Stomatologija, Baltic Dental and Maxillofacial Journal, 18: 9-13, 2016

Hypericum perforatum L. treatment restored bone mass changes in swimming stressed rats

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SUMMARY

Stress, via corticosteroids release, influences bone mass density. *Hypericum perforatum* (Hp) a traditional remedy possess antidepressive activity (serotonin reuptake inhibitor) and wound healing properties. Hp preparation contains mainly hypericin, hyperforin, hyperoside and flavonoids exerting oestrogen-mimetic effect. Cold swimming represents an experimental model of stress associating mental strain and corporal exhaustion.

Objectives. This study investigates the Hp effect on femur and mandible bone mass changes in rats under cold forced swimming procedure.

Methods. 30 male Wistar rats were randomized into three groups.

Group A was treated with Methanolic extract of Hp (Jarsin®) via gastroesophageal catheter, and was submitted to cold swimming stress for 10 min/daily. Group B was submitted to cold stress, since group C served as control. Experiment duration was 10 days. Haematocrite and serum free fatty acids (FFA) were estimated. Furthermore volume and specific weight of each bone as well as bone mass density via dual energy X-Ray absorptiometry (DEXA) were measured. Statistic analysis by t-test.

Results. Hp treatment restores the stress injuries. Adrenals and bone mass density regain their normal values.

Conclusions. Injuries occurring by forced swimming stress in the rats are significantly improved by Hp treatment. Estrogen-like effects of Hp flavonoids eventually may act favorable in bone remodeling.

Key words: swimming stress, bone mass, *Hypericum perforatum*, rats.

INTRODUCTION

Stress affects the physiological corporal activity, as well as mental and psychical function influencing the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS0) (1). Cold swimming represents an excellent experimental model of stress associating mental strain and corporal exhaustion (2).

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It is already documented that stressor stimuli affects Bone Mass Density (BMD). Recent studies indicate that jaws panoramic radiography may serve as an index for BMD and may be useful tools in identifying postmenopausal women or subjects at risk for osteoporosis (low skeletal BMD) high bone turnover rate, or high incidence of osteoporotic fractures (3).

A large number of investigations have focused on the interrelationship between the systemic process of bone mass reduction and the possibility of a significant resorption of the alveolar bone. The degree of alveolar bone loss rise up with age and this may be attributed to systemic conditions that also promote the genesis and the development of osteoporosis. The relationship between the mandible and primary osteoporosis is already established by clinical studies (4). Although alveolar bone loss is independent from local and systemic factors, including osteoporosis, however a cross-sectional study in

post-menopausal women, evaluated the relationship of oral infection and age with osteoporosis and oral bone loss (5).

Hypericum perforatum L. (Guttiferae), a popular folk remedy, commonly known as St John's wort (SJW), is widely been used in the treatment of many disorders.

The plant is known to possess antidepressive activity as it inhibits serotonin reuptake and has recently been reported that it may serve as an effective treatment for mild to moderate depression (6). Studies have shown that SJW exhibits antidepressant like effects in experimental animal models of depression – such as the forced swim test (FST) (7).

The best known standardized extract of herba hyperici is LI 160 (Lichtwer Pharma GmbH, Berlin, Germany) The Hp preparation contains mainly hypericin, hyperforin, hyperoside and flavonoids that can exert oestrogen-mimetic However the exact role these compounds exert regarding the effects of hypericum on mood disorders is elusive (8).

Flavonol glycosides possess spasmolytic activity (9). In addition, they also inhibit monoamine oxidase A (MAO-A) and catechol-omethyltransferase (COMT) enzyme (10). Some flavonoids bind to the benzodiazepine receptor (11) and diverse authors argue that the flavonoids of Hypericum may act in the same way (12).

