-term dental amalgams for at least 10 years (average: 15 years) and they had five amalgams (average). In addition, we have evaluated whether 80 days of nutraceutical supplementation (day 80) may prevent certain oligoelement/s deficency found among patients with long-term dental amalgams as compare their basal levels (day zero, d0: controls before any supplementation). Material and methods: we have compared a plethora of hair's heavy metals/oligoelements among patients with long-term dental amalgams after 80 days of nutritional supplementation (n=17, day 80 (d80), μg/g hair by ICP-MS) as compare their basal levels before any supplementation (day zero, n=17). The mean heavy metals/oligoelements +- S.E.M are shown as control percentage before any supplementation (day 0, controls are 100 % of value). Results: The chronic supplementation with nutraceuticals for 80 consecutive days rised salivary SOD-1 and increased GSH (reduced form) levels and also prevented heavy metal accumulation; in fact, their Hg^{++}, Ag, Sn, Cu^{++}, Al, Pb) levels were significantly reduced after 80 days of supplementation among patients with long-term dental amalgams (average: 5) as compare their basal levels (before any supplementation at day 0). In addition, Sodium (Na^{+}) levels decreased, Boron and Lithium increased after 80 days of supplementation as compare their respective basal levels. Conclusion: These salivary GSH and SOD-1 activity rised could enhance heavy metal detoxification after 80 days of supplementation with nutraceuticals (silimarine, selenium (Se), L-Glutamine, Cordiceps) in these patients. This is the first clinical study in which phytonaturals enhanced heavy metal/s removal and also rised endogenous antioxidant systems (SOD-1 activity or GSH levels) among patients with long-term dental amalgams (five).***

Keywords: Glutathione (GSH); SOD-1; oxidative stress; antioxidants; heavy metals; mercury; oligoelements; Selenium; neurotoxicity; dental clinic; dental amalgam; odontology.

1. Introduction

The toxicity of heavy metal/s is a serious risk for human's health because the relationship between the incidence of metabolic disorders and heavy metal exposure have been reported in humans (i.e.: cardiovascular problems, hypertension) [1-3]; thus, clinical and nontoxic protocols by using nutraceuticals may chelate heavy metals and prevent its detrimental effects in the body a consequence of long-term exposure [4]. Mercury can be released by industrial/anthropogenic human activities, which contribute to environmental contamination [6, 7].

Dental amalgams are the mean chronic contributor to mercury body burden and the safeties of dental amalgams have been questioned in several epidemiological studies [7]. The mercury rises found in the cord blood correlates with the number of maternal amalgam fillings present in women; there was a relationship with the passed time since the last filling replacement [8]. The toxicity of mercury depends on its source (inorganic or elemental form) [9, 10]. Several heavy metals and oligoelements are detected in human samples by inductively coupled plasma-mass spectrometry (ICP-MS), including hair [11, 12].

The chronic exposure to mercury may cause cognitive problems and neurodevelopmental disorders in rodent models and also among patients (autism spectrum disorders, attention-deficit hyperactivity disorder, cerebellar motor problems) [13, 14] [15]. The free radical production (ROS) is enhanced by heavy metals, which can interfere with Super Oxide-Dismutase-1 (SOD-1) enzymatic activity and/or could decrease metallothionein levels [16]. The oligoelement Selenium (Se) is also necessary to remove heavy metals by inducing conjugation with glutathione (GSH). Se is a cofactor for Glutathion Peroxidase (Gpx activity) enzyme, which recycle reduced Glutathione levels (GSH) [17]. The Glutathione S-transferase is a Selenium-dependent enzyme [18].

In addition, Selenium (Se) levels correlates with urinary Se concentration [19]. Other boil trace elements as magnesium ($Mg^{++}$and Zn^{++}) play a role in many enzymatic reactions [20]. In particular, the irreversible incorporation of certain metals into hair has been proposed as an excretion route for certain metals [21]. It is known that autistic children have quite low concentrations of glutathione, which are necessary for the normal excretion processes [22]. Thus, the chronic supplementation with selenium (Se) could remove excessive heavy metals/oligoelements in patients [23]. Several heavy metals as lead (Pb), Mercury (Hg^{++}), Cadmium (Cd), Arsenic (As), Nickel (Ni), Copper (Cu^{++}), Iron (Fe^{++}), Chromium (Cr), Cobalt (Co), Aluminium (Al) are also detected in cosmetic products (colour cosmetics, face and

body care products, hair cosmetics, herbal cosmetics, etc.) [24]. These metals could induce dermatitis/allergic contact by absorption through the skin into the blood. The prevalence of Sarcopenia has been associated with elevated blood lead and mercury levels in the elderly population [1].

The Third NHANES III USA (Health and Nutrition Examination Survey) have reported alterations on bone formation and risk of osteoporosis among humans with elevated lead (Pb) levels [25, 26]; thus, the clinical use of certain nutraceuticals could detoxificate heavy metals by preventing its accumulation into distal organs in the body. **We have found decreased Pb and Hg levels after 80 days of nutraceutical supplementation among patients with long-term dental amalgams. These feature highlight that certain nutraceuticals can chelate heavy metals.** These findings are also supported by increased SOD-1 activity and GSH rised (reduced form) found in these patients, which contribute to detoxificate heavy metal levels; In fact, levels of mercury were significantly reduced after 80 days of chronic supplementation among patients with long-term dental amalgams as compare their respective basal levels (before any supplementation). These nutraceuticals can chelate heavy metal, which is important at the clinical level independently of the mechanism/s by which promote detoxification in patients with long-term dental amalgams (average: 5 amalgams for 15 years).

