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Research Article

Effect of *Hypericum perforatum* Extract in an Experimental Model of Binge Eating in Female Rats

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Purpose. The present study evaluated the effect of *Hypericum perforatum* dry extract in an experimental model of binge eating (BE). *Methods*. BE for highly palatable food (HPF) was evoked in female rats by three 8-day cycles of food restriction/re-feeding and acute stress on the test day (day 25). Stress was induced by preventing access to HPF for 15 min, while rats were able to see and smell it. *Hypericum perforatum* dry extract was given by gavage. *Results*. Only rats exposed to both food restrictions and stress exhibited BE. The doses of 250 and 500 mg/kg of *Hypericum perforatum* extract significantly reduced the BE episode, while 125 mg/kg was ineffective. The same doses did not affect HPF intake in the absence of BE. The dose of 250 mg/kg did not significantly modify stress-induced increase in serum corticosterone levels, suggesting that the effect on BE is not due to suppression of the stress response The combined administration of 125 mg/kg of *Hypericum perforatum* together with Salidroside, active principle of *Rhodiola rosea*, produced a synergic effect on BE. *Conclusions*. The present results indicate for the first time that *Hypericum perforatum* extracts may have therapeutic properties in bingeing-related eating disorders.

1. Introduction

Episodes of binge eating (BE) in humans are characterized by compulsive, nonhomeostatic consumption of an unusually large quantity of highly palatable food (HPF) in a short period of time. Even though not hungry, subjects eat more rapidly than normal until feeling uncomfortably full. These episodes are accompanied by subjective sense of loss of control over eating and are associated with feeling of distress, disgust, depression, being guilty about overeating, and eating alone because of embarrassment [1].

BE represents a central feature of bulimia nervosa, in which episodes of BE are followed by behaviours aimed at avoiding weight gain, such as self-induced vomiting. Intense and persistent BE episodes represent a typical phenomenon occurring also in subjects suffering from binge eating disorder (BED) [2] that is probably the most prevalent eating disorder [3]. It is characterized by repeated episodes of BE in the absence of compensatory behaviours to avoid weight gain. The DMS-IV-TR [1] indicates among diagnostic criteria for BED that BE episodes should occur at least 2 days per week for six months. The BED is associated with significant medical and psychiatric comorbidity [4–6]. It is estimated that BE afflicts approximately 5% of the US adult population at some time in their life [7], and it contributes to aggravate obesity and associated pathologies [3, 8–10].

A large body of evidence suggests that dieting, stress and negative affective states represent possible triggers of BE in patients suffering from BED or bulimia nervosa [11, 12]. Indeed, dieting periods are a common finding in the history of binge eaters, although hunger per se appears to be not enough to induce BE in the absence of stress and negative affective states [13, 14]. Considerable evidence suggests that BE may be caused by a unique interaction between dieting and stress; thus, environmental stress and a history of cyclic food restrictions may be responsible for its precipitation and maintenance [15–17]. Accordingly, recurring food restrictions are consistently

the strongest predictor of overeating in response to stress [11].

Despite a growing recognition of the consequences of bulimia nervosa and of BED on public health, satisfactory treatments are not available at present [18]. Medications that have been suggested to reduce BE in clinical studies, like topiramate [19, 20] or sibutramine [21, 22] are associated with a variety of adverse side effects, which represent a serious problem during chronic treatment [23–25]; in particular, sibutramine has been recently withdrawn from the European market. Fluoxetine has been approved by the FDA for bulimia nervosa, but evidence for its efficacy is reported inconclusive [26]. Apparently, treatment of BED and bulimia nervosa cannot simply rely on pharmacological agents aimed at reducing food intake in general, like serotonergic drugs. Innovative treatments for bulimia nervosa and BED, devoid of severe side effects, are strongly needed.

BE episodes appear to be characterized by uncontrollable urge to obtain and consume food, which is similar to that exhibited by addicted individuals towards drug of abuse. Evidence is accumulating that excessive intake of certain foods under specified conditions produces behaviours and changes in the brain that resemble an addiction-like state [27–31]. Neural systems that motivate and reinforce drug abuse have been proposed to underlie also behaviours associated with compulsive food seeking and food intake [32–36]. In this regard, it is interesting to note that several drugs that influence alcohol addiction have been reported to reduce BE in experimental models (such as naloxone [37], naltrexone, and baclofen [38, 39] and topiramate [40]) as well as in clinical studies [41–43].

Previous studies have shown that acute administration of *Hypericum perforatum* extract attenuates alcohol intake in genetically selected alcohol-preferring rats by influencing the motivational properties of ethanol [44–50].

Extracts of *Hypericum perforatum*, the common plant usually called St. John's wort, are known to exert antidepressant effects in humans [51–55] and antidepressant-like actions in laboratory animals in different experimental models [56–61].