Flavonols present in plants as glycosides can be freed during fermentation and in the case of *Hypericum* flavonoids the quercetin is this phenolic compound. According Mueller *et al.* 2006 (12) LI 160 preparation (Jarsin Tab 300 mg , Lichtwer Pharma Berlin Germany) contains 300 mg of an 80% vol/vol methanolic dried extract hypericum herba *(Pharmacopoea Europea)* per coated tablet, while content per tablet is 24.8 mg of flavonoids, 13.75 mg of hyperforin and 0.43 mg of hypericin (13).

Since in previous study was proved that stress affects the bone mass of the whole skeleton including the jaw bone mass, it is interesting to investigate the role of Hp in the mandible of rats under forced swimming stress.

The aim of the study was to investigate the role of *Hypericum perforatum* on the femur and mandible mass discrepancy in Wistar rats submitted to the cold forced swimming procedure.

MATERIAL AND METHODS

30 male Wistar rats, aged 7-8 weeks, weighted of 230±15 g were randomized into three groups (A, B, C n=10 in each group) and the experiment duration was 10 days: group A was treated with 12.84 mg/kg/daily of dried LI160 *Hypericum perforatum*. Methanolic extract (Jarsin Tab 300®) divided into three doses (0.5 ml each) and administered via a gastroesophageal catheter, and was submitted to cold swimming stress for 10 min/daily.

Group B was submitted to cold stress as above, and group C was not subjected to stress and served as control.

Each tablet was cut and distilled water was added in order to obtain a concentration of 3.21 mg/0.5 ml. Adrenals, femur and mandible were iso-

Table 1. Adrenals weight and bone mass parameters of stressed rats under Hp. treatmentment

	Group A (Stress+Hp)	Group B (Stress)	Group C (Control)	
Adrenals g	0.285±0.09	0.59±0.18*	0.215±0.04	A/B p<0.01, B/C p<0.01
Adrenals weight/body weight mg/g	152±0.3	166±0.4**	130±0.7	
Mandibular weight/BW	0.0056±0.0007	0.0030±0.0003*	0.0031±0.00001	A/B p<0.05, B/C p<0.001
Mandibular specific weight mg/cm^3	2.0285±0.5186	1.3720± 0.2019**	1.4442±0.2026	A/B p<0.01, A/C p<0.01
Femur weight/BW	0.0072 ± 0.0004	0.0046±0.0001*	0.0056± 0.0004	A/B p<0.001
Femur specific weight mg/cm^3	1.4002± 0.0619	1.3849± 0.1234	1.6606± 0.2112	A/B p<0.01, A/C p<0.01, B/C p<0.05
Dexa mandible	0.218±0.0057	0.24±0.0212	0.2105±0.0167	A/C p<0.05, B/C p<0.05
Dexa femur	0.1567±0.0031	0.148±0.03505	0.1238±0.0111	A/B p<0.05

Table 2. VAS and MIO data before and 6 months after treatment

	Group A (Stress+Hp)	Group B (Stress)	Group C (Control)	
Ht%	50±5	52±2	47±2	NS
FFA µEq/l	0.75±0.15	0.88±0.23*	0.60±0.12	A/B p<0.05, B/C p<0.05
Corticosterone ng/ml	102±2.5**	162±3.1**	62±8	A/B p<0.001, B/C p<0.001

lated, removed and weighted. Blood was collected haematocrite (Ht%) serum FFA (Free Fatty Acid ELISA Kit) and corticosterone (Corticosterone Rat ELISA) were estimated. Furthermore the volume and the specific weight of each bone as well as BMD (Bone mass density) were measured via dual energy X-Ray absorptiometry (DEXA) with Lunar DPX, Dual Energy x-ray Bone Densitometer. Statistic analysis was performed with t-test. T-test was performed with QuickCalcs (Graph Pad Software San Diego, California).

Rats were provided the basal cereal diet and water ad libitum and were cared in cages under 12h light/darkness.

The animals were housed and cared according to the" Guide for the Care and Use of Experimental animals" (14) and have permission of the Greek Ethical EL.25BIO 009.