Aim

- We have evaluated whether 80 days of nutraceutical supplementation with several phytonaturals [*Cephalosporium micelle*, L-Glutamine, silimarine, Selenium (Se) among others] could reduce heavy metal levels and/or activate SOD-1 and/or rise Glutathione levels (GSH reduced form) among patients with long-term dental amalgams (average: 5 dental amalgams for 15 years in mouth).
- We have compared several hair's heavy metals/oligoelement levels measured by ICP-MS after 80 days of nutraceutical supplementation in patients who had long-term dental amalgams (number: 5, average: 15 years) as compare their own basal levels (before any supplementation, controls at day cero, day 0).

The dosage of Glutathione Complex formulation (Celsus Lab, Spain) contains several nutraceuticals (mg/day or µg/day depending of each component). These 17 selected patients who had long-term dental amalgams (at least 10 years) were supplemented (oral way) for 80 consecutive days with this formulation; the Glutathione complex formulation contains these bioactive nutraceuticals:

Glutathione Complex Formulation each 3 capsules (GC: Celsus Lab, Spain):
Cephalosporium mmycella: 400 mg (7 % manitol)
N acetyl cysteine (NAC): 350 mg
AlphaLipoic acid: 200 mg
L-glutamine: 200 mg
Calcium ascorbate: 200 mg
Silybum marianum extract: 150 mg (80 % Silimarine)
Dry extract of *Asian Centella*, 3 % of Asiaticosides: 150 mg
Acetil L-carnitine: 150 mg
Dimetil-glicine: 120 mg
L-Gluthatione (GSH): 100 mg
SAMs (s Adenosyl L Methionine): 100 mg
Dry extract of Brecol (*Brassica oleracea*): 50mg, 0,3 % of sulforaphane
Vitamine E (mixure of tocopherols): 60 mg
Vitamine C: 163 mg
Zinc Citrate: 15 mg
Riboflavine: 15 mg
B6 Vitamine (P-5-P): 9,5 mg
Tiamine: 1, 5 mg
Folic acid: 400 µg
Selenium (Sodium selenium): 105 µg
Moligdene (Amonium moligdate): 97, 2µg
B12 Vitamine (Cianocobalamine): 33 µg

2. Materials and Methods

All enrolled patients have been properly instructed and they consent to participate by signing the appropriate informed consent paperwork according to Helsinki Declaration (1974 and updates 2008). All efforts have been made to protect patient privacy and anonymity. The CIROM Clinic has been approved and certificated by AENOR Agency (Spain: CERTIFICATE for dentist and research services: CD-2014-001 number; ER-0569/2014 following UNE-EN ISO 9001: 2008 as well as UNE 179001-2001 Directive from Spain). All dental amalgams were progressively removed each 20 days and replaced by composites (bisphenol-A free) following a safe extraction protocol with nasal filters (active carbon,InspiraHealth) together nutraceutics supplementation [27]; we have progressively replaced these dental amalgams by quadrant during each session (20 days). There are four dental quadrants in the mouth. Thus, most of patients still have at least some dental amalgam/s during 60 days before its complete removal. These 60 days are included within these 80 days of nutritional supplementation. All amalgams have been replaced by composite at the time of collecting hair samples (80 consecutive days of Glutathione Complex supplementation, GC). They have long-term dental amalgams for at least 10 years (average: 15 years) and their average number were five 5 (minimal: 2, maximal: 8)

We have compared levels of a plethora of hair's heavy metals/oligoelements in patients with long-term dental amalgams (n=17). These metals/oligoelements were measured by ICP-MS and expressed as mean µg/of hair +- S.E.M (Standard Error Medium) after 80 days of nutraceutical supplementation (AFTER: day 80). Their heavy metal levels found after 80 days of supplementation (d80) were expressed as control percentage, which are 100 % (controls: before any supplementation at day zero, n=17, d0). We have compared several heavy metals: [µg/g of hair: Al, Hg^{++}, Ba, Ag, Sb, As, Be, Bi, Cd, Pb, Pt, Tl, Th, U, Ni, Sn, Ti] as well as a plethora of oligoelements measured by ICP-MS [µg/g of hair: $Ca^{++,}$ Mg^{++}, Na^{+}, K^{+}, Cu^{++}, Zn^{++}, Mn^{++}, Cr, V, Mo, B, I, P, Se, Sr, P, Co, Fe^{++}, Ge, Rb, Zr] at both times (day 80 and basal control levels at day 0).

