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Potential Pitfalls on the Physiological (Osmotic Fragility) Properties of the Red Blood Cell: Action of a Homeopathic Medicine

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ABSTRACT - *Hypericum perforatum* (Hypericum p.) is a medicinal herb worldwide, primarily as a containing many polyphenolic compounds, popularly called St. John's wort or in homeopathic nomenclature of thousand needles plant. It active principle is the hipericin. Hypericum p. has been used in popular medicine since ancient times for several disorders such as skin wounds, eczema, burns, and diseases of the alimentary tract, insomnia, and mental illness, among others as well as in intolerable pains. 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 hypericum perforatum dinamized homeopathicly is capable of altering the physiological properties as RBC-OF. Briefly, blood samples were incubated with a *hypericum perforatum* 6CH or with sodium chloride solution (0,90% NaCl) as a control for 60 minutes at room temperature. Samples (25µl), were gently mixed with hypotonic NaCl (from 0.12 to 0.90%), centrifuged and the supernatants were isolated to determine the optical density (OD) in a spectrophotometer (540nm). Statistical analysis was performed. Osmotic fragility was significantly ($p<0.05$) changed by the presence of the *Hypericum perforatum* 6CH 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 results of *Hypericum perforatum* 6CH could be responsible for the observed effects.
KEYWORDS - Osmotic fragility; Red blood cell; *Hypericum perforatum* 6CH; Homeopathic medicine.

INTRODUCTION

The use of natural products has increased in all over the world, and popular interest in complementary and alternative medicine (CAM) in Brazil is as large as it is in other countries (1-4) especially with regard to phytotherapy, homeopathy and acupuncture. Medicinal plants are used for the human being however several biological effects and the consequences for the health have not been well established yet. Many plants contain active substances that can induce biological effects and their frequent use has been correlated with a high incidence of diseases or undesired biological effect in the population (5-7). However, various active compounds derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of breast cancer and have been evaluated in clinical trials (8).

Hypericum perforatum (*Hypericaceae*) is a medicinal plant species containing many polyphenolic compounds, popularly called St. John's wort or in homeopathic nomenclature of thousand needles plant, it is a vivacious plant of the family of the *Hyperiaceae*, who grows in hills and dry places, whose active principle is the hipericin. Has been used in popular medicine since ancient times for several disorders such as skin wounds, eczema, burns, and diseases of the alimentary tract, insomnia, and mental illness (9, 10), among others as well as in intolerable pains (10).

Hypericum p. extract contains flavonoids such as rutin, quercetin, and quercitrin, which demonstrated a free radical scavenging activity in a model of autooxidation of rat cerebral membranes (11). Hypericum p., in line with popular credence, also possesses anxiolytic, antiviral, wound healing, antimicrobial, analgesic, and anti-inflammatory effects (12).

Hypericum p. extract has always been referred to as having a benign side-effect profile compared to

tricyclic antidepressants and serotonin-specific reuptake inhibitors (13). Hypericum p. extract, as an efficacious antidepressant medication with a potential antioxidant activity, was therefore hypothesized to be useful in the treatment of pathological situations in which ROS play an important role such us acute inflammation (14).

Homeopathy, recognized as the medicine of the similarities, for using medicines that more resemble the patient; it was enunciated by Hipócrates (450AC): "Similia Similibus Curantur"; or either, *Like Cures Like*' (15). Introduced by the German physician Samuel Christian Frederich Samuel Hahnemann at the beginning of the 19th century, consists of the treatment of the symptoms of a disease by means of ultra-diluted substances. These substances are chosen based on the assumption that they would cause the same symptoms in healthy subjects if ingested in measurable amounts. Minerals, vegetables, or substances obtained from living organisms may be used in the preparation of homeopathic medicines. Supposedly, the effect of these substances becomes stronger the more they are diluted. An important step in their preparation is called the *succussion*: "shakes" that are applied to the substance after each dilution stage. (16,17).