Hypericum perforatum contains a variety of biologically active compounds, including the naphthodianthrones hypericin and pseudohypericin, and the phloroglucinol derivatives hyperforin, adhyperforin, and several flavonoids [51, 62, 63]. A further reason of interest in the effect of *Hypericum perforatum* on BE is raised by the finding that it has been reported to exhibit antistress properties. In this regard, several papers have shown that some of its active principles bind to CRF-1 receptors and exhibit antagonist activity at these receptors [64–67]. Moreover, *Hypericum perforatum* extracts have been shown to reduce the hypothalamic-pituitary-adrenal (HPA) axis activation following chronic treatment [68], to reduce restraint stress-induced increases in plasma ACTH and corticosterone levels following acute administration [69], and to counteract the negative effects of corticosterone on hippocampal cell proliferation [70]. Since stress is a key determinant of BE, a reduction of the response to stress might represent an effective mechanism for suppression of BE. Therefore, we thought it of interest to evaluate whether *Hypericum perforatum* extracts attenuate BE evoked in female rats by combining stress and food restrictions [40].

Moreover, the effect of *Hypericum perforatum* on BE was also evaluated in combination with salidroside, active principle of the dry extract of *Rhodiola rosea* (family Crassulaceae) [71, 72].

Rhodiola rosea roots contain a variety of biologically active compounds, including organic acids, flavonoids, tannins, and phenolic compounds. Phenylpropane and phenylethane phenolic glycosides, such as salidroside, rosavin, syringing, and triandrin are considered the most important active principles [71].

Recently our group reported that *Rhodiola rosea* extract and salidroside suppress BE, interfering with stress mechanisms [73].

In the present study, it was evaluated whether the combined administration of *Hypericum perforatum* and salidroside might offer advantages over their separate administration.

2. Material and Methods

A preclinical model has been recently developed by our group to investigate the neuro- and psychobiology of BE and to identify innovative pharmacological treatments [40]. This model is derived with modifications from the original model developed by Hagan et al. [74]. It uses female rats in relation to the higher prevalence of binge-type eating disorders in women than in men [1, 3] and combines three 8-day cycles of food restriction/refeeding and acute stress (on the 25th day) to evoke BE for HPF in Sprague-Dawley rats.

2.1. Animals. Female Sprague-Dawley rats (Charles River, Calco, Como, Italy) were used. Their body weight was 225–250 g at the beginning of the experiments. Rats were acclimated to individual cages under a 12 h light/dark cycle (lights on at 08:00 a.m.) with ad libitum chow and water for 2 weeks prior to the experiments. They were kept in a room at constant temperature (20–22°C) and humidity (45–55%). Rats were kept in individual cages with metallic walls; the floor and the front wall were made of metallic grid. The dimensions of the cage floor were 30 cm × 30 cm; the cage was 30 cm high. A front door (30 cm × 20 cm) made of metallic grid was present in the anterior wall of the cage to get access to the inside of the cage; the remaining part of the front wall was equipped with a drinking burette.

2.2. Diet. Animals were offered standard rat food pellets (4RF18, Mucedola, Settimo Milanese, Italy (2.6 kcal/g). The HPF was a paste in texture, prepared by mixing Nutella (Ferrero, Alba, Torino, Italy) chocolate cream (5.33 kcal/g; 56%, 31%, and 7% from carbohydrate, fat, and protein, resp.), grounded food pellets (4RF18, Mucedola, Settimo Milanese, Italy), and water in the following weight/weight percent ratio: 52% Nutella, 33% food pellets, and 15% water. The HPF diet had a caloric content of 3.63 kcal/g. HPF was offered in a coffee cup; the handle of the cup was inserted into

the metallic grid of the anterior wall of the cage and fixed to the wall. Standard pellets were offered inside a metallic grid container that was hung on the anterior wall of the cage; it was removed from the cage to measure its weight in order to determine food pellet intake.

2.3. The Stressful Procedure. For 15 min, the coffee cup containing HPF was placed inside a metallic grid container that was hanged up on the anterior wall of the cage. In these conditions, the animal was able to see the cup in which it received HPF on day 5, 6, 13, and 14 of the first two cycles, was able to see the HPF itself, and to smell its odour. In this 15 min period, the rat engaged in repeated movements of the forepaws, head, and trunk aimed at obtaining the HPF, but it was not able to reach it. This procedure was adopted to generate a mild stressful condition that causes a significant increase in serum corticosterone levels [41]. Rats underwent the stressful procedure between 10.00 and 12.00 h. After 15 min, the cup was placed inside the cage of rats of the stress groups, so that HPF became accessible to them.