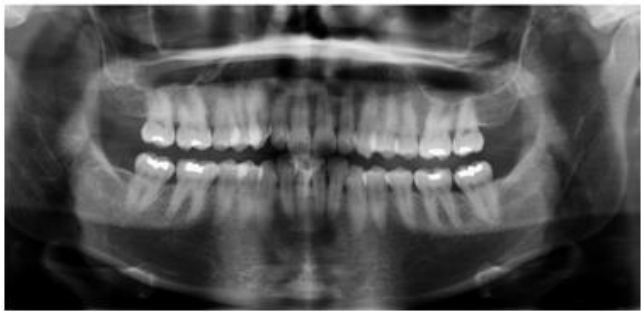
Statistical analysis
All statistical analysis were performed by SPSS-15 version and/or Sigma Plot v-11. The Mann-Withney analysis were used for nonparametric data evaluation and t-Student in case of homogeneity of variance. The homogeneity of variance

was confirmed by Levene test. Statistical significance was accepted if p<0, 05.

***Inclusion criteria*:** we have recruited patients who have long-term dental amalgams for at least 10 years in their mouth (average: 15 years). The average number of dental amalgams was five 5 (min: 2, max: 8 dental amalgams). They have visited a dental clinical for rutinary evaluation; their hair's basal levels were compared after 80 days of nutraceutical supplementation (day 80) as compare their respective basal levels at day zero (d0: before any supplementation). Their average age is 48 years old and they had 5 dental amalgams (average) without titanium/zirconium dental implant/s. Their age have been limited to the 35–65 years old range. The follows panoramic radiography indicated representative case of patient with long-term dental amalgams (3 or 8 dental amalgams).

3 Dental Amalgams

8 Dental amalgams

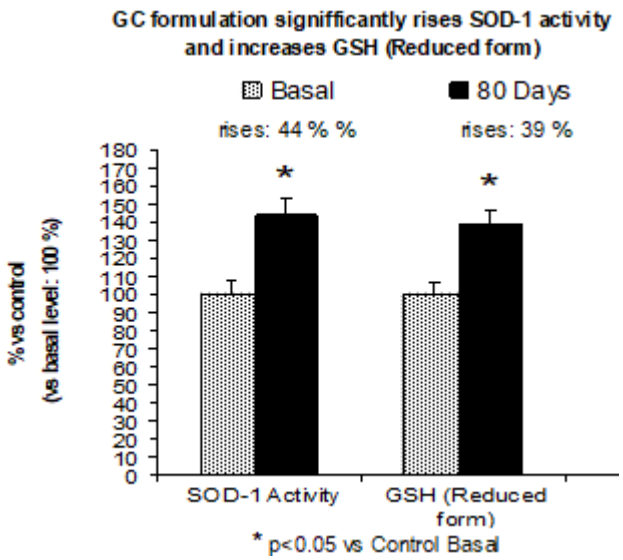


Exclusion criteria: we have excluded whose patients with behavioural problems, psychiatric disease or those taking DMSA/chelators. They have no history of liver/kidney disease/autoimmune disease/renal diseases/Cushing Syndrome/metabolic disease. They are non-smokers and they do not have periodontal disease. Patients whom consump fish over two times/week were also excluded for preventing interferences on mercury levels found in the present study.

3. Results

3.1. Figure 1. The nutritional supplementation for 80 consecutive days with Glutathione complex formulation (GC, d80) rised salivary SOD-1 activity and increased GSH levels (reduced form) as compare their respective basal levels

The chronic supplementation during 80 consecutive days (day 80) significantly rised salivary SOD-1 activity and augmented GSH (reduced form) levels as compare their respective basal levels (control at day0, p<0, 05). SOD-1 activity and reduced glutathione levels (GSH) were measured according to Cabaña-Muñoz et al., 2015 procedure [12]. These antioxidant systems were expressed as control percentage (controls before any supplementation, d0), which were 100 %. The SOD-1 activity rised 44 % and GSH augmented at 39 % after 80 consecutive days of nutraceutical supplementation with GC formulation (p<0, 05).

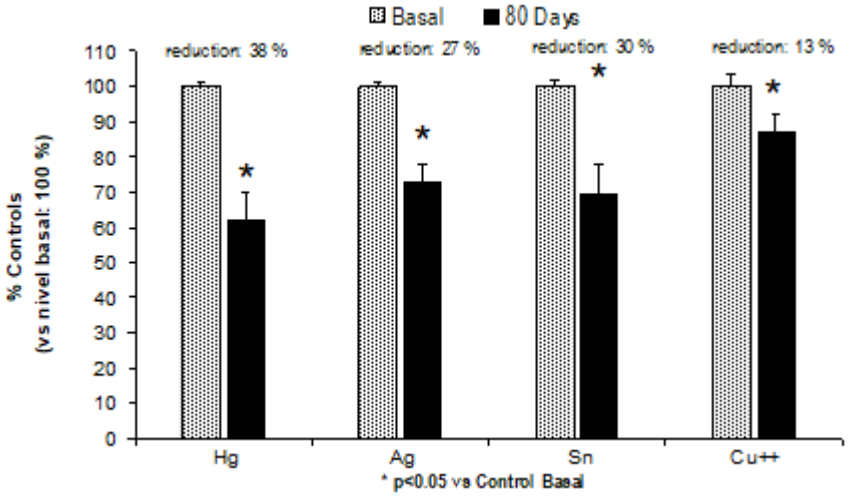


Day zero (d0: Basal level, gray color): control: 100 %, n=17).

