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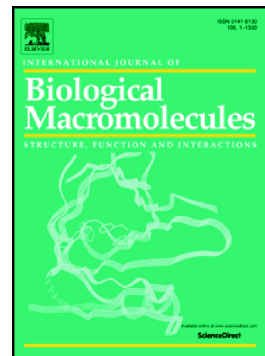


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In vivo evaluation by oral administration of chitosan combined with bioactive glass against cadmium-induced toxicity in rats

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ABSTRACT

Bioactive glass and chitosan are biomaterials widely used for orthopedic applications, notably as bone grafts. Although these biomaterials show promising therapeutic properties, no research has yet examined their potential for oral administration in soft tissue protection, particularly against metal toxicity. The aim of our study was to evaluate the potential of chitosan from cuttlefish (CHS) bone combined with bioactive glass (BG) against Cadmium-induced toxicity in rats. Cadmium (Cd), a heavy metal that accumulates in tissues, causes various disorders. Experiments were carried out on rats intoxicated acutely by oral administration of Cd (20mg/kg body weight) and/or concomitantly with oral administration of CHS/BG (100 mg/kg body weight) for 7 days. Using pathophysiological and biochemical tests, we evaluated the detoxifying effect of orally administered CHS/BG against Cd toxicity. Our results showed, for the first time, a significant detoxifying effect of CHS/BG against Cd-induced toxicity in rats. Treatment with CHS/BG protected rats against the harmful effects of Cd by reducing lipid peroxidation levels and enhancing antioxidant enzyme activities. In addition, it helped restore phosphocalcic balance and protect liver, kidney and brain function. Remarkably, it also reduced Cd levels in the liver, kidneys and brain, as well as in the bones of rats. These results show that oral administration of CHS/BG has a strong therapeutic potential on tissues through detoxification of cadmium-exposed rats.

Keywords: Chitosan, Bioactive glass, Oral administration, Cadmium, Toxicity.

1. Introduction

Cadmium (Cd) is considered to be one of the most toxic heavy metals. It is implicated in Itai-Itai disease, which causes bone disorders [1]. It is a widely distributed metal in the environment, emitted mainly by industrial activities, posing a harmful threat to human health as an endocrine disruptor [2-3]. The harmful effects of this metal on various tissues, including the kidneys, liver, brain, bone marrow, lungs and testicles, have been the subject of extensive research [3-4]. The accumulation of Cd in these organs leads to numerous disorders caused by reactive oxygen species (ROS), inducing oxidative stress [4-5]. In addition, Cd causes lipid peroxidation, which is considered to be the main cause of its destructive effect on membrane tissues [6], increasing the susceptibility of tissues to cadmium toxicity. Chronic or acute exposure to cadmium is associated with severe toxic effects, particularly on the liver, brain and bone marrow. Although the use of medicinal plants to treat metal-related disorders, including cadmium, is widely studied [7-8], finding innovative therapeutic strategies to reduce cadmium toxicity remains a major challenge in modern toxicology.

It is widely recognized that the environment is a rich source of chemical compounds that are biologically active and can have many beneficial effects. Chitosan (CHS) (β -(1 \rightarrow 4)-2-amino-D-glucose) is a linear polysaccharide derived from the partial deacetylation of chitin by the deacetylation reaction with a degree of more than 50% [9]. Numerous marine and terrestrial chitin source species have been explored to produce CHS [9, 10]. CHS is used in many applications such as food, medicine and textiles because of its solubility, biocompatibility, non-toxicity and biodegradability [11, 12]. The characteristics of the finished product are determined by these factors. Its purity, molecular weight (Mw), crystallinity and deacetylation level (DD) are crucial in biomedical applications [13]. The biological characteristics of CHS, such as its antifungal, antibacterial, antioxidant, anticancer and anti-inflammatory activities, are overshadowed by these factors [10, 14]. CHS has been widely used in previous studies for the complexation and chelation of heavy metals, in particular the in vitro use of its oligomers to complex copper ions [15]. The application of chitosan to a polluted effluent shows its selective capacity towards various metals, including cadmium, demonstrating variable efficacy depending on the cations considered, independently of their size and hardness [16]. These characteristics represent a potential route

for cadmium detoxification. Bioactive glass (BG) known also as a third-generation biomaterial, is widely used clinically in hard tissue because of its ability to bind to bone tissue [17]. It releases pharmacologically active ions into the physiological environment, inducing various biological effects such as a therapeutic ion carrier, anti-osteoporotic, osteogenic, anti-inflammatory, anticancer and antibiotic agent [18]. Subsequently, with advances in the field of inorganic biomaterials, BG is beginning to attract attention for the regeneration and repair of non-bone and non-calcified tissues [19]. Recently, novel toxicological evaluations involving oral administration of bioactive glasses have been undertaken, demonstrating that a lethal dose (DL_{50}) of 45S5 bioglass was greater than 2000 mg/kg body weight (bw) in rats, without observing histological toxicity or neurobehavioral disturbances [20]. In addition, oral administration of bioactive glass incorporating barium oxide demonstrates anti-ulcer efficacy by forming a protective physical barrier and neutralising acid, indicating promising therapeutic potential against peptic ulcers [21].

Although the promising regenerative properties of bioglass have been demonstrated in preclinical studies, no research has explored the oral and systemic use of these biomaterials in the treatment of disorders associated with heavy metal toxicity. In addition, numerous investigations have focused on the joint use of chitosan and bioactive glass for bone filling applications. Their combination has shown positive and encouraging results, particularly through local implantation in the bone [22, 23, 24, 25]. However, to our knowledge no study has yet examined the therapeutic effects of oral combination, particularly in the context of disorders induced by heavy metals such as cadmium. In this context lies the novelty of our work, aiming to explore, for the first time, the efficacy of orally administering chitosan treatment in combination with bioactive glass for treating disorders caused by cadmium-related toxicity in rats. This research goes beyond contributing to our knowledge of cadmium toxicity and detoxification mechanisms. It seeks to propose innovative therapeutic strategies by exploiting natural resources as a direct source of chitosan and promoting oral administration of the bioactive glass.

2. Materials and Methods

2.1. Chitosan preparation

To prepare chitosan, we adopted the process described by Ait Hamdan *et al.*, [10]. This process involves the use of a mixture composed of 50% potassium hydroxide, 25% ethanol (96°), and 25% mono-ethylene glycol, applied to chitin extracted from cuttlefish bone by

demineralization (0.55 M HCl) and deproteinization (NaOH, 0.3 M at 80-90°C), with stirring at 120°C for 10 hours. The chitosan was then filtered, washed with water until neutral and then with ethanol/acetone (1/1, wt %) before being dried at 40°C.

2.2. Bioactive glass preparation

The bioactive glass 46S6 has been prepared from sodium metasilicate (Na_2SiO_3), silicon oxide (SiO_2), calcium metasilicate (CaSiO_3), and sodium metaphosphate ($\text{Na}_3\text{P}_3\text{O}_9$) as indicated in our previous studies [26]. The composition composition is shown in the Table 1. The premixed mixtures were melted in platinum crucibles that were placed in an electric furnace. The first rise of temperature rate was $10\text{ }^\circ\text{C min}^{-1}$, and it was held at $900\text{ }^\circ\text{C}$ for 1 h to achieve the decarbonation of all products. The second rise of temperature rate was $20\text{ }^\circ\text{C min}^{-1}$, and it was held to $1350\text{ }^\circ\text{C}$ for 3 h. The samples have been casted in preheated molds, in order to form cylinders of 13 mm in diameter and annealed at $565\text{ }^\circ\text{C}$ for 4 h near the glass transition temperature of each glass.