2.4. Drug Treatment. Hypericum perforatum dry extract, containing 0.1% hypericin and 3.8% hyperforin, was a generous gift of Indena, Milano, Italy. It was dissolved in 2% ethanol and water and administered by gavage (2 ml/kg) at doses of 125–500 mg/kg [75] 1 h before access to HPF. Salidroside, an active principle of *Rhodiola rosea* extract, was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China). It was dissolved in 2% ethanol and water and administered by gavage (2 ml/kg) 1 h before access to HPF at the dose of 312 μg/kg. Control rats received vehicle administration by gavage (2 ml/kg). In experiment 4, the volume of administration was 1 ml/kg for both compounds.

2.5. Experimental Procedure

Experiment 1. Effect of Repeated Food Restrictions and Acute Stress of HPF Intake in Female Rats. Forty female rats were used. They were divided in 4 groups of 10 animals, matched for body weight and daily food intake: (1) the nonrestricted and not exposed to stress group (NR + NS), (2) the restricted and not exposed to stress group (R + NS), (3) the non-restricted and exposed to stress group (NR + S), and (4) the restricted and exposed to stress group (R + S).

Rats were subjected to 3 consecutive 8-day cycles followed by the final test on day 25. Each 8-day cycle was as follows: (a) the control group (NR + NS) had chow ad libitum for 4 days, on days 5-6, it received chow ad libitum + HPF for 2 h (from 10:00 a.m., i.e., 2 h after the beginning of the light phase of the cycle); on days 7-8, it had chow ad libitum; on day 25, it was not exposed to stress; (b) the second group (R + NS) had chow restricted to 66% of the usual intake for 4 days, was offered chow ad libitum and HPF for 2 h on days 5-6 and only chow on days 7-8; on day 25, it was not exposed to stress; (c) the third group had chow and HPF as controls (NR + NS), but on the test day (day 25), it was exposed to stress (NR + S); (d) the fourth group (R + S) had food available like group R + NS and on day 25, it was exposed to stress.

The 8-day cycle was repeated three times, but in the third cycle, the animals did not have access to HPF on day 21 and 22.

It has been recently reported by our group that in the estrous phase of the ovarian cycle, female rats do not exhibit BE in the adopted model [76], while in all the other three phase of the ovarian cycle they exhibit BE without significant differences in intensity. Therefore, immediately after the test on day 25, vaginal smears were collected and analysed under microscope to assess the ovarian phase, and data from rats in the estrous phase were not included in the statistical analysis. Vaginal smears were analysed by an experienced experimenter blind to treatment conditions.

Food intake was expressed as mean kcal/kg ingested ± S.E.M.; it was measured for 2 h, since previous experiments showed no differences among groups after this period. HPF intake was measured at 15, 30, 60, and 120 min after access to it. Food pellet intake was measured only at 2 h, in relation to the findings of previous studies showing that the food pellet intake was very small and to avoid disturbance to the animals during the test.

Experiment 2. Effect of Hypericum perforatum Extract on BE Evoked by Cycles of Food Restriction and Exposure to Acute Stress. Eighty female rats were used. They were divided in 2 groups of 40 animals, matched for body weight and daily food intake: (1) the nonrestricted and not exposed to stress group (NR + NS) and (2) the restricted and exposed to stress group (R + S). Only these two groups were used since Experiment 1 confirmed that BE is not expressed in the R + NS and NR + S groups. Rats were subjected to 3 consecutive 8-day cycles followed by the final test on day 25, as reported in Experiment 1.

Each group of 40 rats was divided in 4 subgroups of 10 rats, treated, respectively, with vehicle or with *Hypericum perforatum* dry extract (125, 250, or 500 mg/kg) given by gavage 1 h before access to HPF. Food intake was expressed as mean kcal/kg ingested ± S.E.M. HPF intake was measured at 15, 30, 60, and 120 min after access to it. Food pellet intake was measured only at 2 h.

Experiment 3. Effect of Hypericum perforatum Dry Extract Given in Combination with Salidroside on BE in Female Rats. Additional eighty female rats received the same procedures of the NR + NS and R + S rats in the Experiments 1 and 2. On the test day (day 25), NR + NS and R + S rats were treated by gavage as follows (10 rats per group): (a) vehicle + vehicle, (b) vehicle + Salidroside 312 μg/kg, and (c) *Hypericum perforatum* extract 125 mg/kg + vehicle, *Hypericum perforatum* extract 125 mg/kg + Salidroside 312 μg/kg. Food intake was expressed as mean kcal/kg ingested ± S.E.M. HPF intake was measured at 15, 30, 60, and 120 min after access to it. Food pellet intake was measured only at 2 h.