Days 80 (black): 80 days of nutritional supplementation with GC formulation (n=17)
GS: Glutathione Complex formulation (GC).

3.2. Figure-2: Hair Mercury (Hg^{++}), Silver (Ag), Tin (Sn) and Cupper (Cu^{++}) levels were significantly decreased after 80 consecutive days of supplementation among patients with long-term dental amalgams as compare their respective basal levels (controls).

These measured heavy metal/oligoelements were expressed as control percentage +- S.E.M (Standard Error Media), which were 100 % (day zero, d0). Levels of Hg^{++}, Ag, Sn and Cu^{++} were significantly decreased after 80 days of supplementation as compare their own controls levels (before any supplementation d0, p<0,05, n=17).



All data were expressed as control percentage, which were 100 %. The specific reduction on each heavy metal/s were indicated within this figure (n=17).

3.3. Figure 3: 80 days of supplementation reduced hair's Aluminium (Al), Lead (Pb) levels (metals of environmental exposure) and also reduced Selenium (Se) levels as compare their respective basal levels (control at day cero, d0, n=17).

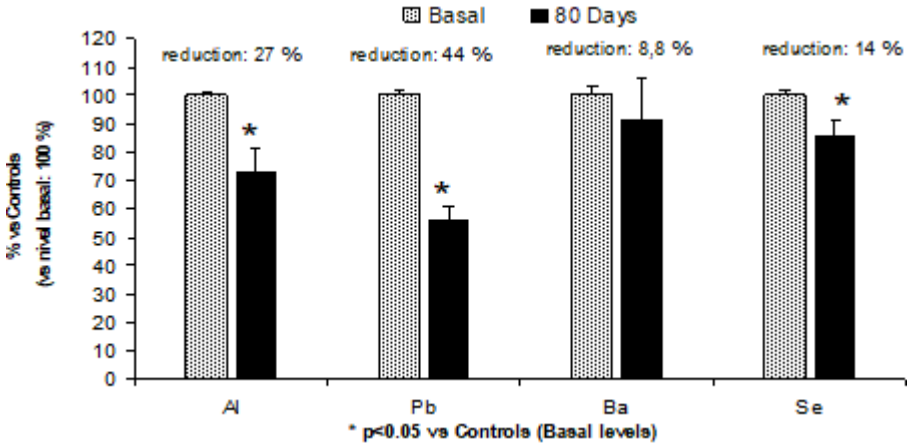
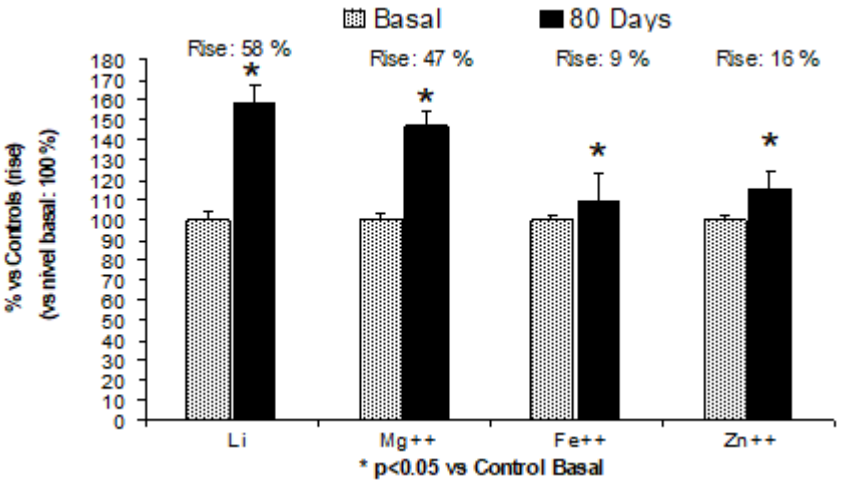
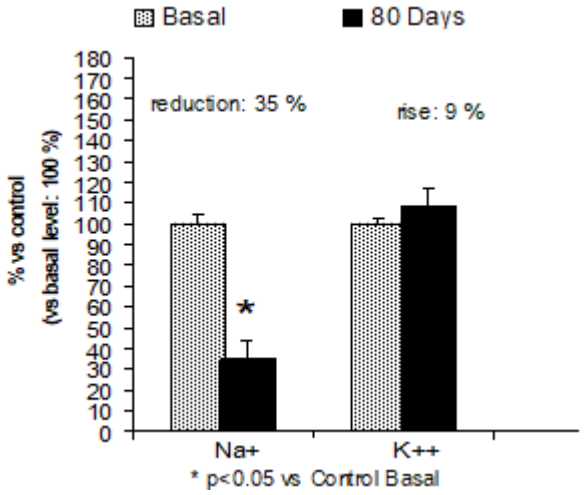


