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Comparative Evaluation of Antidepressant Effects of Two *Hypericum* Species (*H. perforatum* L. and *H. maculatum* C) in Swiss Mice

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Abstract. *H. perforatum* L. is widely used for the treatment of mild to moderate forms of depression. The aim of the present study is to compare the antidepressive effect of the *H. perforatum* to *H. maculatum*, the most widely spread species from Romania. The powdered aerial parts of vegetal product were extracted with ethanol 70°, minimum content in total hypericins for the medicinal drug were determined by a spectrophotometer method. The experiment was carried out on 15 female Swiss mice, divided in three equal groups, *H. perforatum* group, *H. maculatum* group and control. The both extracts were injected 30 minutes before the experiment, 70 mg DS/kg body weight. Forced Swimming Test (FST) was performed by using the classic Porsolt's method, with minor changes. Both extracts (*H. perforatum* and *H. maculatum*) increase the immobility time as compared to control, the difference were distinct statistically significant; so, unexpectedly *Hypericum sp.* alcoholic extracts provide similar sedative effect.

Keywords: *Hypericum sp.*, Forced Swimming Test, antidepressant, Swiss mice, sedative.

INTRODUCTION

Hypericum perforatum L. (*Hypericaceae*), commonly known as St. John's wort, is one of the best investigated medicinal plants. *H. perforatum* is widely used for the treatment of mild to moderate forms of depression. Some well documented clinical studies (Brenner *et al.*, 2000, De Vry *et al.*, 1999) had shown that alcoholic extract from these plants have at least the same efficiency as the conventional drugs but with far less side effects. It has been suggested that *H. maculatum* has antipanic and anxiolitic effects on human subjects (Bach-Rojecky *et al*., 2004).

Aerial parts of *Hypericum species* contain the following active substances: naphtodiantrons 0.1-0.5% (hypericin, pseudohypericin and their isomers), flavonoids 4-5% (hyperoside, rutoside, quercitroside, biapigenin), tannins 10-20%, hyperforin, proanthocyanins, caffeic acid derivatives, xanthons, essential oil 0.1-1% etc (Herman and Gheorgiu, 1961). The main active components of *Hypericum sp.* are thought to be hypericin and hyperforin, but other common plant constituents (e.g., flavonoids and flavonoid derivatives, xanthone derivatives, amentoflavone, biapigenin, volatile oil) that may have antidepressant effects. Although additional research is needed to definitively understand the effects of these components alone and in combination, most available *Hypericum sp.* formulations are now standardized to include hypericin (range: 0.1 to 0.4 %) and hyperforin (range: 2.0 to 4.0 %) because these constituents have been studied the most extensively.

In Romania's spontaneous flora are mentioned 12 species of *Hypericum* (Stănescu *et. al.*, 2004), the most widely spread, and used species are *H. perforatum* L. (in the hills regions) and *H. maculatum* C. (in the mountain regions) Tămaş *et al.* (2001); *H. maculatum* is also harvested in order to obtain *Hyperici herb* (Ciulei *et al.* 1993). Both Romanian Pharmacopoeia (R.Ph. 1993) and European Pharmacopoeia (E. Ph. 2008) accept as officinal only *H. perforatum* L, therefore the aim of the present study is to compare the antidepressive effect of the *H. perforatum* to *H. maculatum*, the most widely spread species from Romania.

Among all animal models, the *Forced Swimming Test* (FST) (Porsolt *et al.*, 1977) remains one of the most used tools for screening antidepressants (Petit-Demouliere *et al.*, 2005). This experiment is aiming to test the antidepressant efficiency of *H. maculatum* and *H. perforatum* alcoholic extract (70^0) on Swiss mice trough FST.

MATERIALS AND METHODS

Plant materials: the aerial parts of *H. perforatum* were harvested from Chiribiş (Bihor County) and the aerial parts of *H. maculatum* were harvested from Ciucea (Cluj County) in the blossom period, in June 2008. The vegetal products were dried and grounded to a fine powder (sieve VI - R. Ph. 1993) (Istudor, 1998).

*Preparation of tinctures***:** 10g of powdered aerial parts of vegetal product were extracted with 100 g of ethanol 70° at room temperature, as described in Romanian Pharmacopoeia X^{th} Edition.

In E. Ph. 2008 it is mentioned as minimum content in total hypericins for the medicinal drug *Hyperici herb* 0.08%, determined by a spectrophotometer method and a qualitative analysis by TLC (York *et al.*, 1990) in order to identify the flavonoids and hypericin

To prevent toxic effect of the alcohol, often more toxic than plant compounds dissolved in it, alcoholic solution was maintained at 70˚C until half of the content evaporates, than filed with sterile saline solution up to 5 ml for each mouse. The aqueous solution was administrated intraperitoneally immediately in order to prevent the bacterial and fungus contamination.

The animals were caged in groups of 5 per cage, at controlled temperature of 21-22ºC, humidity (40-60%) and reversed 12/12h light/dark cycle (light off at 10 a.m.). Standard lab chow, provided by National Institute for Research and Development "Cantacuzino" Bucharest (Batch no. 2 / 26.03.2010), and water were freely available. The permission for the animal tests and experiments has been given by the Bioethical Board of the Faculty of Veterinary Medicine Cluj-Napoca.