2.3. Bioglass and chitosan characterization

Biomaterials are characterized by FTIR (Jasco 4600A Gemini sampling spectrometer), and X-ray diffraction (Rigaku III diffractometer) with scan angle from 5° to 80° (1° min^{-1}). To calculate the DDA of chitosan, we used $^1\text{H-NMR}$ (400 MHz Bruker) according to the formula [27]:

$$\text{DDA (\%)} = \frac{(\text{H}-1-\text{D})}{(\text{H}-1-\text{D} + 1/3 \text{HAc})} \times 100$$

With H-1-D is the integration of the H1 proton of glucosamine and H-Acetyl (CH_3) is the integration attributed to the proton peak assigned to the acetyl group. To determine the viscosity and molar mass Ubbelohde capillary viscometer ($0.5\text{-}3\text{ mm}^2/\text{s}$, 20 ml) was used of chitosan at $25\text{ }^\circ\text{C}$ using the Mark Houwink equation [28]:

$$[\eta] = \mathbf{K} \cdot [\mathbf{M}]^a$$

With $[\eta]$ the intrinsic viscosity (ml/g); M the viscometric molar mass; $\mathbf{K} = 0.070$ and $a = 0.081$. The solvent is a mixture of 0.3 M acetic acid and 0.2 M sodium acetate.

2.4. Antioxydant activity evaluation:

2.4.1. DPPH Activity

The antioxidant activity of the chitosan sample was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) according to similar method described by Sahin [29] with some modifications. Briefly, 2.9 mL of a methanolic solution of DPPH (0.1 mM) was mixed with 100 μ l of chitosan solution at different concentrations of (0.2; 0.5; 1 and 2 mg/mL). The resulting mixture was kept in the dark for 30 minutes. The percentage of inhibition was calculated by the formula below:

$$\% \text{ Inhibition} = \frac{(\text{DO control} - \text{DO sample})}{\text{DO control}} \times 100$$

Where the absorbance of the sample ($\text{DO}_{\text{sample}}$) is measured at 517 nm against a control ($\text{DO}_{\text{control}}$) consisting of DPPH and methanol solution. Commercial chitosan with Quercetin, BHT (butylated hydroxyanisole) and ascorbic acid were used as standard positive control. The activity (IC_{50}) is expressed in mg/mL.

2.4.2. FRAP assay

The ferric reducing ability power (FRAP) test was carried out using method described by Oyaizu's [30]. The technique involves preventing the development of Fe (II)-Ferrazine complexes when samples are incubated with ferrous iron. Briefly, the sample and control substance were mixed with phosphate buffer (2,5 ml, 0,2M, pH= 6,6) and potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (2.5 ml, 1 %). Thirty minutes later, trichloroacetic acid (2,5 ml of 10 % (w/v)) and of FeCl_3 (ferric chloride, 0.5 mL, 1%) was added respectively to the mixture after it had been incubating for 30 minutes. The absorbance is measured at 517 nm. The quercetin, BHT and ascorbic acid were employed as a positive control. Three replicates were performed to have statistical results of IC_{50} and the activity (IC_{50}) is expressed in mg/mL.

2.5. Animals and treatments *in vivo*

Sprague-Dawley rats (18–20 weeks of age) housed in transparent cages under controlled environmental conditions of temperature (22 ± 2 °C) and humidity ($50 \pm 10\%$) and maintained on a 12 h: 12 h light/dark cycle. They had free access to food and water. Animal procedures were performed in accordance with the guidelines of European Council Directive EU2010/63 on the care and use of laboratory animals. Approval for animal experimentation was obtained from the Institutional Review Board of the Faculty of Science, Cadi Ayyad University, Marrakech, Morocco. The animals were divided into four groups: (1) control

group (treated with 0.9% saline), (2) Cd group (20 mg/kg), (3) group received chitosan (100 mg/kg) and (4) group received chitosan + bioactive glass (CHS+BG: 100 mg/kg). The dose of 20mg/kg was chosen on the basis of studies on the acute toxicity of cadmium [4]. The experiment included 7 days of treatment with six animals per group. The anesthesia rats were sacrificed by decapitation and the blood was collected. The serum was recovered by centrifugation (1500×g, 15min, 4°C), frozen and stored at -20°C until analysis. Kidneys, liver, spleen, brain, heart and lungs were removed. Femur bones were removed from each group for analysis. All samples were stored at -80°C until further use.

2.6. Oxidative stress measurements

Lipid peroxidation in liver, brain, kidney and bone marrow of all animal groups was measured by quantification of thiobarbituric acid reactive substances (TBARS) determined according to a method described by Buege & Aust [31]. Superoxide dismutase (SOD) activity was assessed spectrophotometrically using an earlier protocol cited by Marklund & Marklund [32]. Catalase (CAT) was measured by calorimetry at 240 nm and expressed as moles of H₂O₂/min/mg protein according to Aebi [33]. Total protein content was determined by the Bradford method [34], using bovine serum albumin as a standard at 595 nm.

2.7. Morphometric study

The organ coefficient, a crucial parameter for assessing the toxic effect of cadmium by measuring variations in organ weight [35], was determined after sacrifice. The organs were dissected and weighed immediately and accurately, and the organ coefficient was calculated using the following formula:

$$\text{Organ coefficient} = \left[\frac{\text{Weight of the organ (g)}}{\text{Total body weight(g)}} \right] \times 100$$

2.8. Tissue cadmium assay

Samples of organs such as brain, liver, kidneys and bones from animals in the different groups were etched with nitric-perchloric acid (69%) at 80°C for 12 hours. Cadmium levels were determined using the flame atomic absorption spectrophotometer technique, graphite furnace (S.A.A.F.F), SHIMADZU: AA type 6300/GFA-EX7i.

2.9. Biochemical analysis

Biochemical analysis was performed on serum obtained from centrifugation of blood samples collected, to determine phosphorus and calcium levels, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (PAL), creatinine and urea using a

biochemical apparatus (Cobas 6000, Roche).

2.10. Histological study

For the histopathological study, vital organs such as the brain, liver and kidneys were dissected and fixed in a 10% formalin solution for 12 hours. They were then subjected to dehydration in a series of graded alcohols and embedded in paraffin wax. Sections 4-10- μm thick were stained with haematoxylin and eosin for pathological studies, as described by Malatesta [36].

2.11. Statistical analysis

Statistical analysis of the data was carried out using GraphPad Prism version 9.00 software (San Diego, California, USA). Results are presented as mean \pm SEM. An analysis of variance (ANOVA) was performed, followed by Tukey post-hoc tests with a significance level of $p=0.05$.

