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Effects of the *Matricaria recutita L.* (German Chamomile) on sheep erythrocyte osmotic fragility

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ABSTRACT : German chamomile is a medicinal herb widely used, primarily as a sedative, anxiolytic, antiseptic, anti-inflammatory and antispasmodic, and for the treatment of nightmares, hemorroids, mastitis, rheumatic pain, oral mucositis induced by methotrexato therapy, conjunctivitis, colds and flu, flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, vomiting, colic, fevers, induce menstruation, strengthen the uterus and vulnerary (wound healing). Its active constituents are terpenoids (bisabolol, chamazulene, and others), flavonoids (apigenin and others). The capability of red blood cells (RBC) to resist hemolysis characterizes what is called the osmotic fragility (OF) of the membrane. We have investigated the possibility that German chamomile is capable of altering the physiological properties of the RBC. The osmotic fragility evaluations of the RBC were performed with blood samples incubated with a chamomile extract (0.032 mg/mL) or with sodium chloride solution (0.90% NaCl) as a control for 60 minutes at room temperature. Briefly, RBC samples (25 μL), were gently mixed with hypotonic NaCl (from 0.12 to 0.90%), centrifuged, the supernatants were isolated to determine the optical density (OD) in a spectrophotometer (540 nm). Statistical analysis was performed. Osmotic fragility was significantly ($p<0.05$) changed by the presence of the chamomile extract in the isotonic concentrations. Finally, the alterations to RBC membrane transport mechanisms were sufficient to promote modifications in osmotic fragility. Our results suggest that, the chemical properties of German chamomile could be responsible for the observed effects.

KEYWORDS: Osmotic Fragility; Red Blood Cell; German chamomile.

INTRODUCTION

Chamomile is a daisylike, aplle-scented flower that has been used medicinally for thousands of years. In contemporary Germany, it is considered a cure-all (1). German chamomile is a medicinal herb widely used, primarily as a sedative, anxiolytic, antiseptic and anti-inflammatory and for the treatment of nightmares, hemorroids, mastitis, rheumatic pain, oral mucositis induced by methotrexato therapy (2), conjunctivitis, colds and flu, flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, vomiting, colic, fevers, induce menstruation, strengthen the uterus and vulnerary (wound healing) (3).

Blood contains many types of cells with very different functions, ranging from the transport of oxygen to the production of antibodies. The sodium-potassium pump has a direct role in regulating red blood cell (RBC) volume: It controls the solute concentration inside the cell, thereby regulating the osmotic forces that can make a cell swell or shrink (4).

The capability of RBC to resist hemolysis characterizes what is called the osmotic fragility (OF) of the membrane. The osmotic fragility is classically used as a general screening procedure (5, 6). The "fragility curve" reflects the structural and geometrical changes in RBC. Hemolytic results from a structural perturbation of the RBC and its cytoskeleton caused by its high partition in the membrane (5, 7). The aim of this study was to investigate the possibility that German chamomile is capable to altering the physiological properties of the red blood cells (RBC).

MATERIAL AND METHODS

Reagents

The reagent sodium chloride (NaCl) (Merck S.A., Brazil) was used to prepare the solutions to evaluate osmotic fragility. German chamomile was obtained of the Hikary Indústria e Comércio LTDA, Brazil.

Preparation of the chamomile extract

German chamomile extract was prepared by infusion of one 3.2 mg of German chamomile dust in a total

volume of 100 mL of 0.9% NaCL. The solution obtained was considered 0.032mg/mL of German chamomile and denominated chamomile extract.

The experimental procedure

Blood was withdrawn from a female sheep with a heparinized syringe. The osmotic fragility evaluations of the RBC were performed with sheep blood samples incubated with chamomile extract or with 0.90% NaCl as a control, for 60 minutes at room temperature. According to Dacie's modified method (7), a drop of blood samples (25 μL), incubated with chamomile extract or with sodium chloride solution, were gently mixed with 5mL of hypotonic NaCl solutions with concentrations from 0.12 to 0.90%. After 30 min, these tubes were centrifuged (1500 rpm, 15 min). The supernatants were isolated to determine the optical density (OD) of the hemoglobin in a spectrophotometer (540 nm). The optical density of each supernatant was compared with that corresponding to stronger hypotonic solution (0.12% NaCl) that was considered 100% of hemolysis. The supernatant of the tube, which contained 0.90% NaCl, was considered the blank "tube" for the reaction, because it has no hemolysis.

According to Cavalcanti and collaborators (7), three intervals were determinates: interval 1 between 0.12 and 0.36% NaCl, interval 2 between 0.36 and 0.60% NaCl and interval 3 between 0.60 and 0.90% NaCl, according the curve tendency. The means and SD of each interval was determinate and the statistical analysis was performed. The experiments were carried out in compliance with guidelines on the use of live animals in scientific investigations.

Statistical analysis

The results were compared with the control samples, and statistical analysis was performed by independent t-test (at significance level of 0.05) to determine the significance of the difference between incubated with chamomile extract and control samples.

RESULTS

The osmotic fragility evaluations of the RBC were performed with blood samples incubated with German chamomile (0.032 mg/mL) or with sodium chloride solution (0.90% NaCl) as a control, for 60 minutes at 37°C. Briefly, RBC samples (25 μL), treated or not, were gently mixed with hypotonic NaCl (from 0.12 to 0.90%) solutions according to Dacie`s modified method. After 30 min, at 37°, these tubes were centrifuged (1500 rpm, 15 min). The supernatants were isolated to determine the optical density (OD) in a spectrophotometer (540 nm). The optical density of each supernatant was compared with that corresponding to 100% lyses (0.12% NaCl). The supernatant of the tube, which contained 0.90% NaCl, was considered the blank "tube" for the reaction.