Experiment 4. Effect of Hypericum perforatum Extract and Salidroside on Serum Corticosterone Levels Following Cycles of Food Restriction and Exposure to Acute Stress

Experiment 4*a.* To assess if the doses of *Hypericum perforatum extract* (250 and 500 mg/kg) that reduced BE have an effect on the increased corticosterone levels in the R + S group, additional fifty-four Sprague-Dawley rats were used. They were divided in two groups (NR + NS and R + S) of 27 animals, were subjected to three cycles of 8 days with the same procedure described under Experiment 1. At the end of the third cycle (on day 25) in each group, 9 animals received vehicle, 9 animals received *Hypericum perforatum* dry extract, 250 mg/kg, and other 9 rats received *Hypericum perforatum* dry extract, 500 mg/kg. The R + S group received drug administration 45 min before exposure to the stressful procedure and was sacrificed at the end of the 15 min period of stress. The NR + NS group was administered vehicle or *Hypericum perforatum* dry extract, 250 or 500 mg/kg and then sacrificed 60 min later.

Experiment 4*b.* Additionally forty-five Sprague-Dawley rats, divided in two groups: NR + NS ($n = 9$) and R + S ($n = 36$), were subjected to three cycles of 8 days with the same procedure described under Experiment 1. At the end of the third cycle (on day 25), 9 NR + NS rats received vehicle + vehicle, R + S rats were divided in four groups of 9 animals that received: vehicle + vehicle, vehicle + Salidroside 312 μg/kg, *Hypericum perforatum* dry extract (125 mg/kg) + vehicle, and *Hypericum perforatum* dry extract (125 mg/kg) and salidroside 312 μg/kg. The R + S group received drug administration 45 min before exposure to the stressful procedure and was sacrificed at the end of the 15 min period of stress. The NR + NS group was administered vehicle and then sacrificed 60 min later.

Blood samples were collected from the rat trunk after decapitation. To improve serum separation from whole blood, samples were allowed to clot at room temperature before centrifugation (1000 ×g for 10 min). Serum was transferred into clean tubes and stored at −20°C until the assay. Taking into account the circadian rhythm of corticosterone, all sacrifices were carried out between 12.00 and 2.00 p.m., that is, during the diurnal period when its concentrations are relatively constant [77, 78]. Assessment of serum corticosterone level was done by means of enzyme immunoassay (EIA) using a commercially available kit (Arbor Assays, Ann Arbor, MI, USA), which utilizes microplate reader set at 450 nm. Serum samples were diluted 1 : 100 in appropriate assay buffers in order to be within the calibration curve range and assayed in duplicate. The detection limit of the assay was 16.9 pg/mL; intra- and interassay coefficients of variations were, respectively, 5.1 and 7.9%.

2.6. Statistical Analysis. Results are expressed as means ± S.E.M. Data were analyzed by two-ways analysis of variance (ANOVA) with between-subject comparisons for experimental groups or drug treatments and within-subject comparison for time of observation when appropriate (Systat 13.0). Post hoc comparison was carried out by the Bonferroni test. Statistical significance was set at $P < 0.05$.

3. Results

Experiment 1. Effect of Repeated Food Restrictions and Acute Stress of HPF Intake in Female Rats. Thirty female rats (of the 40 used in the experiment) proved not to be in the estrous phase at the moment in which the experiment was carried out. Only data from these animals (6–8 per group) were subjected to statistical analysis.

As shown in Figure 1, body weight of rats was reduced during the 4 days of food restriction, but immediately afterwards the animals increased their food intake and rapidly recovered their body weight to control levels by the end of each cycle. On the test day, body weight of the 4 groups of animals, as well as their food intake in the previous 24 h, was not significantly different.

The ANOVA revealed a highly significant difference in 2 h HPF intake in the 4 groups of rats ($F(3, 26) = 19.32$; $P < 0.001$). As shown in Figure 2 and in Table 1, HPF intake in the R + S group was markedly higher than that of the control (NR + NS) group. HPF intake of R + S rats was very pronounced in the first 15 min of access to it; these animals never engaged in competing behaviours, but continuously remained over the cup containing HPF and focused their attention on the intake. Cumulative HPF intake in the R + S group was significantly higher than in controls up to 120 min after access to it. HPF intake of the NR + S group was not significantly different from that of controls (NR + NS), indicating that stress was not enough to induce BE. Moreover, also HPF intake of the R + NS group was not significantly different from that of controls (NR + NS), indicating that cycles of food restriction are not enough to induce BE.

The intake of standard food pellet was very small (about 3–5% of the overall calories intake in the 2 h test), and it was affected neither by food restriction, nor by stress, and nor by the combination of both. In the 2 h test, rats of the R + S group ate 176.2 kcal/kg of HPF and only 6.5 kcal/rat of food pellets.

Experiment 2. Effect of Hypericum perforatum Extract on BE Evoked by Cycles of Food Restriction and Exposure to Acute Stress. Fifty-seven female rats (of the 80 used in the experiment) proved not to be in the estrous phase at the moment in which the experiment was carried out. Only data from these animals (6–8 per group) were subjected to statistical analysis. As shown in Figure 3, following vehicle administration, HPF intake in the R + S group was markedly higher than that of the NR + NS group. The ANOVA revealed a highly significant difference in 2 h HPF intake in the 2 groups of rats following vehicle administration ($F(1, 12) = 16.88$; $P < 0.01$).