3. Resultats and discussion

3.1. Bioglass and chitosan Characterization

The XRD model of chitosan and bioglass is shown in Fig.1. CHS shows (Fig.1A) two peaks are observed at 10° (2θ) and 20° (2θ), characteristic of chitosan corresponding to type II orthorhombic crystallization [37]. The BG reveals an amorphous character, manifested by a diffraction halo between 25° and 35° (2θ) (Fig.1B). The infrared spectrum of chitosan and bioglass is shown in Fig.2. Spectrum of chitosan reveals several characteristic bands. Vibrations of $-\text{NH}_2$ and $-\text{OH}$ groups are observed in the 3000 to 4000 cm^{-1} range, while bands at 1657 , 1597 and 1320 cm^{-1} correspond to amides I, II and III respectively. The $-\text{CH}$ -bending vibrations are noted at 2926 , 2880 and 665 cm^{-1} (Fig.2A). Skeletal vibrations of the C-O stretch are detected at 1075 and 1033 cm^{-1} , and vibrations of the C-O-C groups in the saccharide structure appear around 1153 and 890 cm^{-1} [38-39]. For bioglass (Fig.2B), it reveals four broad absorption bands. The first band at around 498 cm^{-1} is characteristic of Si-O-Si vibration. Three other characteristic bands at 741 , 911 and 1025 cm^{-1} attributed to the stretching vibrations of the Si-O bond in each SiO_4 tetrahedron are detected. In addition, only a faint band at 613 cm^{-1} is characteristic of bending vibrations of the P-O group, indicating the presence of a small amount of phosphate bound to the silica lattice [40-41]. $^1\text{H-NMR}$ is considered to be the best and most accurate method currently available to accurately calculate the DDA of chitosan [42], [43], [44]. The DDA determined a by NMR is 96.15% (Fig.3), this

verifies that the N-deacetylation reaction according to the Brussignac process was successfully performed with a molecular weight of 94,712 g/mol (95 KDa) and a measured viscosity of 472 ml/g

3.2. Antioxidant activity of Chitosan and Bioglass

Bioactive glass, renowned for its pharmacologically active ions, exhibits significant oxidative potential, particularly when doped with other elements [45]. Chitosan is widely known for its antioxidant mechanisms, such as free radical scavenging capacity, chelating capacity and reducing capacity [46]. To assess their antioxidant properties, two tests were carried out, FRAP and DPPH (Fig.4). In fact, the IC₅₀ values of cuttlefish-derived chitosan by DPPH and FRAP tests were 2.11 and 4.33 mg/mL respectively, while bioactive glass presented 7.11 mg/mL and 9.7 mg/mL respectively, indicating that they possess a high free radical neutralizing capacity. This activity is comparable to the values of standard antioxidants such as quercetin, BHT and ascorbic acid for the DPPH and FRAP tests. Furthermore, the 50% radical scavenging activity attributed to cuttlefish chitosan is in agreement with literature results for marine species comprising mainly cuttlefish bone [47]. Chitosan can scavenge free radicals thanks to its free amine function, and this scavenging capacity is therefore strictly dependent on its DDA. Consequently, the interaction of radicals is with the hydrogen ion of ammonium ions (NH³⁺) to give a stable molecule [48]. For bioactive glass, the synthesized formulation of 46S6 chelates free radicals thanks to the scavenging activity of SiO₂ towards HO[•] radicals. A combination of these two molecules would therefore be effective and interesting.

3.3. Effect of CHS/BG on body weight during the acute Cd toxicity

During the 7-day treatment period, body weight monitoring revealed distinct trends among the experimental groups (Fig. 5). The group administered with Cd exhibited a significant decline in body weight compared to the control and treatment groups ($p < 0.05$). This suggests a potential effect of Cd on weight loss over the short-term observation period. Cd exposure induced a substantial decrease in body weight correlated with anorexic behavior in rats, a phenomenon widely debated in the literature [49-50]. On the other hand, after administration of the biomaterials, the groups showed no weight loss, with a significant correction and recovery of body weight (on day 7 of the experiment, Cd: 108.4g vs Control: 161g; Cd+CHS: 165.6g; Cd+CHS+BG: 154.6g, $p < 0.05$), indicating a stimulation of appetite compared with the Cd-treated group.

3.4. Effect of CHS/BG on morphometric parameters during the acute Cd toxicity

The influence of Cd intoxication on body weight prompted further investigation into its effects on vital organs. A morpho-functional study was conducted to examine the potential therapeutic impact of CHS and the combination of CHS/BG (Fig. 6). The findings unveiled noteworthy hepatomegaly and nephromegaly, indicating significant tissue damage and functional disturbances linked to cadmium intoxication (Kidneys: Cd: 0.67% vs Control: 0.49%; Liver: Cd: 4.66% vs Control: 3.31%, $p < 0.05$ respectively). These results align with prior research emphasizing the vulnerability of the liver and kidneys to cadmium's effects, characterized by its slow elimination through urine [51]. Treatment with CHS or in combination with BG has been shown to alleviate this organ imbalance. This alteration was alleviated by the administration of CHS and CH/BG (Kidney: Cd+CHS: 0.53%; Cd+CHS+BG: 0.44%. Liver: Cd+CHS: 3.62%; Cd+CHS+BG: 3.33%, $p < 0.05$ respectively). CHS has been shown to have beneficial effects on the liver and kidney. Studies have found that when low molecular weight CHS is absorbed, it can extend to several organs including the liver, kidney [52]. CHS has been found to have a protective effect on the reproductive system, including increasing antioxidant levels in the liver and kidney [53]. To our knowledge, no study has demonstrated if there is a protective effect of BG alone, but it was proved to not have a toxicological effect on the liver nor kidney [54]. We may explain the better results in the combination by the fact that bioactive glass helps with the bioactivity of chitosan [55]. However, no significant changes were observed in the brain, spleen, lungs, or heart following exposure. This can be explained by the fact that the liver and kidneys are particularly sensitive to cadmium toxicity and accumulate high levels of the metal [56].

3.5. Effect of CHS/BG on biochemical statut during the acute Cd toxicity

When a toxic compound is administered repeatedly, assessing its impact on liver and kidney function becomes crucial. Our experimental data revealed a significant increase in serum levels of creatinine, urea, AST, ALT, and PAL in Cd-treated rats compared with the control group ($p < 0.05$) (Table 2), consistent with other studies demonstrating chronic exposure-induced hepatic and renal toxicity through elevated enzymatic activity, as indicated by increased serum levels of creatinine, AST, ALT, PAL, and urea in Cd-exposed rats [57]. Treatment with CHS, either alone or in combination with BG, significantly reduced the levels of hepato-renal markers, mitigating toxicity in these vital organs. Notable hepatoprotective and nephroprotective effects of chitosan were observed, aligning with previous studies [58-

59-60].

Cd also induced phosphocalcic disorders, evidenced by a decrease in serum calcium levels and an increase in serum phosphorus levels compared to the control group. This exposure led to phosphocalcic disorders, attributed to a significant exchange of cadmium with calcium in the bones, indicating ionic competition based on charge ($\text{Cd}^{2+}/\text{Ca}^{2+}$) [61]. Treatment with CHS alone or in combination with BG effectively counteracted these disturbances, restoring serum calcium and phosphorus levels to normal, consistent with previous findings suggesting chitosan as a potential non-calcium phosphorus binder for patients intolerant to prescription phosphorus [62]. The combination of CHS/BG maintained serum calcium and phosphorus levels, consistent with literature results using bioactive glass/chitosan as bone grafts [62]. Furthermore, the combination of CHS/BG demonstrated a superior protective effect on organs compared to chitosan alone. This enhancement may be attributed to the presence of bioactive glass particles in chitosan, which can intensify absorption bands related to C-O and CO_3 vibrations, as well as the amide II vibration, indicating that bioactive glass enhances the bioactivity of chitosan [63].