The homeopathic performance of Hypericum p. has been observed in the terminations of the sensitive nerves, in intolerable pains (10). Hypericum p. has been considered by some authors as antidepressant, so the homeopathic medicine is not used specifically for this. But considering its pathogenesis, described in the homeopathic materia medica, Hypericum p. has indicative to depression state (18).

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 (19).

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 (20,21). 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 (20, 22).

The aim of this study was investigated the possibility that a medicine homeopathic Hypericum p. 6CH (diluted and dinamized homepathicly) is capable of altering the physiological properties as well as osmotic fragility of the red blood cells (RBC).

MATERIAL AND METHODS

Reagents

The reagent NaCl (Merck S.A., Brazil) was used to prepare the solutions to evaluate osmotic fragility. Hypericum p. was obtained of the Novo extrato Farmácia homeopática Ltda, Brazil as a NaCl 0.90% dilution. Heparinized whole blood was withdrawn from a female sheep. The osmotic fragility evaluations of the RBC were performed with blood samples incubated with Hypericum p. dinamized homeopathicly as 6CH.

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 Hypericum p. 6CH or with sodium chloride solution (0.90% NaCl) as a control for 60 minutes at room temperature. 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 (Cavalcanti *et al*, 2003). After 30 min, at room temperature, these tubes were centrifuged (3500 rpm, 15 min). The supernatants were isolated to determine the hemoglobin optical density (OD) 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.

There are 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 (Cavalcanti *et al*, 2003). 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

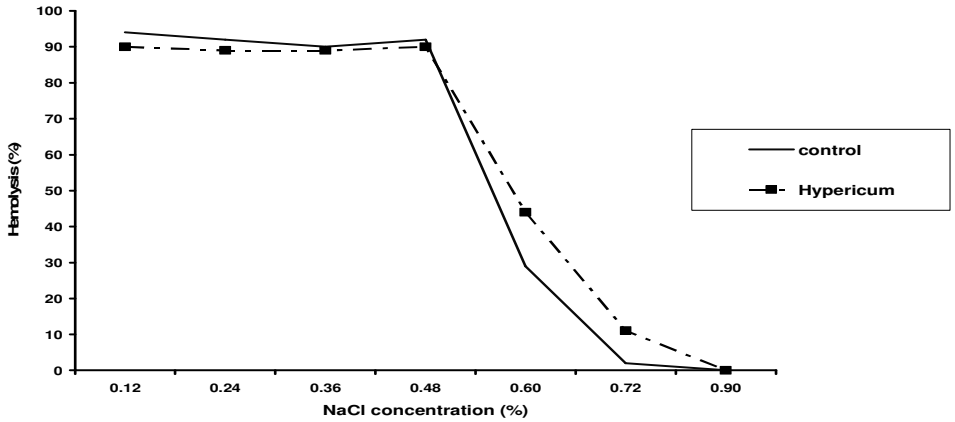
The results were compared with the control samples, and statistical analysis was performed by independent test ($p<0,05$) to determine the significance of the difference between incubated with Hypericum p. 6CH and control samples.

RESULTS

Figure 1 shows the osmotic fragility of the sheep erytrocyte incubated with Hypericum p. 6CH when