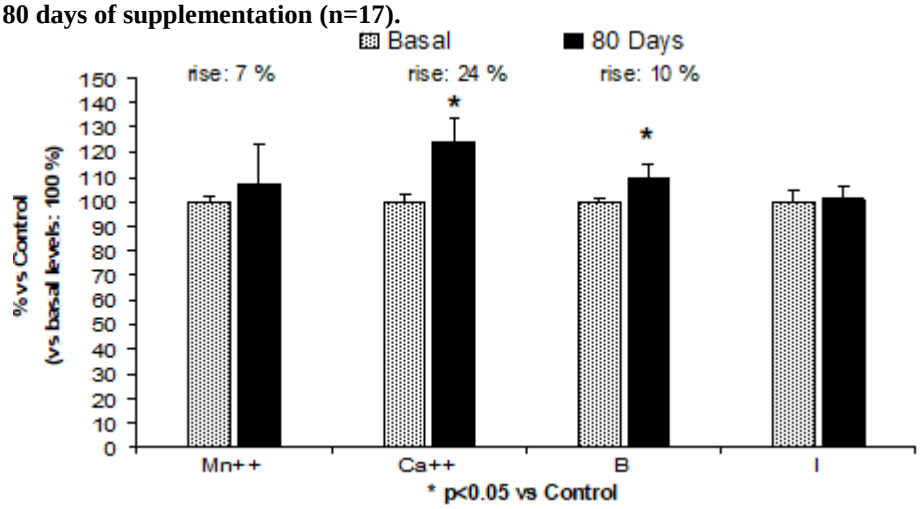
Figure 4: 80 days of supplementation (day 80) increased Lithium (Li), Magnesium (Mg^{++}), Iron (Fe^{++}) and Zinc (Zn^{++}) levels among patients with long-term dental amalgams as compare their respective basal levels (controls d0, n=17).



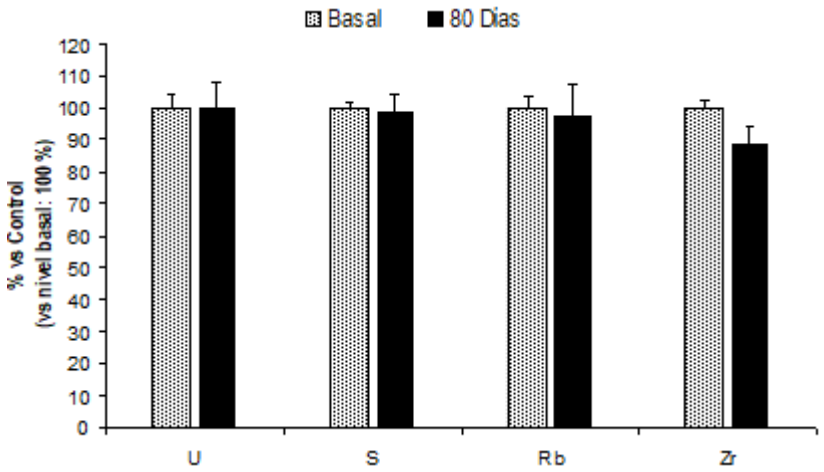
3.5. Figure 5: The supplementation for 80 consecutive days with GC formulation decreased Sodium (Na^+) levels without affecting Potassium (K^+) levels as compare their respective basal levels (controls, day0, n=17).



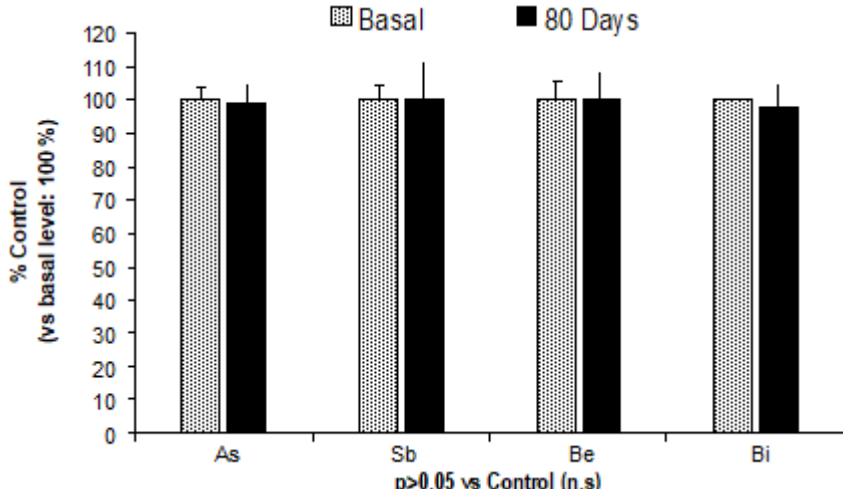
3.6. Figure-6: Boron (B) and Calcium (Ca^{++}) levels were augmented without changes on Iodine (I) or Manganese (Mn^{++}) levels after 80 days of supplementation (n=17).