3.6. Histopathological Study of CHS/BG in acute Cd toxicity

Microscopic examination of rat organs after exposure to 20 mg/kg Cd showed obvious changes in kidney, liver and brain tissue (Fig.7). The kidneys showed congestion and disorganization of Bowman's space, as well as destruction of the glomeruli (Fig. 7B1). Examination of the liver revealed severe dilatation and congestion of the portal vein and artery, accompanied by diffuse vacuolar degeneration in the hepatocytes (Fig.7 B2). The biochemical changes observed correlate with histological analyses, revealing Cd's ability to induce tissue changes, including glomerular destruction in kidneys and hepatocyte degeneration, as mentioned previously [64]. In brain tissue, particularly the cortex, intoxication reveals clear signs of cellular inflammation (Fig.7 B3), confirming earlier discussions on the neurotoxic effects of cadmium [65]. After treatment with CHS/BG, organ tissues showed remarkable tissue restoration, particularly in the combined CHS/BG treatment compared with CHS. This protective efficacy was further elucidated by histological examination, highlighting the notable hepatoprotective and nephroprotective effects of chitosan [66-67-68], reinforced by the pharmacological action of the ions released by the bioactive glass.

3.7. Effect of CHS/BG on oxidative stress status during the acute Cd toxicity

Acute administration of Cd to rats led to a significant reduction in CAT and SOD activities in the liver, kidneys, brain, and bone marrow compared to the control group ($p < 0.05$) (Table 3). This reduction is attributed to disrupted enzymatic defense systems due to excessive ROS production [69-70]. In cadmium-induced kidney injury, the activation of certain factors such as Nrf2 amplifies oxidative stress and inhibits autophagy, leading to aggravated kidney damage [71]. Moreover, Cd exposure significantly elevated TRAPS levels in these organs compared to the control group ($p < 0.05$), indicating redox imbalances in the brain as reported previously [72]. Treatment with CHS, or CHS/BG effectively regulated Cd-induced oxidative effects at the organ level ($p < 0.05$). This underscores their potency as antioxidants, mitigating ROS levels induced by Cd toxicity. Notably, antioxidants like chitosan and its derivatives are known for neutralizing free radicals and preventing oxidative damage by interrupting radical chain reactions [73]. Numerous *in vitro* and *in vivo* studies have demonstrated that CHSs exhibit powerful antioxidant activity through a moderate redox regulatory mechanism. They inhibit the production of ROS and prevent lipid oxidation [74-75]. The radical trapping mechanism of chitosan relies on a radical interaction with hydroxyl and amine groups. Several studies have indeed highlighted that the antioxidant activity of chitosan may be limited by its solubility, viscosity, and strong hydrogen bonding both within and between molecules [76-77].

They reduce serum free fatty acids and MDA levels while enhancing antioxidant enzyme activities and lowering lipid hydroperoxides and uraemic toxins in the gut [78], possibly explaining chitosan's role against Cd-induced oxidative stress. Similar to chitosan, other trace elements, such as selenium, have been shown to alleviate brain damage induced by exposure to mercuric chloride (HgCl_2) [79]. The combined effect of CHS and BG aligns with previous studies on their joint application at bone levels, demonstrating significant antioxidant effects [25]. Notably, treatment with CHS and CHS/BG administered locally as bone grafts offered significant protection against oxidative stress [24-25]. In another study, the antioxidative properties of chitosan-doped bioactive glass were evaluated. Researchers carefully assessed oxidative stress levels by examining grafted bone tissues, and they found that chitosan and bioactive glass effectively reduced ROS levels while enhancing antioxidant activity [22-24]. These findings shed light on the protective efficacy of CHS alone or CHS/BG against Cd-induced oxidative stress, whether administered orally or topically.

3.8. Tissue Cd Levels and detoxifying effect of CHS/BG

In this study, we investigated the relationship between tissue cadmium accumulation and the severity of biochemical and histopathological disturbances. Our results demonstrate substantial cadmium accumulation of 30% in the liver, 67% in the kidney, 58% in the brain, and 50% in bone tissues, significantly higher than the control group ($p < 0.05$) (Fig. 8). This accumulation pattern may contribute to the observed histopathological lesions in the liver, kidney, and brain. These findings are consistent with prior research [4]. Notably, treatment with CHS/BG resulted in a significant reduction in cadmium levels in these organs ($p < 0.05$), confirming the hepatoprotective, nephroprotective and neuroprotective roles of these biomaterials, which could account for the weight gain in these organs. The impact of chitosan has extended to renal function, preventing renal failure by decreasing renal dipeptidase activity [50].

Conclusion

In the present study, we evaluated *in vivo* the detoxifying potential of CHS/BG against cadmium exposure (20 mg/kg). These biomaterials revealed antioxidant activity *in vitro* by FRAAP and DPPH assays. Combined oral administration of CHS/BG (100mg/kg) showed significant protective effects. Organic coefficients and biochemical markers (AST, ALT, ALP, creatinine and urea) showed a possible significant effect of CHS/BG on vital organ function and body weight recovery in rats. Similarly, histological examinations and Cd assays in tissues confirmed the protective and detoxifying role of CHS/BG. In addition, CHS/BG corrected phosphocalcic disorders and enhanced antioxidant enzyme activities by reducing lipid peroxidation. In addition to their local application as bone grafts, these biomaterials have provided new experimental evidence for an oral therapy against metal toxicity for soft tissue regeneration. However, further investigations into pharmacokinetics (metabolism and elimination) and biodistribution are required to better understand the potential clinical applications of this treatment.

Ethical approval

The authors declare that this manuscript complies with the Elsevier Ethical Guidelines for Journal Publication.

Conflict of interest

The authors declare that they have no competing interests

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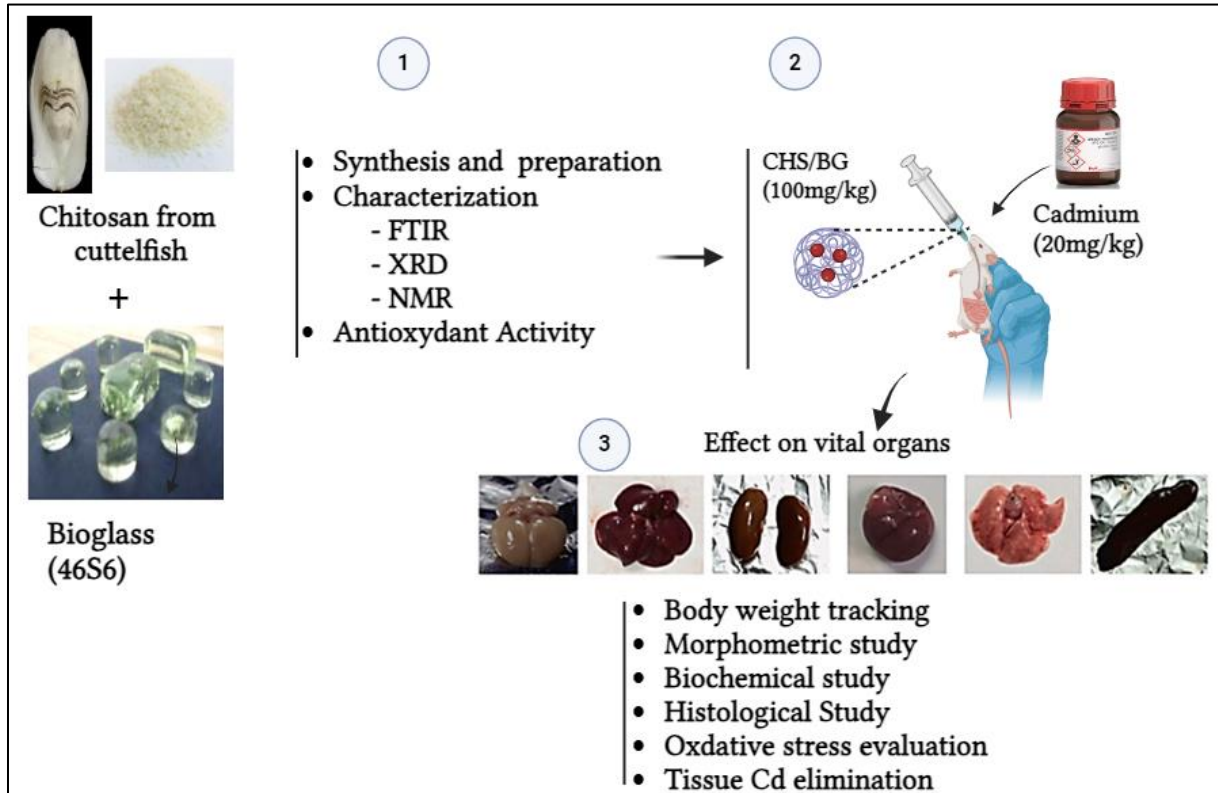
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Graphical abstract