The experiment was carried out on 15 female Swiss mice, 3 months old and 30±3 g average body weight. The animals were divided in three equal groups, *H. perforatum* group, *H. maculatum* group and control. The extracts were injected 30 minutes before the experiment, 70 mg DS/kg body weight, for each extract in study, while control mice received ethanol 70°, subject to the same procedure of evaporation. .

Forced Swimming Test, (FST) was performed by using the classic Porsolt's method (Porsolt *et al.*, 1977), with minor changes as follows; the glass recipients, 15 l each, have had 30 cm in diameter, they were wrapped in black plastic foil so the animal, could not see outside the recipient. In every recipient it was poured taped water at a controlled temperature of 23±1˚C and 20 cm height. Subsequently it was introduced one mouse at a time in each one, giving it 2 min to adjust to the environment, then it was clocked the immobility time for the

next 4 min, meaning the time in which the mouse sat still or made only little movement necessary to keep its head above the water (Bach-Rojecky *et al*., 2004, Petit-Demouliere *et al.,* 2005, David *et al.,* 2003, Porsolt, 2000,). After that the mouse was removed from the recipient, dried up with a towel and put in a dry cage.

Statistics - the data were expressed as the mean and standard deviation. T Student multiple range test from Excel Windows Software was used to assess the differences among groups. Differences at $p<0.05$ and $p<0.01$ were considered significant and respectively distinct significant.

RESULTS AND DISCUSSION

Surprisingly, both extracts (*H. perforatum* and *H. maculatum*) increase the immobility time as compared to control, the difference were distinct statistically significant ($p<0.01$) (fig.1); therefore, in the present study, it had been shown that alcoholic extracts of both *Hypericum sp.,* induce an depressant-like effect in FST.

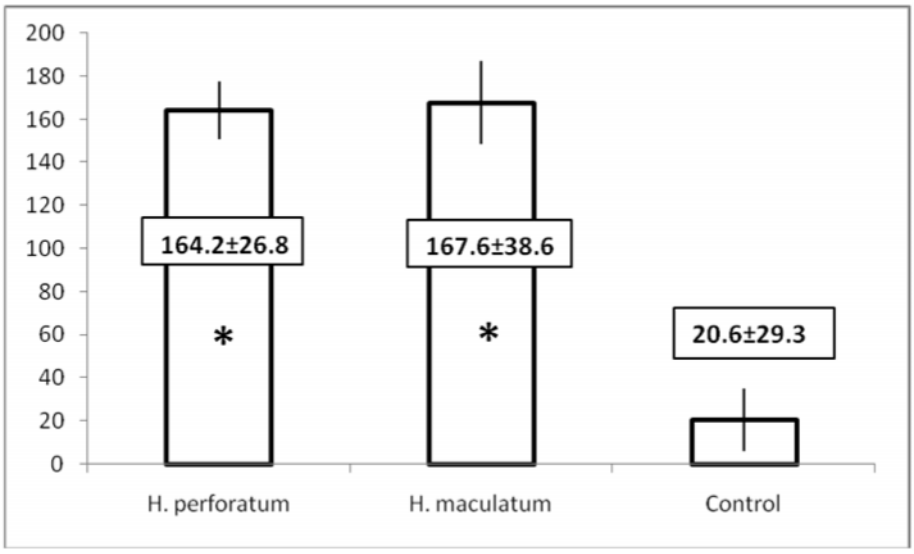

Fig. 1:The immobility time (mean ± S.D.) (sec).
*= statistically distinct significant at $p<0.01$ as compared to Control group

The immobility displayed in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. In FST, a prolonged exposure to an aversive situation induces immobility, interpreted as an expression of "behavioral despair", which could also be related to a depression syndrome (Porsolt, 2000, Porsolt *et al.,* 1977). Other studies found that after a single administration of the extract in doses of 7, 35, 70 mg kg b.m. suspension, showed that the immobility time of animals decreased dose-dependently, namely, the animals were more active, which means that the antidepressant effect was stronger (Bach-Rojecky *et al.,* 2004).

However, *H. perforatum* seems to have different effects according to the doses, it increase motor activity at 10 mg/kg and to decrease it at the highest dose injected (200 mg/kg). The results do not confirm other findings, according to which high doses of *H. perforatum* increase activity in Open Field Test (Giovanni *et al.,* 2007).

Muller *et al.* (2003) have suggested that *Hypericum sp.* acts via inhibition of the reuptake of serotonin, dopamine and noradrenaline, along with activation of gamma-aminobutyrate and glutamate receptors. At high dosages, hypericin is a monoamine oxidase inhibitor; however, these effects have not been demonstrated with the consumption of *Hypericum sp.* at dosages recommended for the treatment of depression (Lawvere and Mahoney, 2005). On the other hand, it has been reported that hyperforin, increases the release of striatal acetylcholine (Mennini and Gobbi, 2004); if confirmed by analytical studies, a substantial concentration of hyperforin might explain not only the reduced locomotor activity induced by *H. perforatum*, but, also, in *H. maculatum*.

CONCLUSIONS

Hypericum sp. alcoholic extracts (*H. perforatum* and *H. maculatum*) provide similar sedative effect; both of them significantly increase the immobility time in FST.

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