As shown in Figure 3, in the NR + NS group, the ANOVA revealed a nonsignificant treatment effect ($F(3, 25) = 2.82$; $P = 0.059$). Following the highest dose of 500 mg/kg of *Hypericum perforatum* extract, a marked trend to the

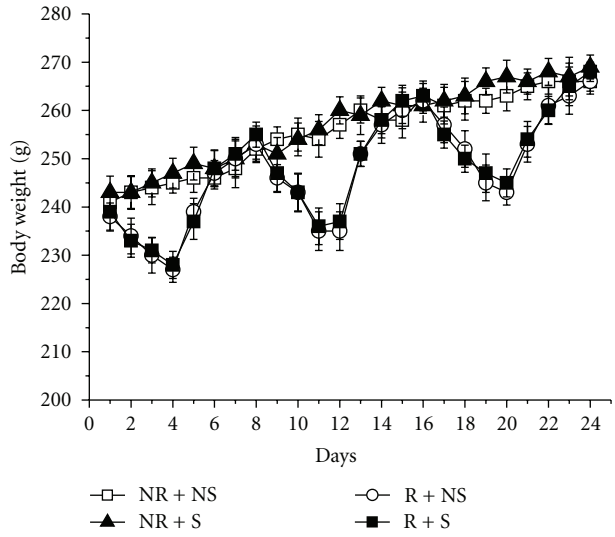

Figure 1: Body weight of female rats during the three 8-day cycles of food restriction/refeeding. Values are means ± S.E.M.

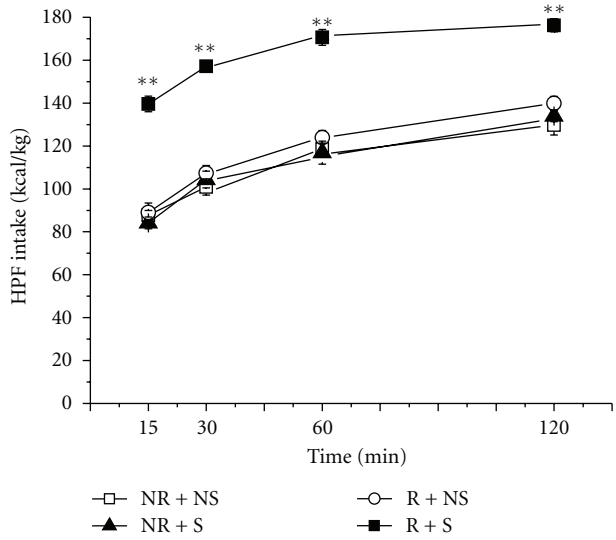

Figure 2: HPF intake (kcal/kg) on the test day in female rats exposed to either cycles of food restriction or stress or the combination of both. Data are means ± S.E.M. Statistical difference from controls (NR + NS): $^{**}P < 0.01$.

Table 1: HPF intake in grams on the test day in female rats exposed to either cycles of food restriction or stress or the combination of both. Data are means ± S.E.M. Statistical difference from controls (NR + NS): $^{**}P < 0.01$.

Group	15 min	30 min	60 min	120 min
NR + NS	6.6 ± 0.2	7.6 ± 0.3	9.0 ± 0.3	9.8 ± 0.3
NR + S	6.2 ± 0.1	7.7 ± 0.6	8.6 ± 0.6	9.8 ± 0.4
R + NS	7.6 ± 0.6	8.1 ± 0.6	9.4 ± 0.8	10.6 + 1.2
R + S	10.5 ± 0.4**	11.9 ± 0.4**	12.9 ± 0.4**	13.3 + 0.5**

reduction of HPF intake was observed, but the difference was not statistically significant.

The *Hypericum perforatum* extract significantly reduced HPF intake in the R + S group ($F(3, 24) = 7.41$; $P < 0.001$). The effect was statistically significant only at 15 min following administration of 250 mg/kg and up to 60 min following administration of 500 mg/kg; the dose of 125 mg/kg did not significantly reduce BE.

Experiment 3. Effect of Hypericum perforatum Dry Extract Given in Combination with Salidroside on BE in Female Rats. Sixty female rats (of the 80 used in the experiment) were not in the estrous phase (6–9 per group). Following vehicle administration, a highly significant difference in 2 h HPF intake in the 2 groups of rats following vehicle administration was observed (data of NR + NS group were not shown).

The ANOVA revealed a highly significant treatment effect only in R + S group ($F(3, 28) = 5{,}98$; $P < 0.01$).