Tables

Table 1. The chemical composition (mol %) of Bioglass (46S6)

Element	SiO ₂	CaO	Na ₂ O	P ₂ O ₅
Composition (%)	46	24	24	6

Table 2. Effect of Cd on biochemical parameters of rat serum after acute exposure to Cd and the effect of treatment with CHS and/or combined with BG.

	Control	Cd	Cd + CHS	Cd + CHS/BG
ASAT (UI/L)	217,09 ± 7,57	641,75 ± 52,49*	308,15 ± 8,46 [#]	276,34 ± 15,63 ^{#/μ}
ALAT (UI/L)	74,19 ± 4,36	103,23 ± 8,79*	86,67 ± 3,03 [#]	82,75 ± 1,75 [#]
PAL (UI/L)	112,29 ± 14,01	481,71 ± 18,05*	247,42 ± 12,06 [#]	148,08 ± 9,07 ^{#/μ}
Creatinine (μmol/l)	3,29 ± 1,08	6,21 ± 2,11*	5,39 ± 1,09	5,75 ± 1,06
Urea (mmol/l)	0,16 ± 0,01	0,34 ± 0,05*	0,22 ± 0,06 [#]	0,19 ± 0,07 [#]
Ca (mmol/l)	95,70 ± 24,01	84,49 ± 18,05*	93,03 ± 20,16 [#]	95,68 ± 19,07 [#]
P (mmol/l)	89,13 ± 14,01	83,36 ± 18,05*	86,29 ± 12,03 [#]	91,22 ± 9,08 ^μ

Values are presented as means ± standard deviation. The biochemical parameters aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (PAL), phosphorus, calcium, creatinine and urea were measured in the serum of intoxicated and treated rats. Statistically significant differences ($p < 0.05$) compared with the control group are indicated by *, compared with the CHS group by # and compared with the CHS/BG group by (μ). Statistical evaluation was performed using ANOVA with $p < 0.05$ considered statistically significant between control and treated groups.

Table 3. Effect of Cd on oxidative stress parameters in kidney, liver, brain and bone marrow and treatment with CHS and/or combined with BG.

Tests	Organs	Control	Cd 20mg/kg	Cd+CHS	Cd+ CHS/BG
SOD (U/mg of proteins)	Liver	305,19 ± 4,41	111,43 ± 1,52 [*]	263,57 ± 1,15 [#]	213,67 ± 1,15 [#]
	Kidney	320,39 ± 2,96	180,72 ± 0,88 [*]	291,12 ± 0,88 [#]	298,64 ± 0,57 [#]
	Brain	411,85 ± 3,75	233,98 ± 2,30 [*]	368,62 ± 2,90 [#]	293,56 ± 1,45 ^{#/μ}
	Bone Marrow	345,19 ± 1,76	257,80 ± 1,20 [*]	321,93 ± 2,90 [#]	355,18 ± 2,33 ^{#/μ}
CAT (μmol/mi of protein)	Liver	3214,30 ± 1,73	2011,38 ± 4,16 [*]	2340,92 ± 0,88 [#]	2547,38 ± 4,41 ^{#/μ}
	Kidney	4328,75 ± 5,77	2080,60 ± 7,42 [*]	3604,50 ± 5,77 [#]	3215,60 ± 2,88 ^{#/μ}
	Brain	3363,25 ± 18,5	1929,90 ± 2,96 [*]	2135,60 ± 5,77 [#]	3369,59 ± 5,77 ^{#/μ}
	Bone Marrow	4371,47 ± 5,85	1300,96 ± 5,20 [*]	3447,98 ± 5,77 [#]	3613,26 ± 5,77 [#]
MDA (nmol/mg of protein)	Liver	1,26 ± 0,14	3,09 ± 0,05 [*]	1,73 ± 0,01 [#]	2,86 ± 0,04 ^{#/μ}
	Kidney	0,71 ± 0,01	1,12 ± 0,01 [*]	0,75 ± 0,02 [#]	0,86 ± 0,01 ^{#/μ}
	Brain	0,24 ± 0,01	0,64 ± 0,00 [*]	0,28 ± 0,02 [#]	0,44 ± 0,02 ^{#/μ}
	Bone Marrow	0,61 ± 0,00	1,00 ± 0,02 [*]	0,82 ± 0,02 [#]	0,74 ± 0,01 [#]

Values are presented as means ± standard deviation. Superoxide dismutase (SOD) activity expressed as U/mg protein, catalase (CAT) expressed as μmol/min/mg protein and malondialdehyde (MDA) levels expressed as nmol/mg protein in liver, brain, kidney and bone marrow. Statistically significant differences ($p < 0.05$) from the control group are indicated by*, from the CHS group by # and from the CHS/BG group by (μ). Statistical evaluation was performed using a one-way ANOVA ($p=0.05$).