Figure1: Osmotic fragility tendency of red blood cell, treated with German chamomile

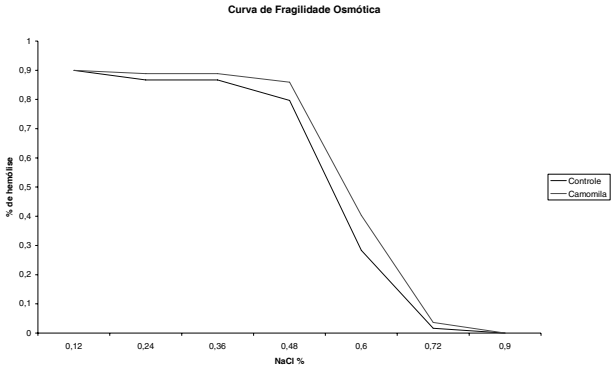
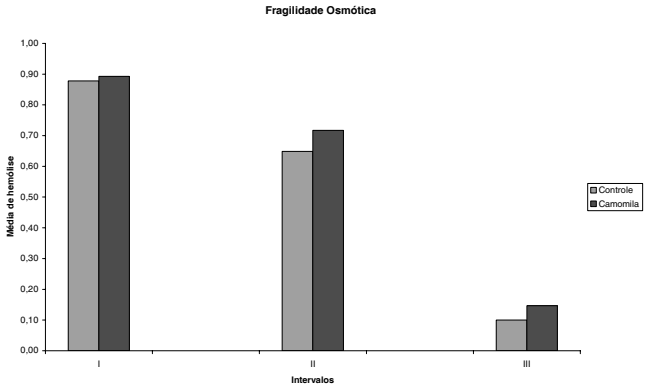


Figure 1 shows the osmotic fragility of the sheep erythrocyte incubated with chamomile extract when reacted with different NaCl hypotonic solutions. The curve tendency shows that in small concentrations of the salt the hemolysis increased.

Figure 2: the osmotic fragility of red blood cells treated with German chamomile



Data in each interval represent mean ± SD. Three intervals were determinates, interval 1 between 0.12 and 0.36% NaCl, interval 2 between 0.36 and 0.60% NaCl and interval 3 between 0.60 and 0.90% NaCl, according the curve tendency. The means and SD of each interval was determinate and the t-test ($p<0.05$) was used to analyze potential differences between the percentage of hemolysis of each interval.

Figure 2 presents the mean of the osmotic fragility after analyses of the three NaCl concentrations intervals obtained of the osmotic curve of the figure 1. The analysis of the results showed a significant statistical

increase ($p<0.05$) on osmotic fragility of erythrocyte incubated with chamomile extract in the intervals 2 (0.36 to 0.60% NaCl) and 3 (0.60 to 0.90% NaCl).

DISCUSSION

A large number of drugs that cause alterations on the shape and physiology of the red cells have been cited by some authors (8, 9). Our group has demonstrated that the natural products *Thuya occidentalis* (10), tobacco (11), *Peumus boldus* (12), *Maytenus ilicifolia* (13), *Mentha crispa* (14), *Hypericum perforatum* (15) are able to interfere on the labeling of red blood cells with 99mTc and alter the fixation of this radionuclide to the precipitated blood proteins.

We have previously reported that *Mentha crispa* and *Piper Methysticum* alter morphologicaly the RBC by expressing an increased morphometric factor (16). Moreover, the results obtained with the quality comparison of the shape of the RBC (non treated and treated with natural extracts) under optical microscopy could justify the modifications in the uptake of 99mTc by red blood cells in the presence of *Mentha crispa* extract, similar to that observed with the extract of *Maytenus iliciofolia* (13). The achieved results have revealed important morphological alterations due to the treatment of blood with *Mentha crispa* extract (14). Hiperico extract also produced modifications in the uptake of 99mTc by RBC and in the uptake of the radiopharmaceutical sodium pertechnetate ($99mTcO_4Na$) by thyroid, stomach and pancreas (15).

Erythrocyte osmotic fragility is the resistance of RBC hemolysis to osmotic changes that is used to evaluate RBC friability (17). In the present study we have found that the RBC osmotic fragility was changed by presence of the chamomile extract in the studied concentration. The results showed that there was a significant statistical increase ($p<0.05$) in the osmotic fragility of those cells treated with chamomile extract (72%) relative to the control (65%) in the interval 2 (0.36 to 0.60% NaCl) that is related to the hypotonic interval of the osmotic curve (Figure 1). In the interval 3 (0.60 to 0.90% NaCl) that is related to the isotonic interval, the osmotic fragility also increased significantly ($p<0.05$) with chamomile extract (15%) relative to the control (10%). Components present in chamomile extract could be altering (i) the erythrocyte membrane morphology, (II) the erythrocyte membrane ions transport or (iii) the osmotic transport balance. The different alterations could be inducing the stronger osmotic fragility in isotonic concentrations of NaCl.

In conclusion, the experiment shows that the osmotic fragility of the RBC can be increased in the presence of the chamomile extract and we can suggest that this effect may be due to the properties of this natural product.

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