Applied Clay Science 103 (2015) 62–66

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Research paper

Efficacy of orally administered montmorillonite for acute iron poisoning detoxification in rat

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ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 3 November 2014
Accepted 5 November 2014
Available online 21 November 2014

Keywords:
Geophagy
Montmorillonite
Iron toxicity
Iron adsorption

ABSTRACT

Iron overload in the amount of 10–20 mg/kg of body weight can induce toxicity. Iron causes its toxic effects in a form of oxidative stress and inhibition of key metabolic enzymes. It seems that montmorillonite clay can reduce absorption of elemental iron by iron fixation in the gastrointestinal tract. The potential effect of montmorillonite in decontaminating the gastrointestinal tract was investigated based on serum iron concentration. In the present experiment, ferrous sulfate (100 mg/kg) was administered to Wistar male rats followed by oral gavage of montmorillonite suspensions at three different doses (0.5, 1.0 and 1.5 g/kg). Blood for determination of serum iron concentrations was drawn at 1 h after montmorillonite administration. The results showed that montmorillonite clay with doses of 0.5 and 1.0 g/kg were the best for significantly decreasing the elevated serum iron concentration compared to 1.5 g/kg ($p < 0.05$), probably through the proper adsorption of elemental iron. The activity of control and 1.5 g/kg treated groups diminished compared with other groups. Rats in the control group also defecated loser fecal matter than others.

1. Introduction

Geophagy, the habit of eating clay or earth, has been practiced by humans for a long period of time (Abrahams et al., 2006; Gichumbi et al., 2011; Hooda et al., 2004; Mahaney et al., 2000; Reilly and Henry, 2000). Examination of the diets of certain aboriginal tribes of South America, Central Africa, and Australia, showed that people use clay to keep away from getting stomachache, dysentery, and food infections (Droy-Lefaix and Tateo, 2006). Sailors traditionally carried out similar practice on board ships (Droy-Lefaix and Tateo, 2006). Geophagia has also been considered as a source of mineral nutrient supplementation such as calcium, magnesium, zinc, manganese and iron (Hooda et al., 2004; Tateo and Summa, 2007; Tateo et al., 2001), but many researchers have introduced soil consumption as a risk factor of anemia by decreasing metabolic iron absorption (Dreyer et al., 2004; Minnich et al., 1968; Tateo and Summa, 2007; Young et al., 2010, 2011a, 2011b).

The human body can not directly increase iron excretion, so regular iron monitoring is done through the digestive tract. Acute iron toxicity is induced by ingestion of 10–20 mg/kg of elemental iron per kg of body mass (Tokar et al., 2013). Serious symptoms include alterations in level of consciousness, persistent vomiting, hematemesis, diarrhea, hemodynamic instability, metabolic acidosis, lethargy, tachycardia, hypovolemia, shock and coagulopathy occurring in acute iron toxicity (Hosking, 1971; Manoguerra et al., 2005; Perrone, 2011). Oxidative stress and inhibition of key metabolic enzymes are two important mechanisms in iron poisoning (Papanikolaou and Pantopoulos, 2005; Tokar et al., 2013).

Approximately 5000 cases of iron supplement poisoning occur annually in the United States, and most of them occur in children by consuming iron tablets, capsules and syrup or drop ingestion. According to American Association of Poison Control Centres report, iron is one of the leading causes of poisoning deaths in children <6 years old (Brown and Gray, 1955; Eshel et al., 2000; Litovitz et al., 1986; Manoguerra et al., 2005; Whittaker et al., 2002). The mainstay of acute iron toxicity management includes early gastrointestinal decontamination by gastric lavage or whole gut irrigation; chelating therapy with deferoxamine (or deferipirone) and organ failure treatment. Activated charcoal is a first-line treatment for poisonings and standard method of gastrointestinal decontamination but it has no good effect on iron absorption. Whole bowel irrigation also has not altered the clinical outcome of iron-poisoned patients (Eshel et al., 2000; Tennebein et al., 1991).

Montmorillonite is a clay mineral with substantial isomorphic substitution of structural cations, which lead to negative charges on the basal layers. Exchangeable cations in the 2:1 layers balance the

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http://dx.doi.org/10.1016/j.clay.2014.11.010

negative charges generated by isomorphic substitution. The kinetics of cation exchange in montmorillonite is fast and the cations are easily exchanged with solute ions by varying the cationic composition of the solution (Bhattacharyya and Gupta, 2008). The technological properties of clays are directly related to their colloidal size and crystalline structure in layers, meaning a high specific surface area, optimum rheological characteristics and/or excellent sorptive ability and a high affinity to various heavy metal ions (Bhattacharyya and Gupta, 2008; Chantawong, 2004; Kraepiel et al., 1999; López-Galindo et al., 2007; Shirvani et al., 2006). Based on these characteristics, montmorillonite has been used in biomedicine and clinical therapies (Han et al., 2012) as well as to prevent the accumulation of heavy metals in organs (Han et al., 2012; Kim and Du, 2009; Wei et al., 2010).

In the last decade few studies have been conducted in relation to iron adsorption by clay minerals in the gastrointestinal tract and their potential on preventing iron entry to blood (Hooda et al., 2004; Minnich et al., 1968); therefore the hypothesis of clay minerals as the most active part of soil was evaluated for the treatment of acute iron poisoning. Finally, because there is no known study regarding the efficacy of montmorillonite on iron absorption in acute toxicity, we made considerable effort to investigate the quantity of Fe adsorbed by montmorillonite in iron intoxicated animals.