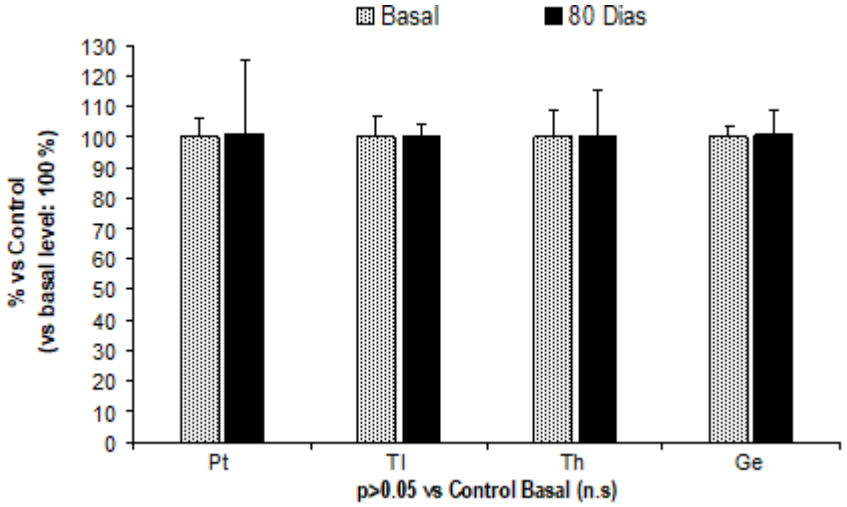
3.7. Figure-7: 80 days of nutritional supplementation did not affect Uranium (U), Sulfur (S), Rubidum (Rb) or Zirconia (Zr) levels as compare their respective basal levels (p>0, 05, n.s, n=17).



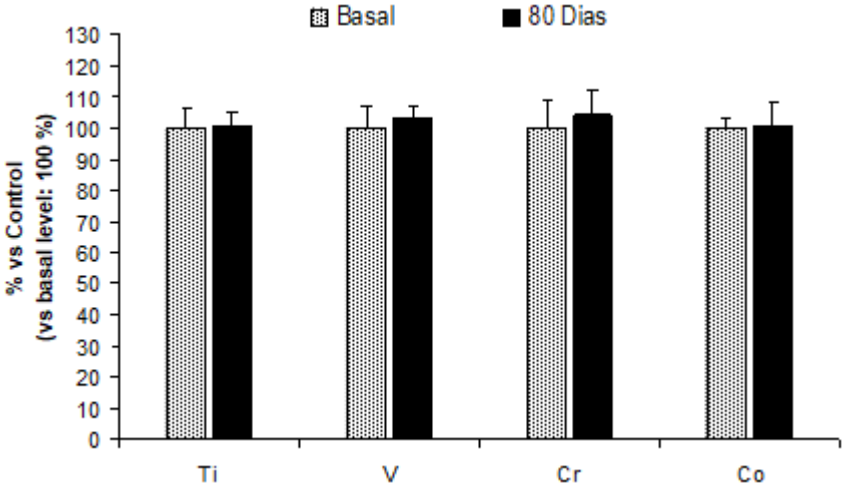
3.8. Figure-8: Other heavy metals such as As, Sb, Be, Bi did not differed after 80 days of nutraceutical supplementation as compare their respective basal levels (p>0, 05, n.s, n=17).



3.9. Figure-9: Levels of Pt, Tl, Th, Ge, Cd did not differed among patients after 80 days of supplementation as compare their basal levels (p>0, 05, n.s, n=17).



3.10. Figure-10: 80 days of chronic supplementation did not affect heavy metal levels from titanium dental alloy (Ti-6Al-4V) in these patients as compare their basal levels (p>0, 05, n.s).



These figure 2-9 show mean percentage +-S.E.M (Standard Error Media: variance/root n; n=17) for each heavy metals/oligoelement measured by ICP-MS in hair samples from patients with long-term dental amalgams (n=17); their levels were compared after 80 days of nutritional supplementation (AFTER, day 80) and compare with their respective basal levels (controls at day cero, d0, n=17). These means were expressed as control percentage, which were 100 %.

The Fig-1 indicates SOD-1 activity and GSH levels (reduced form percentage), Fig-2: (Hg^{++}, Ag, Sn, Cu^{++}), Fig-3 (Al, Pb, Ba, Se), Fig-4 (Li, Mg^{++}, Fe^{++}, Zn^{++}), Fig-5 (U, S, Rb, Zr), Fig-5 (Na^{+}, K^{+}), Fig-6 (B, Ca^{++}, I, Mn^{++}). Fig-7 (U, S, Rb, Zr), Fig-8 (As, Sb Be, Bi), Fig-9 (Pt, Tl, Th, Ge, Cd –non shown-), Fig-10 (Ti, V, Cr, Co) levels.
Basal: day cero (basal control levels: 100 %, d0, n=17).
Day 80: 80 days of nutraceutical supplementation with GC (d80, n=17).
GC: Glutathione Complex formulation.

4. Discussion

The toxicity of mercury is characterized by a long-lasting latency period of five to seven years before symptoms manifestation/s would be detected in patients [9].The aim of this study has been to test whether a CLINICAL protocol by using several nutraceuticals for 80 consecutive days could detoxificate heavy metals by reducing its levels among patients with long-term dental amalgams as compare their basal levels (controls), independently of its mechanistic action.
The Cephalosporium mycella mushroom, present in the formulation, has been used for bioremendation of organic pollutants in metal-organic mixed environment areas [28], Thus, this evidences support it possible capacity to chelate heavy metals in the present human study. The formulation also contains Asiaticoside (AS), a triterpenoids isolated from the Indian medicinal herb *Centella asiatica*, which protected in a rotenone-induced hemiparkinsonism model [29]. The therapeutic role of Asianticosides by inducing CYP enzymatic activities have been demonstrated (CYP450, CYP3A4, CYP2D6, CYP2C9) without inducing harmful effect/s [30]. In addition, silimarine can activate phase-II hepatic enzymes [31], which could enhance heavy metal removal in patients with long-term dental amalgams after 80 days of supplementation with silimarine and asiaticosides (present in this formulation). However, the exactly mechanism/s cannot be elucidated given the synergia among bioactive components of this formulation.