Figures

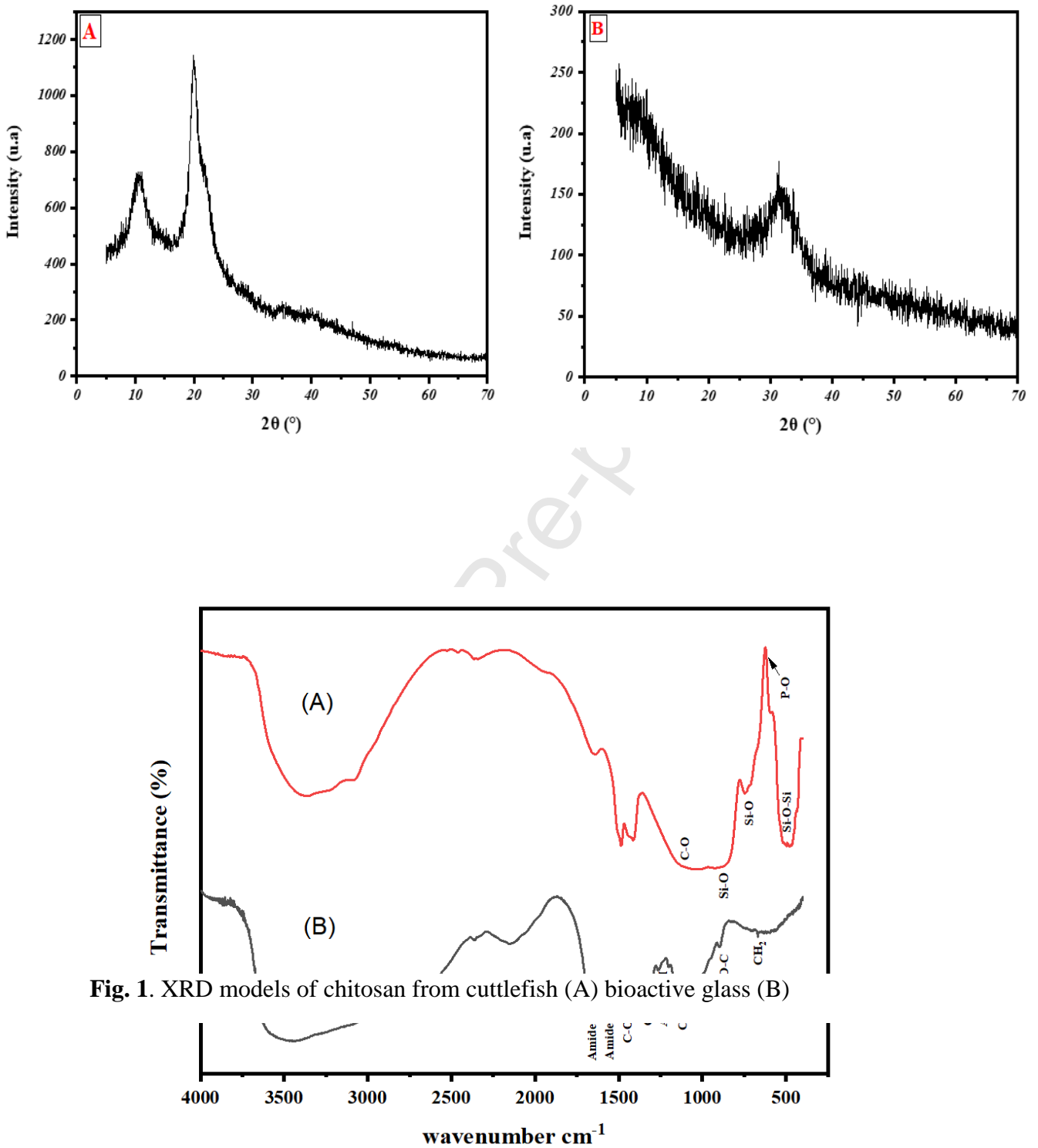


Fig. 1. XRD models of chitosan from cuttlefish (A) bioactive glass (B)

Fig. 2. FTIR spectra of bioactive glass (A) and chitosan from cuttlefish (B)

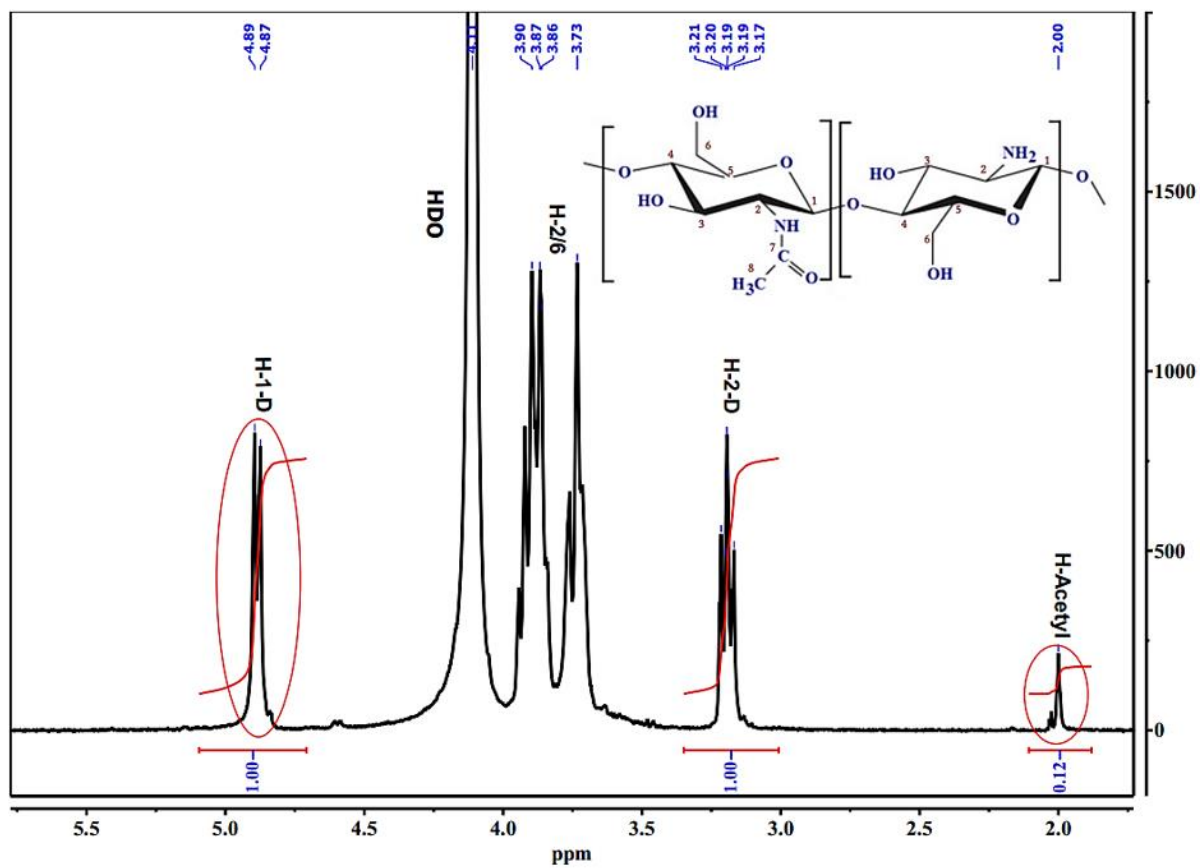


Fig. 3. $^1\text{H-NMR}$ of chitosan prepared from Cuttlefish

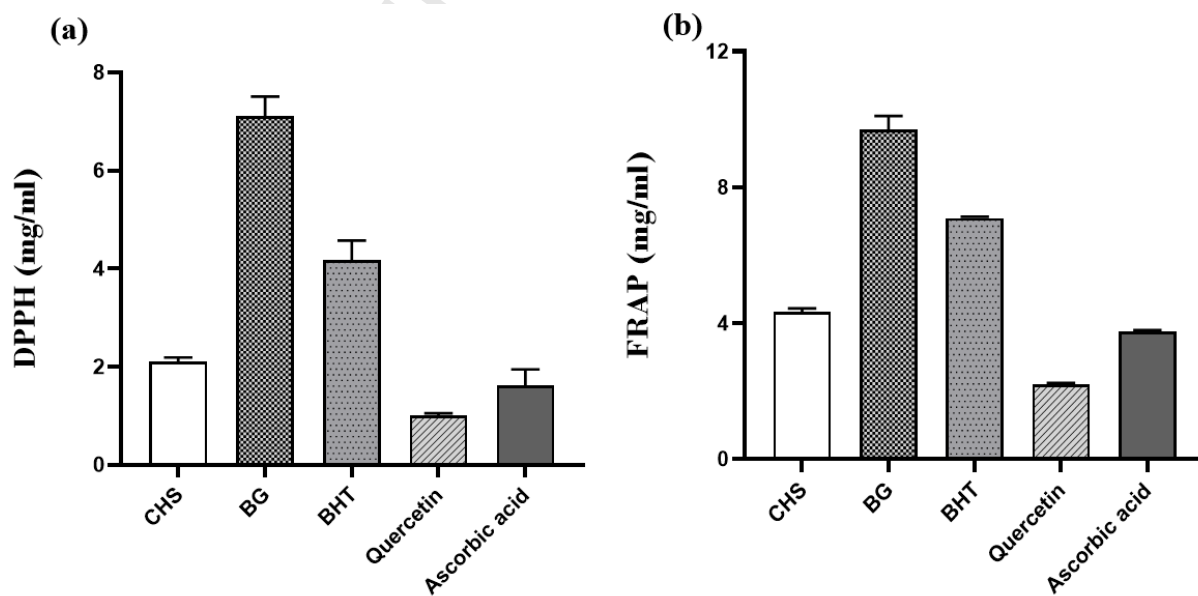


Fig. 4. Antioxidant activity of Chitosan (CHS) and bioactive glass by DPPH (a) and FRAP (b) assay