2. Experimental

2.1. Montmorillonite purification and preparation

Montmorillonite samples were obtained from raw bentonite. Raw bentonite was prepared by Ghaen Zarin Khak Company (with trade name of Zarin binder). The bentonite mine is located in the city of Ghaen in the South Khorasan province and it includes more than 80% montmorillonite and also has some impurities such as quartz, feldspars, halite, gypsum and mica. It is very important that quartz and feldspars, which have large amounts of silica, are removed from clay mineral. The mean particle size of the raw bentonite was 325 mesh (44 μm diameter). To remove unwanted microorganisms and organic matter from samples, thermal processing at about 350 °C was performed. A combination of purification methods were carried out including wet sieving sedimentation, centrifugation and ultrasonification. Purification carried out according to the method described by Ha Thuc et al. (2010) with some modifications.

In order to separate the pure montmorillonite particles (particle size-fractions required: <2 μm) from associated minerals (quartz, feldspar, etc.), 50 g of bentonite was suspended in 1000 mL of distilled water by laying the cylinder for 24 h only for separation of clay particles, then clay suspension was stirred again and the <2 μm fraction of a clay with density 2.65 g/cc was collected after 4.5 h from a distance of 6.1 cm (Burt, 2004). The sand fraction (>20 μm) was separated from the silt fraction using a 20 μm sieve. The supernatant liquids containing clay-sized particles of <2 mm particles were siphoned into separate 100 mL centrifuge bottles.

Chemical treatments were applied to remove impurities such as salts, gypsum and calcium carbonate from samples (Ha Thuc et al. (2010). Sodium acetate–acetic acid (pH 5) buffer solution was used to remove carbonates from samples (Duman and Tunc, 2009; Ha Thuc et al., 2010). At the final stage, purified samples were cation exchanged with sodium chloride to produce homogeneous interlayer cations. The purified and cation exchanged samples were then dried at 110 ° C for 24 h and ground to powder.

The efficiency of purification was determined based on X-ray, particle size, cation exchange capacity (CEC) and ratio peak of the quartz/montmorillonite analysis pre and post experiments. For XRD analysis montmorillonite samples were characterized by X-ray diffraction (XRD, λCuK$\alpha = 1.54$) using a Bragg–Brentano (θ, 2θ) and XRD pattern collected from $2\theta = 4°$ to 60°. X-ray diffraction (XRD) measurement was performed using a Philips PW1800 model diffractometer. The XRD pattern of purified montmorillonite was given in Fig. 1. The morphology of the samples was observed by scanning electron microscopy (SEM). CEC determination was done with ammonium acetate (NH4OAc) method. The particle size distribution of purified montmorillonite clay was determined by laser granulometry to determine the efficiency in separating the small particles from the whole materials by CORDOUAN model of particle size analyzer in Central Laboratory of Ferdowsi university of Mashhad.

2.2. Animal study

All animal experiments were approved by the Animal Care Committee of the Mashhad University of Medical Sciences. Twenty male Wistar rats, 250–270 g weight, were divided in four groups. All animals received 100 mg/kg body weight of ferrous sulfate by applying ferrous sulfate drops (FSD) (Mad Pharmaceutical Company, Iran) by oral gavage.

Montmorillonite suspensions were prepared by mixing various amounts of clay in distilled water, gavaged to the rats at three doses (0.5, 1.0 and 1.5 g/kg).

Animals were deprived of food 12 h before the test. Five minutes after iron administration, rats were orally gavaged with clay suspensions. The control group received only the iron solution in distilled water. The rats were lightly anesthetized with ether during blood sampling. Blood samples were collected from orbital sinus before and 1 h after iron gavage. Blood serum samples were separated by centrifuge. Serum iron level (SIL) was measured by Pars Azmoon Kit according to the instructions of the manufacturer. The absorbance was measured at 578 nm using enzyme-linked immunosorbent assay (ELISA) reader (Statfax 2100, USA).

The stool consistency and activity scores of the animals were all monitored. Stool consistency was scored as follows: 1: constipation, 2: stool with normal consistency, 3: loose stool and 4: defecation incontinence. The activity score of animals was evaluated according to our previous work. Grade 1: no voluntary movements after painful stimulation, Grade 2: stretch movements after painful stimulation, Grade 3: stretch movements after stimulation and Grade 4: spontaneous normal mobility (Moshiri et al., 2013). All animals were evaluated by an individual unaware of the groups.

2.3. Statistical analysis

Results are expressed as mean ± standard deviations and were analyzed with ANOVA and Mann–Whitney test by using Spss11.5 software. Statistical significance was set at $p < 0.05$ for all tests.

3. Results and discussion

There was no significant difference among the mean SIL of different groups of rats before FSD gavages ($p < 0.05$). The average serum iron concentrations of the control group were significantly higher than SIL among rats treated with 0.5 and 1.0 g/kg of montmorillonite 1 h after FSD administration (Fig. 2). However, there was no significant difference between SIL of rats that received 1.5 g/kg montmorillonite and control groups ($p > 0.05$).

The animal activity in control and 1.5 g/kg of montmorillonite groups diminished in comparison to others (Table 1). The animals of the control group also defecated looser and darker fecal matter than others. All groups treated by montmorillonite had normal light stools without any constipation (Table 1). Constipation is the most common adverse effect of activated charcoal (Osterhoudt et al., 2004) but in our experiments we did not observe any constipation, therefore it seems that montmorillonite in the concentrations given can be more effective against constipation, however, it could be hazardous to ingest far more clay than is necessary—eventual constipation will occur at high doses. If montmorillonite leads to constipation we can consider a