As shown in Figure 4, *Hypericum perforatum* extract (125 mg/kg) did not significantly modify HPF intake. Salidroside (312 μg/kg) reduced HPF intake; the combination of *Hypericum perforatum* extract (125 mg/kg) and Salidroside (312 μg/kg) increased the reduction of HPF in comparison to that observed after Salidroside alone. Following Salidroside (312 μg/kg) treatment, the difference from vehicle-treated rats was statistically significant only at 15 min ($P < 0.05$); with *Hypericum perforatum* extract (125 mg/kg) and Salidroside (312 μg/kg), the effect was statistically significant up to 60 min ($P < 0.05$).

Thus, when the dose of 125 mg/kg of *Hypericum perforatum* extract (i.e., per se inactive) was administered together with Salidroside 312 μg/kg, the antibinge effect proved to be more intense, longer lasting, and reached the level of $P < 0.01$ as statistical significance in comparison to the effect observed following Salidroside 312 μg/kg alone ($P < 0.05$).

Experiment 4. Effect of Hypericum perforatum Extract and Salidroside on Serum Corticosterone Levels Following Cycles of Food Restriction and Exposure to Acute Stress

Experiment 4a. Forty-two female rats (of the 54 used in the experiment) were not in the estrous phase at the moment in which the experiment was carried out (6–8 per group).

Two-way ANOVA showed significant group effect ($F(1, 36) = 20.7$, $P < 0.01$). As reported in Figure 5, exposure to HPF without access to it significantly increased corticosterone levels in the serum samples obtained from R + S rats, in comparison to those from NR + NS rats (Bonferroni post hoc test, $P < 0.05$).

On the other hand, the ANOVA revealed neither a drug treatment effect ($F(2, 36) = 3.3$, $P > 0.05$), nor a group-drug treatment interaction ($F(2, 36) = 0.1$, $P > 0.05$) on serum corticosterone levels. The administration by gavage of *Hypericum perforatum* extract at either dose used (250 or 500 mg/kg) failed to reduce the increase in serum corticosterone levels in the R + S group. Post hoc tests showed that serum corticosterone levels in R + S rats, treated with the 2 doses of *Hypericum perforatum* extracts, were comparable to those of R + S rats treated with vehicle ($P > 0.05$), but

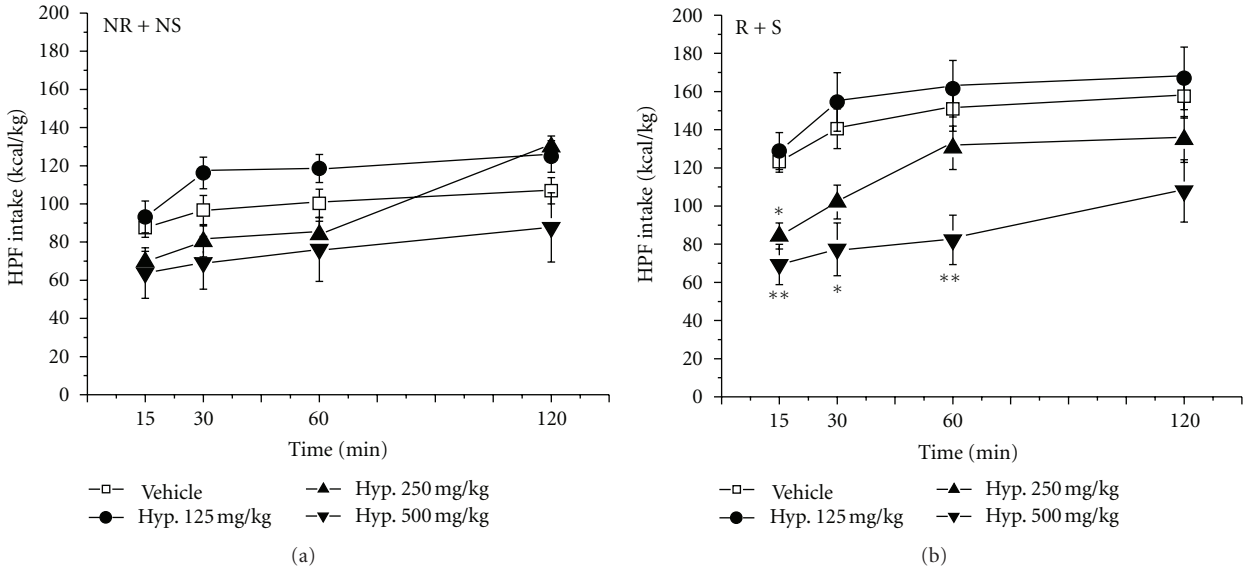

Figure 3: Effect of intragastric administration of different doses of *Hypericum perforatum* extract or vehicle on HPF intake on the test day in R + S and NR + NS rats. Data are means ± S.E.M. Statistical difference from vehicle-treated rats: $^{*}P < 0.05$; $^{**}P < 0.01$.