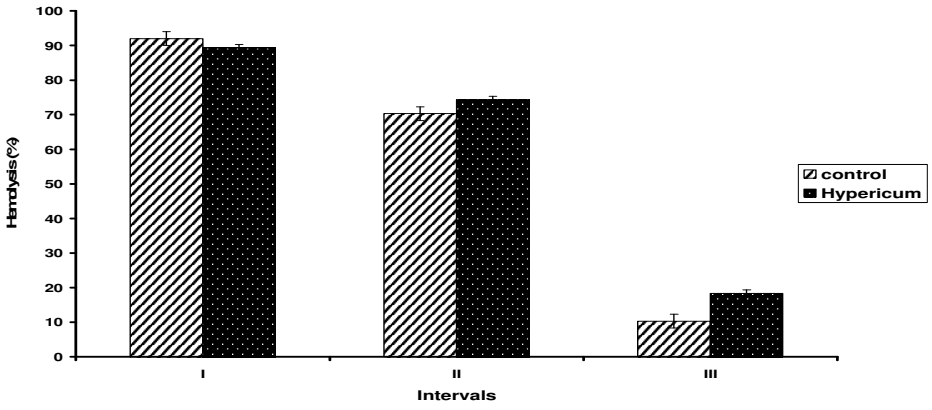
reacted with different NaCl hypotonic solutions. The curve tendency shows that in hypotonic and isotonic solutions the hemolysis increased significantly ($p<0.05$). Figure 2 presents the mean of the osmotic fragility after analysis of the three NaCl concentrations intervals obtained of the osmotic curve of the figure 1. The analysis of the results confirmed a significant statistical increase ($p<0.05$) on osmotic fragility of the erythrocyte incubated with Hypericum p. 6CH in the intervals 2 (0.36 to 0.60% NaCl) and 3 (0.60 to 0.90% NaCl).

Figure 1: Osmotic fragility tendency of red blood cell, treated with Hypericum perforatum



The osmotic fragility evalutions of the RBC were performed with blood samples incubated with hypericum perforatum 6CH (diluted and dinamized homepathicly) or with sodium chloride solution (0.90% NaCl) as a control, for 60 minutes at 37º. Briefly, RBC samples (25µl), 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 37º, these tubes were centrifuged (1500rpm, 15 min). The supernatants were isolated to determine the optical density (OD) in a spectrophotometer (540nm). 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.

Figure 2: The osmotic fragility of red blood cells treated with Hypericum perforatum



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.

DISCUSSION

Many authors have cited a large number of drugs which cause alterations on the shape and physiology of the red cells (23, 24). The evidence that drugs can interfere on the labeling of RBC has been demonstred with various natural products, like *Thuya occidentalis* (25), tobacco (26), *Peumus boldus* (27), *Maytenus ilicifolia,* (28), *Mentha crispa* (29), *Hypericum perforatum* (30).

Previously, we have reported that *Mentha crispa* and *Piper Methysticum* alter morphologicaly the RBC by expressing an increased morphometric factor (31). 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* (28). The achieved results have revealed important morphological alterations due to the treatment of blood with *Mentha crispa* extract (29). Either, *Hipericum p*. extract produced modifications in the uptake of 99mTc by RBC and in the uptake of the radiopharmaceutical sodium pertechnetate (99mTcO4Na) by thyroid, stomach and pancreas (30).

The homeopathic Hipericum p. has indicative to depression state (18), and intolerable pains (10). However, we could not find report of natural products diluted and dinamized homeopathicly could interfere on the labeling of RBC.

Erythrocyte osmotic fragility is the resistance of RBC hemolysis to osmotic changes that is used to evaluate RBC friability (32). In the present study we have found that the osmotic fragility of RBC's was changed by presence of Hypericum p. 6CH in the studied concentrations. The results showed that there was a significant statistical ($p<0.05$) increase in the osmotic fragility of those cells treated with Hypericum p. 6CH (71%) relative to control (68%) in the interval 2 (0.36 and 0.60% NaCl) that is hypotonic interval of the osmotic curve (Figure 1). In the interval 3 (0.60 and 0.90% NaCl) that is isotonic interval, the osmotic fragility also increased significantly ($p<0.05$) with Hypericum p. 6CH (16%) relative to control (10%).

These experimental data show that the osmotic fragility of the RBC can be increased in the presence of Hypericum p. 6CH solution and we can suggest that this effect may be due to the properties of this medicine that may (i) alter the RBC physical properties, (ii) have a direct or an indirect effect on intracellular sodium ion concentration and (iii) the medicine homeopathic could not be considerate *a placebo effect*.

Conclusion

The experiment shows that the osmotic fragility of the RBC can be increased in the presence of the Hypericum p. 6CH and we can suggest that this effect may be due to the properties of this homeopathic medicine.

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