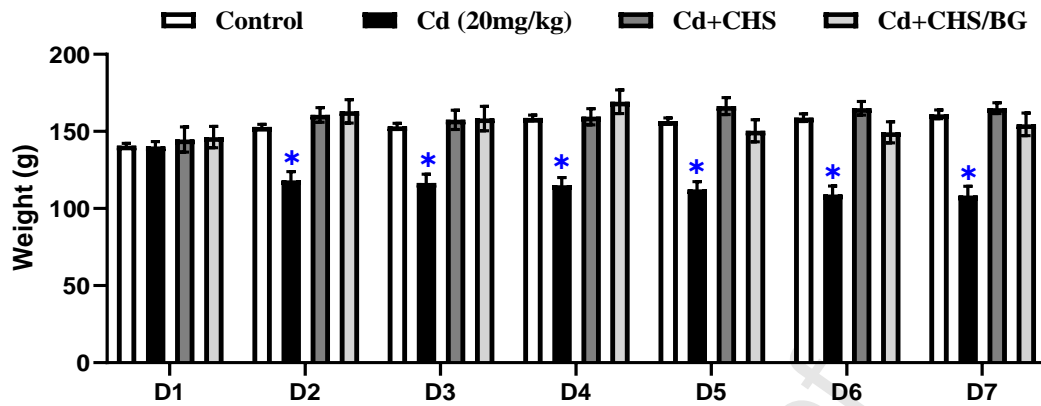
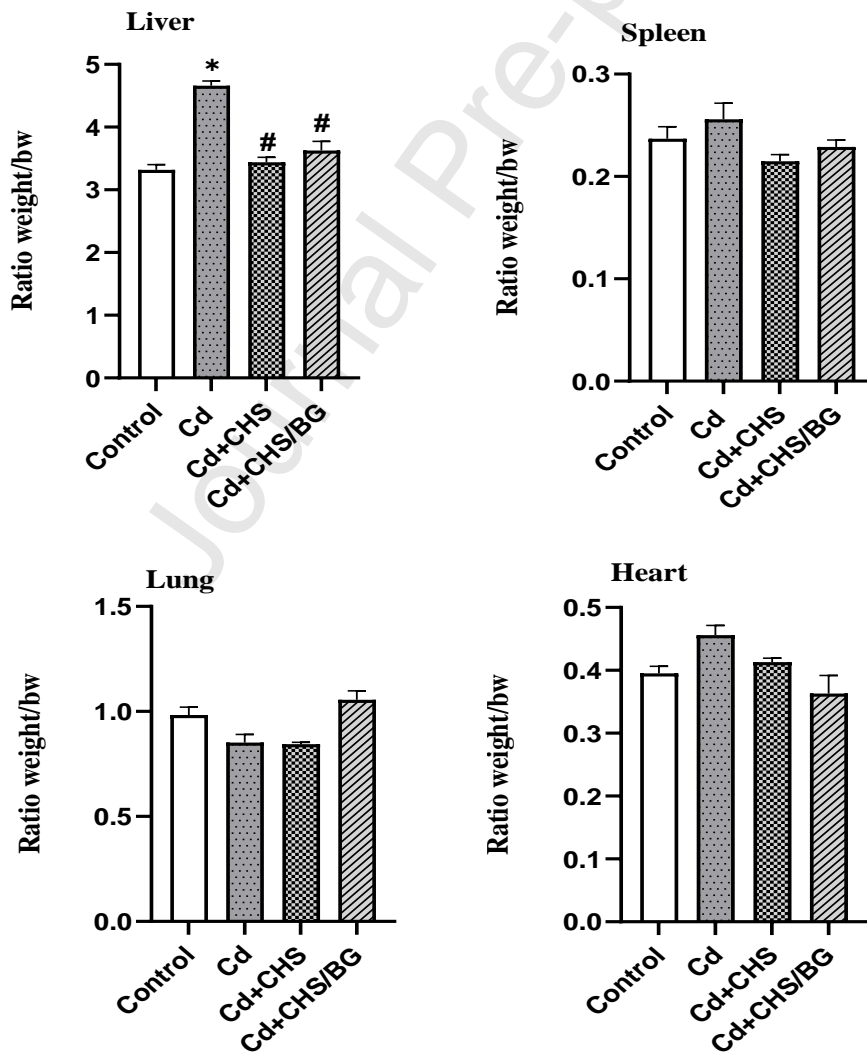


Fig. 5. Evolution in body weight of rats intoxicated with Cd and treated with CHS and CHS+BG. Values are presented as means \pm standard deviation. Statistically significant differences ($p < 0.05$) from the control group are indicated by *.



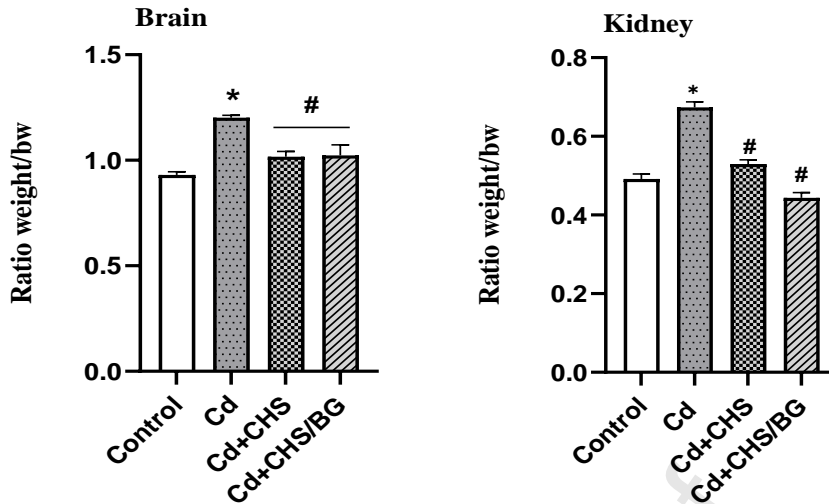


Fig. 6. Graphic representation of different organ weight ratios (Liver, Kidneys, Heart, Lungs, Brain and Spleen). Results are expressed as a percentage of the ratio of organ weight to animal weight (%). Values are presented as means \pm standard deviation. Statistically significant differences ($p < 0.05$) from the control group are indicated by * and from the CHS group by #. Statistical evaluation was performed using a one-way ANOVA ($p=0.05$).