RESULTS

Adrenals absolute weight and the ratio adrenals weight/body weight was increased in group B demonstrating the influence of the stress process , both parameters seem to be ameliorated under the administration of *Hypericum perforatum* (Table 1).

Similarly serum FFA levels and haematocrite %, as expected in stress, were enhanced in group B in comparison to control group while the group A tends to be restored by the *Hypericum treatment* (Table 2).

Stress induced serum corticosterone rise tend to be normalized by *Hypericum* (Table 2).

Concerning the bone parameters, absolute, specific mandible and femur weights and DEXA, were decreased by stress and restored by *Hypericum perforatum* (Table 1).

DISCUSSION

The phloroglucinol derivative hyperforin has been recently shown to be a major antidepressant component in the extract of *Hypericum perforatum*. Experimental studies clearly demonstrated its activity in different behavioral models of depression. Moreover clinical studies linked the therapeutic efficacy of *Hypericum* extracts to their hyperforin content, in a dose-dependent manner. The molecular mechanism of action of hyperforin is still under investigation. Hyperforin has been shown to inhibit, like conventional antidepressants, the neuronal uptake of serotonin, norepinephrine and dopamine. However, hyperforin inhibits also the uptake of gamma-aminobutyric acid (GABA) and L-glutamate (15). Hypericin has also become the subject of intensive biochemical research and is proving to be a multifunctional agent in drug and medicinal applications. Recent studies report antidepressive, antineoplastic, antitumor and antiviral (human immunodeficiency and hepatitis C virus) activities of hypericin. These effects are intriguing even if confirmation of data is incomplete and mechanisms of these activities still remain largely unexplained. In other recent studies (16) screening hypericin for inhibitory effects on various pharmaceutically important enzymes such as MAO (monoaminoxidase), demonstrated therapeutic potential. Moreover the flavonoids presence in the active substances of *Hypericum perforatum L*. possess a substantial oestrogenic property that may be beneficial to the bone structure.

The cold swimming procedure affects essentially the animals pathophysiology increasing laboratory finding as FFA and haematocrite (17, 18).

The increase of adrenal gland weight is used as an indirect parameter of hypothalamic-pituitary-adrenal axis activation and it is obvious that is in accordance to the corticosterone values, which are enhanced under stress and tend to return to baseline after Hp treatment (19).

The interaction of the sympathetic nervous system (SNS) and the skeleton has received increasing attention in recent years. The deleterious effects of stress on skeletal integrity, whether mild or severe, may be attributed, to the elevated production of catecholamines and glucocorticoid (20).

Stress decreased both the femur specific weight and the ratio femur weight /body weight, as compared to controls, regained both indices their normal values following the Hp treatment as expected. Similarly, the Dual Energy X-Ray Absorptiometry (DEXA) of the femur showed changes in the stressed animals versus control that were ameliorated by Hp administration.

Likewise the mandible specific weight was decreased in the stress group versus control, Hp treatment restored mandible weight. On the other hand in stress condition the ratio mandible weight/ body weight showed no difference (21).

The mandible parameters may be characterized by discrepancies that are due to the permanent movement of the jaws and the tension strength of the masseter. Moreover the enhancement of mandible DEXA values in Hp treated animals could be attributed to differences between lingual and buccal cortex that may be responsible for this. In the rats mandibles are "V" shaped in superior/inferior view and separate at the midline as opposed to the "U" shaped singular construction of the human mandible.

The rat mandible is composed of approximately 10% trabecular bone and 90% cortical bone (22). Therefore the thickness of the cortical bone may have obscured any possible changes in trabecular bone (23). The difference in BMD was possibly below DEXA detection levels.

