

Once the eligibility of the subjects to enter the study was confirmed, 6 male subjects were chosen to proceed with the procedure of the study.

A single oral dose of diacerein 50 mg capsule was administered to the 6 healthy subjects, succeeded by 500 mg glucosamine capsule orally twice daily for 5 days to the same subjects. Another single oral dose of diacerein 50 mg capsule was administered once more to the subjects. Drug administration was performed by the Principal investigator and supervised by another medical professional to confirm drug intake and to verify compliance of the subjects. The administration was followed by a mouth and hand check.

Blood samples for rhein analysis, the active metabolite of diacerein, were collected pre-drug administration and at the following times post drug administration: 0.16, 0.33, 0.50, 0.75, 1.00, 1.33, 1.66, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00 hours.

Analysis of plasma concentrations was performed by means of high performance liquid chromatography with fluorescence detection method with selective quantization of rhein in plasma.

## 2. Discussion of study design

The study is divided into two stages, the details of which are:

- The first stage of the study: subjects were hospitalized the night before the start of the study and remained there for 12 hours post drug administration, they were examined for alcohol and drug consumption and received a standard dinner before 7.30 pm and they fasted overnight for ten hours, and given a single oral dose of 50 mg diacerein inserted in a labeled plastic pack.

Blood samples were withdrawn through a cannula placed into a suitable forearm or hand vein, pre-drug administration and at the following times post drug