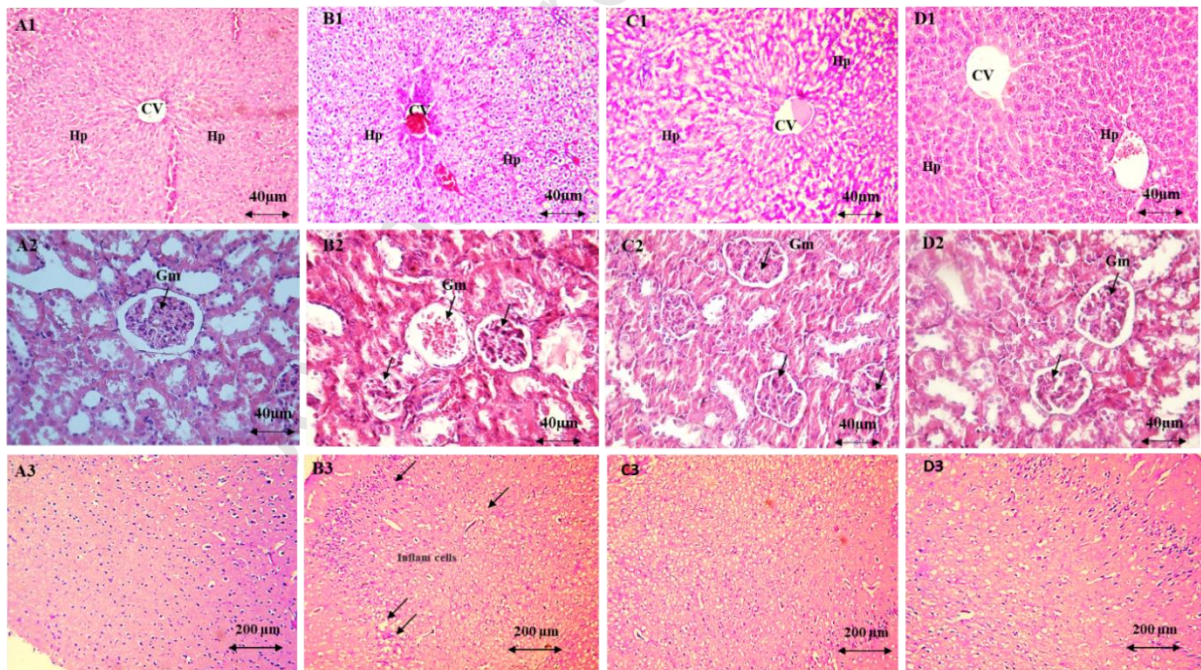


Fig. 7. Histopathological examinations of the liver and kidneys after Cadmium toxicity following treatment with CHS and BG. **Liver tissue:** Control (A1), Cd-intoxicated Group (B1), Group treated with Cd+CHS (C1), and Group treated with Cd+CHS/BG (D1). **Kidney tissue:** Control (A2), Cd-intoxicated Group (B2), Group treated with Cd+CHS (C2), and Group treated with Cd + CHS/BG (D2). **Brain tissue:** Control (A3), Cd-intoxicated Group (B3), Group treated with Cd+CHS (C3), and Group treated with Cd+CHS/BG (D3), Inflamm cells inflammatory cells, (100 \times , H&E stained).

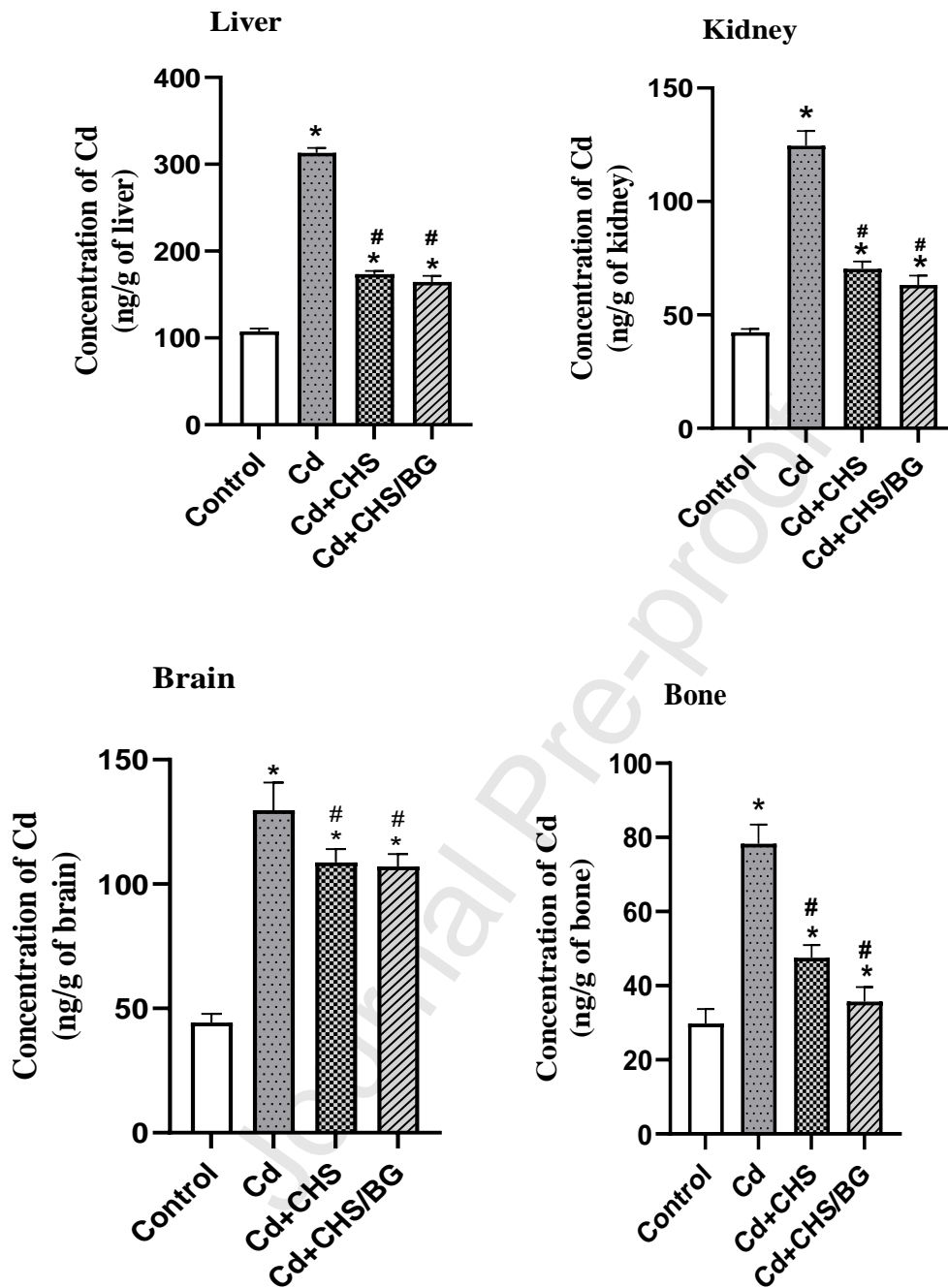


Fig. 8. Histograms showing Cd levels in the different control, intoxicated and treated groups.

Results are expressed in ng/g of organ (liver, kidney, brain and bone marrow). Values are presented as means \pm standard deviation. Statistically significant differences ($p < 0.05$) from the control group are indicated by * and from the CHS group by #. Statistical evaluation was performed using a one-way ANOVA ($p=0.05$).

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Abdelfatah Ait BABA: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation.

Hajar AZRAIDA: Review, Writing – Original draft.

Hamid KABBDY: Investigation, Review

Hassane OUDADESSE: Supervision; Validation; Visualization, Review & editing.

Abderrahman CHAIT: Supervision & Validation

Mohammed RHAZI: Conceptualization, Methodology, Supervision; Validation; Visualization, review & editing.