Many studies suggest an association between depression and osteoporosis. In a mouse model, depression induces bone loss, mediated by brain-to-bone sympathetic signaling. Depression and bone antianabolic sympathetic tone are alleviated by increasing central serotonin (5-hydroxytryptamine, 5-HT) levels. However, selective serotonin reuptake inhibitors (SSRIs), the first-line treatment for depression increase extracellular 5-HT levels but have deleterious skeletal effects (24).

Osteoclasts derive from hematopoietic cell precursors and a relationship between the bone and the immune system has been established (25). Then it is possible that neuroendocrine mechanisms, in particular those related to the serotonin (5-HT) system, may also regulate osteoclast differentiation/activation. In this regard, fluoxetine (Prozac), the most widely prescribed (SSRI) has been reported to reduce bone resorption in mice with adjuvant-induced arthritis (26). Such observations suggest that there may be a significant relationship between the 5-HT system and bone remodeling.

The proposed mechanism of action seems to be multiple. It is suggested that Hp. blocks serotonin, noradrenaline and dopamine reuptake non selectively, increases the density of serotonergic and dopaminergic receptors and the affinity for GABAergic receptors. Moreover, the inhibition of monoaminoxidase- A (MAO-A) activity cannot be excluded. In any case, the increase of monoamine concentrations in the synaptic cleft resembles several actions exerted by clinically effective antidepressants (27).

However, antidepressants (mainly SSRIs), or diazepam (28) should be evaluated in view of the causal relationship between stress/depression and bone loss, and *vis-à-vis* their skeletal adverse effects. Patients with depressive disorders should undergo a routine skeletal evaluation and receive timely antiosteoporotic therapy, especially when SSRI treatment is prescribed.

On the other hand SJW has be used to ameliorate the climacteric symptoms as depression and osteoporosis probably to its contents in phytoestrogens (29).

According the pharmacological effect of *Hypericum perforatum* the above results may be attributed to the smoothness of the stresses stimuli since the plant constitutes exerts clinically antidepressive action. It may be hypothesized that the observed additive synergistic effects induced by the administration of Hp extract is due to its different compound. These results and this general approach may have a significant impact on the understanding of phytomedicines in general and *H. perforatum* specifically.

Herbal medicines have been used for centuries to ease hormonal concern (16). *Hypericum perforatum* (St John's wort) was traditionally used for nervous afflictions including psychological symptoms of menopause. The herb has shown significant improvement in symptoms such as lowered mood, loss of interest, sleep disturbance and concentration.

Although antidepressant drugs are increasingly prescribed for menopausal women, they are associated with several adverse short-term effects and their long-term effects are not well understood. Numerous studies have shown St. John's Wort to be as effective as low doses of antidepressants in treating mild to moderate depression such as that associated with menopause.

The elevated concentration of corticosterone due to excessive or prolonged stress suppresses synaptic plasticity as well as, the ability to change the electrical connectivity between neurons in the hippocampus, which is thought to be the cellular mechanism of learning and memory. During stress procedures rats are involved in more chewing movement that probably the secretion of ACTH is decreased, in spite of stressor stimuli. Therefore it can be postulated that in mandible stress affects neither the ratio mandible weight/body weight nor the Dexa results.

One mechanism that may explain this ameliorative effect is the inhibition of systemic stress responses by mastication, which has been shown in various psychological and physical types of stress stimuli such as immobilisation, novelty exposure and tail pinch. First, mastication suppresses stress-related increases in core body temperature, blood pressure and the level of plasma adrenaline, suggesting that mastication ameliorates the compromised functioning of the stress-activated sympathetic-adrenalmedullary system. Second, mastication during stress prevents immune activation of pro-inflammatory cytokines such as interleukin-1b and interleukin-6 (30).

CONCLUSIONS

It may be concluded that the injuries occurring by forced swimming stress in the experimental animals can be significantly improved by Hp extract treatment and eventually its content in flavonoids may play an important role due to their estrogenic properties.

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Received: 03 03 2014
Accepted for publishing: 28 03 2016