Levels of heavy metals as Aluminium (Al) were signifficantly reduced after 80 days of asiticosides, selenium (Se), L-Glutamine, L-Gluthathione, and alpha lipoic acid supplementation as compare their respective controls (before any supplementation). The possibility that GSH may enhance detoxification should not be excluded since its precursor amino acids (NAC, L-Glu, Gly) and Selenium (Se) were also included in the formulation. The Glutathione rised (reduced form) observed after 80 days of nutraceutical supplementation contribute to enhance heavy metal removal [(Hg), as Silver (Ag), Tin (Sn)], maybe by inducing SOD-1 activity/GSH levels in patients with long-term dental amalgams as compare their respective basal levels. Previously, we have demonstrated elevated mercury (Hg^{++}) as well as Aluminium (Al) levels in women with long-term dental amalgams (average: 15 years) [12]. It is known that Cupper (Cu^{++}) and Aluminum (Al) contribute to Alzheimer-disease pathology [32]. Thus, the clinical use of phytonaturals able to reduce Cu^{++} levels could be beneficial among patients with long-term dental amalgams. The role of Al in Alzheimer Disease pathology (AD) is controversial [33.34] and Al levels did not differed in plasma from AD patients as compare to those with minor cognitive impairment [35]. We have selected healthy patients without cognitive deficit/s, whereby this observed Al removal seem to be attributable to the chronic nutraceutical supplementation after 80 consecutive days here.

The exactly mechanism/s by which these nutraceutical supplementation detoxificate heavy metals after 80 days of supplementation are unknown among patients with long-term dental amalgams; however, several hypothesis could explain this enhanced removal of heavy metals; (1) the reduced Se uptake can affect glutathione peroxidase activity [18] and selenomercurials might chelate mercury by formation mercury-selenium (Se) complex. Certain heavy metals are accumulated in the brain of autistic children who had low GSH levels; surpresively they have high hair's Se levels [14, 36]. The possibility that low Se observed in the present study after 80 days of supplementation could reflect enhanced detoxification of heavy metals should not be excluded in patients with long-term dental amalgams; this features suggests that oral intake with Se may enhance mercury or Silver removal here. In fact, these heavy metals are removed by conjugation with Gluthatione (GSH: reduced form) [18, 37]. Other metals from titanium dental alloy (Ti-6Al-4V) did not differed here since all recruited participants did not have dental titanium implant alloy/s. The role of selenium as antidote against mercury toxicity is a hot topic in medical toxicology. The formulation used here contains all amino acids precursors neccesary for Glutathione (GSH) synthesis (NAC, Glu, Gly) together selenium (Se). The striking low Se levels found here after 80 days of supplementation could reflect enhanced removal of heavy metals because Se is used during the conjugation process with GSH. This features explains why salivary GSH rises after 80 days of nutritional supplementation in the present study. As Se is also required for selenoprotein formation by binding to mercury [38], these low Se levels observed together rised SOD-1 activity would remove heavy metals in patients with long-term dental amalgams. Collectively, these mechanisms prevent heavy metal accumulation in patients with long-term dental amalgams here [12].

The Aluminium (Al) reduction observed after 80 days of supplementation agree with a study in which Al levels decreased in patients after 30 days of EDTA chelation therapy (twice/day). Our findings agree with data of other authors in rabbits, which demonstrated neuroprotective effects after treatment with two different Al chelators [39].

Other heavy metals as silver (Ag) were significantly decreased after 80 days of supplementation here. This feature agrees with decreased hepatic Malonaldehide and

urinary mercury levels found in methyl mercury poisoned rats [40]. The enhanced detoxification by rising SOD-1 activity could enhance heavy metals removal after 80 days of supplementation. Moreover, GSH (reduced form) rised also contribute to detoxificate heavy metals among patients with long-term dental amalgams; in fact, women who have four long-term dental amalgams significantly rised their GSH levels [12]. Moreover, the alpha lipoic acid, also present in the formulation, can chelate heavy metals by rising GSH levels in these patients with long-term dental amalgams. In fact, alpha lipoic acid also prevents oxidative stress-induced cadmium (Cd) and regenerates GSH levels *¨in vitro¨* [41, 42]. Other bioactive nutrceuticals present in the formulation can prevent heavy metal accumulation. Interestingly, levels of Hg^{++}, Ag, Sn, Cu^{++}, Al, or Pb were significantly decreased after 80 days of supplementation among patients with long-term dental amalgams. It is worthy to mention the reported relationship among sarcopenia and high mercury (Hg^{++}) or Lead (Pb) levels described in 704 ederly Korean population [1]. These findings highlight the detoxificant role of these nutraceutics after 80 days of supplementation since Lead (Pb) or mercury (Hg^{++}) levels were reduced in these patients with long-term dental amalgams. The beneficial role of selenium against mercury toxicity has been reported among 269 Korean patients with high consumption of fish [43]. The fish consumption is 1 time/week in the present study, whereby mercury detection in hair samples by ICP-MS is not methylmercury. As Sodium (Na^{+}) contributes to hypertension [44] and Se may exhibit a protective effect against cardiovascular disease [3], these reduced sodium (Na^{+}) levels observed after 80 days of supplementation, suggest antihypertensive effects by these phytonaturals in the present study.