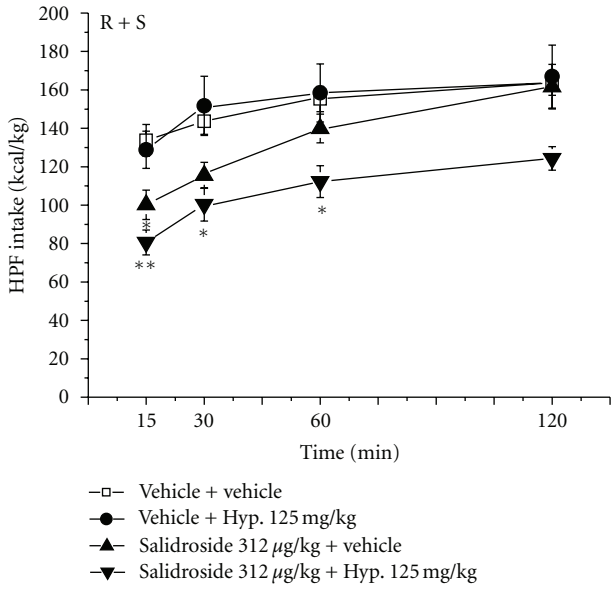

Figure 4: Effect of the combined administration of *Hypericum perforatum* extract, 125 mg/kg, and Salidroside 312 μg/kg on HPF intake on the test day in R + S rats. Data are means ± S.E.M. Statistical difference from vehicle + vehicle-treated rats: $^{*}P < 0.05$, $^{**}P < 0.01$.

were statistically different from those of NR + NS rats, either treated or not treated with *Hypericum perforatum*.

Experiment 4b. Thirty-six female rats (of the 45 used in the experiment) were not in the estrous phase at the moment in which the experiment was carried out (6–9 per group).

Two-way ANOVA showed significant group effect ($F(1, 34) = 93.4$, $P < 0.01$). As reported in Figure 6, exposure to HPF without access to it significantly increased corticosterone levels in the serum samples obtained from R + S rats, in comparison to those from NR + NS rats (Bonferroni post hoc test, $P < 0.01$).

On the other hand, the ANOVA revealed no drug treatment effect in R + S group ($F(3, 23) = 1.64$, $P > 0.05$) on serum corticosterone levels.

4. Discussion

The present study confirms that stress or repeated food restrictions, given separately, are not enough to induce BE, but the combination of both determinants is required. Previous work by Cifani et al. [40] has shown that the experimental model of BE adopted in the present study possesses, in addition to face and construct validity, also predictive validity, since both topiramate and sibutramine abolish BE.

The administration by gavage of a dry extract of *Hypericum perforatum*, 250 mg/kg, significantly reduced the increase in HPF intake in the R + S group (subjected to both stress and repeated food restrictions), and the dose of 500 mg/kg completely reduced it. On the other hand, 125 mg/kg did not significantly influence HPF intake in the R + S group. While suppressing the increase in HPF intake in the R + S group, the three doses tested of *Hypericum perforatum* extract did not significantly reduce HPF in the control group (NR + NS) even if a clear trend of reduction was shown by the highest tested dose (500 mg/kg).

The mechanisms accounting for this selective effect on BE remain to be elucidated. As mentioned in the Introduction, *Hypericum perforatum* extracts have been reported to influence stress mechanisms. Several papers have shown that some of its active principles bind to CRF-1 receptors and

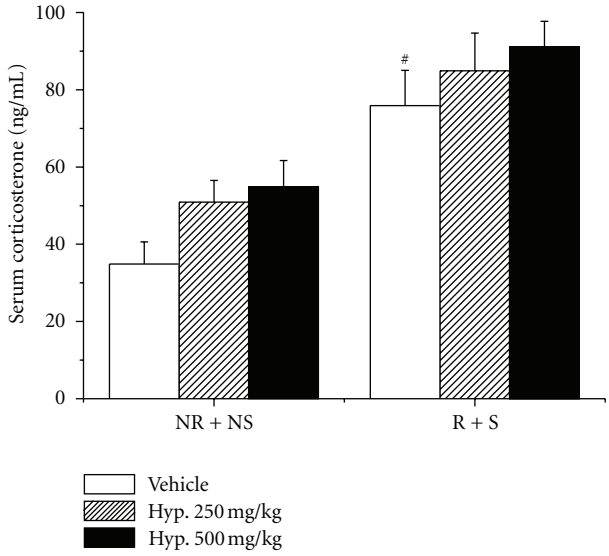

Figure 5: Effect of *Hypericum perforatum* extract (250 or 500 mg/kg) or its vehicle on serum corticosterone levels in R + S rats and in NR + NS rats. Data are means ± S.E.M (n = 6–8 rats per group). Statistical difference vehicle R + S rats versus vehicle NR + NS: $^{\#}P < 0.05$; statistical difference from vehicle-treated rats in each group was never statistically significant.