The interaction among metals as lead (Pb), cadmium (Cd) can aggravate its toxicity [4, 45]. The relationship between osteoporosis and blood mercury rises were demonstrated among postmenopausal women [46]. In addition, patients whom have long-term dental amalgams increased their Boron (B) and Calcium (Ca^{++}) levels after 80 days of supplementation as compare their respective basal levels. The blood mercury levels may differ among ethnias and can be influenced by fish consumption [47]. Thus, these phytonaturals could prevent lead (Pb), silver (Ag) or mercury (Hg^{++}) heavy metal accumulation in Caucasian patients with long-term dental amalgams.

It is known that heavy metals are augmented in the cerebrospinal fluid of 38 patients with Amyloid Lateral Sclerosis (ALS) [48]. This CLINICAL protocol with nutraceuticals supplementation enhanced detoxification of heavy metals in patients with long-term dental amalgams without inducing toxicity. Surpresively, hair's mercury (Hg^{++}), zinc (Zn^{++}), and copper (Cu^{++}) levels did not significantly differed among autistic children (74 participants: 2-4 and 5-9 years) as compare with age-matched controls; conversely, older autistic children had 45 % of hair Se rise in this study. These findings suggest that high hair's Se levels could indirectly reflect a poor detoxification capacity and lead to heavy metal accumulation in patients (Hg^{++}, Pb). Thus, these reduced Se levels observed after 80 days of supplementation may indirectly reflect enhanced chelation of mercury (other heavy metals) in patients with long-term dental amalgams.

On the other hand, the oligoelement lithium (Li) increased after 80 days of supplementation among patients with long-term dental amalgams, which suggest that heavy metals may interfere with lithium action/s. In addition, Magnesium (Mg^{++}), Zinc (Zn^{++}) and Boron (B) levels increased after 80 days of supplementation, which may reflect beneficial effects; in fact, we have previously described Zinc rised among women with long-term dental amalgams [12]. The formulation also contains s-Adenosyl-Metionine that increases serotonine levels [49] and also contains vitamine B-6, B-12, C and E with antioxidant properties [50].

Finally, this study has several limitations. First, it did not evaluate the causality between heavy metal exposure and toxicity among patients who have long-term dental amalgams along the time. Prospectively, designed studies are mandatory to clarify these relationships. Levels of hair heavy metals have been measured before and exactly after 80 days of supplementation without data at early times. Second, lead, mercury and cadmium can differ among ethnic groups [51]. They are caucasian patients (Spain, Europe) although the present findings cannot be extrapolated to other ethnic groups. Third, although total mercury may be a reliable biomarker of mercury exposure, the mercury measured by ICP-MS is not methylmercury [52] given their low fish consumption/week. The inorganic Hg is often removed by urin [7]. The electromagnetic contamination can aggravate the toxicity of mercury [51].

The enhanced detoxification by nutraceuticals must be confirmed in further clinical trials. These findings indicate that synergic nutraceutical supplementation enhanced GSH levels and rised SOD-1 activity, which detoxificates heavy metals in this pilot study. The supplementation for 80 days with selenium (Se), *Cordiceps*, silimarine, L-Glutamine among other nutraceuticals prevented heavy metal accumulation in patients with long-term dental amalgams (average: 5 dental amalgams for 15 years).

5. Conclusion

The supplementation with nutraceuticals for 80 consecutive days [L-Glutathione and its aminoacid precursors, Se, L-Glutamine, silimarine, Cordiceps (Glutathione Complex, Celsus Lab$^{@}$)] enhanced detoxification of heavy metals in patients with long-term dental amalgams by reducing $Hg^{++,}$ Ag, Sn, Cu^{++}, Al, Pb) levels. In addition, these phytonaturals enhanced salivary SOD-1 activity and also rised Glutathione (GSH) levels after 80 days of supplementation as compare their respective controls (basal: before any supplementation). Finally, deficits on Lithium (Li), Boron (B) or Sodium (Na^{+}) were corrected after 80 days of supplementation with this formulation.

6. Conflict of interest

None to declare by all authors

The authors declare no conflict of interest.

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8. Abbreviations

ALS: Amyloid Lateral Sclerosis SOD-1: SuperOxide Dismutase-1 GSH: Reduced Glutathione form
Al: Aluminium Hg^{++}: mercury Cu^{++}: Cupper Zn^{++}: Zinc
Se: Selenium NAC: N-Acetil-Cystein Gly: Glycine
Cys: cysteine Glu: glutamate

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