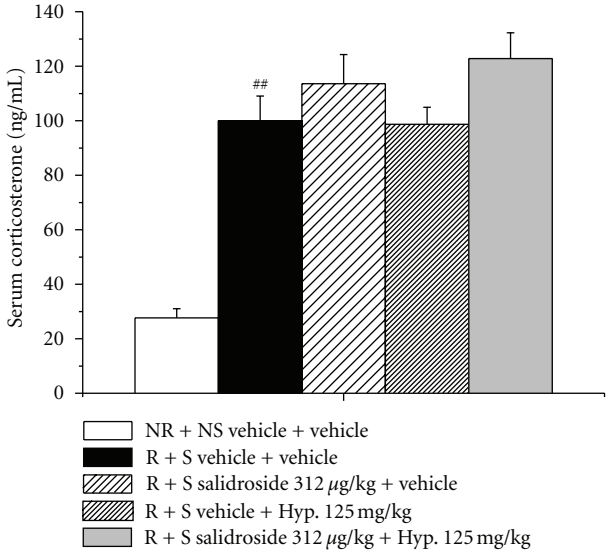

Figure 6: Effect of the combined administration of *Hypericum perforatum* extract, 125 mg/kg, and Salidroside 312 μg/kg on serum corticosterone levels in R + S rats. Data are means ± S.E.M (n = 6–8 rats per group). Statistical difference vehicle R + S rats versus vehicle NR + NS: $^{\#\#}P < 0.01$; statistical difference from vehicle-treated rats in R + S group was never statistically significant.

exhibit antagonist activity at these receptors [64–67]. Moreover, *Hypericum perforatum* extracts have been reported to reduce HPA axis activation following chronic treatment [68] and to reduce restraint stress-induced increases in plasma ACTH and corticosterone levels following acute administration [69]. Corticosterone has been proposed to have motivational properties and to influence drug-seeking behaviour in rats [79–82], suggesting that it may have a role in the control of compulsive behaviour, like that exhibited in episodes of BE. However, a recent study of our group has shown that peripheral administration of corticosterone is not able to induce BE in rats exposed to repeated cycles of food restrictions; moreover, metyrapone, a glucocorticoid synthesis inhibitor, does not affect BE [83]. On the other hand, the same study has shown that a CRF-1 receptor antagonist completely blocks BE in the experimental model adopted [83]. Taken together, these findings suggest that CRF controls BE through CRF-1 receptors mainly in extrahypothalamic brain areas, rather than in the hypothalamic structures controlling the HPA axis. The results of Experiment 3 indicate that in our experimental conditions *Hypericum perforatum* does not influence HPA axis activation; however, it cannot be excluded that it abolishes BE by blocking central CRF-1 receptors in extrahypothalamic brain area.

On the other hand, the inhibition of the BE may be achieved also by suppressing addictive-like behaviours, in particular those related to the binge/intoxication stages of addiction [84]. In this regard, it is interesting to note that *Hypericum perforatum* has been reported to suppress voluntary alcohol intake, ethanol self-administration and the alcohol-deprivation effect in genetically selected alcohol-preferring rats [44–50, 75], including the Marchigian Sardinian alcohol-preferring rats that represent an interesting experimental model of alcohol abuse [85]. Therefore, *Hypericum perforatum* may influence motivation and compulsive behaviours towards HPF in BE episodes, as it does towards alcohol.

Among the active principles identified in *Hypericum perforatum* extracts, apparently pseudohypericin is the most potent CRF-1 receptor antagonist [68], but also hypericin exhibited high binding affinity for human CRF-1 receptors [65]. Other *Hypericum perforatum* constituents with high affinity for the CRF-1 receptor are also bisanthraquinone glycosides [64] and flavonoids [68]. Hyperforin and hyperforin derivatives do not bind with high affinity at CRF1 receptors and are not involved in the control of HPA axis function [86]. On the other hand, hyperforin has been proposed to be the main active principle responsible for the effect of *Hypericum perforatum* in the control of alcohol abuse [75]. The extract used in the present study had a very low hypericin content (0.1%) and a rather high hyperforin content (3.8%). These data favour the hypothesis that hyperforin may have a major role in the suppressive effect of *Hypericum perforatum* on BE; accordingly, the mechanism of action may be more likely related to suppression of addictive-like behaviours than to interference with stress mechanisms.

It is interesting to note that *Hypericum perforatum* was able to increase the effect of Salidroside, that in our previous study, showed to be the active principle of *Rhodiola rosea* extracts responsible for the selective effect in reducing BE [73].

Recently, the effect of Salidroside on expression and secretion of neuropeptide Y (NPY) in neuroglia cells has been demonstrated [87]. Since it is well documented that NPY reduces consummatory ingestive behaviour by about

40% [88, 89], it has been suggested [87] that the effect of Salidroside on BE is mediated by NPY.

In conclusion, the present findings suggest that *Hypericum perforatum* extracts and Salidroside may be useful for treatment of bingeing-related eating disorders.

Conflict of Interests

The authors have no conflict of interests to declare.

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