

The effects of *Spirulina (Arthrospira) platensis* on morphological and hematological parameters evoked by social stress in male rats

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ABSTRACT

Stress is a complex phenomenon and exposure to stress results in a series of reactions in the organism, including alterations in behaviour and various physiological changes. Role of nutrition in the maintenance of homeostatic mechanisms, including the stress, is very dense. The current study aimed to evaluate the potential effects of *Spirulina (Arthrospira) platensis* against mix stress models. For this purpose, 36 Sprague-Dawley male rats were allocated into four groups; 1. Control(C), 2. Stress(S), 3. *S.platensis* (Sp) and 4. *S. platensis* + Stress (SpS). *S. platensis* was applied to Sp and SpS groups by oral gavage (1500 mg/kg/day) for 28 days. All rats were exposed to light : dark cycle (long lightening period; 18h light : 6h dark) stress for 14 days. Also, S and SpS groups were stressed with additional mix stress by leaving in crowded environment and hosting alone under long lightening period. The animals which fed with *S. platensis*, shown significant changes in the numbers of circulating leukocytes, % of neutrophils, and the neutrophil : lymphocyte ratio. However, there were no significant differences in the morphological parameters. In conclusion, the possible preventive effect of *S. platensis* on hematological parameters was shown in a rat's stress model of social stress which was included mix stress under long lightening period.

Keywords: *Spirulina (Arthrospira) platensis*, social stress, neutrophil : lymphocyte ratio, rats

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Introduction

Stress, depending on the type and intensity of stress, can lead to death in animals, because of adaptation problems, pathological changes and failure to cope with the new situation due to severe disorders in homeostasis (Benyo et al., 2007; Sejan et al., 2011). Many factors, such as the environmental temperature changes that exceeds the limits of thermoneutral

zone, hosting in crowded environments, and leaving animals alone, which were group hosting, can cause stress and may lead physiological changes in the organism. Especially crowded hosting and high temperature may adversely affect feed intake, intestinal health and thereby growth (Meddings and Swain, 2000; Mawdsley and Rampton, 2005).

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Stress factors can influence either the intestinal flora or feed efficiency and weight gain in rats (Marin et al., 2007). Although rats can adapt to the environmental temperature between 10-30°C, homeostatic mechanisms can be forced due to changes in the environmental temperature, and thereby stress occurs. Some researchers determined that increase in environmental temperature or crowdedness and stuffiness may constitute to the decrease both in feed consumption and growth in several animals (Peng et al., 1989; Keeling et al., 2003). In addition, the light and dark periods are important throughout the day for rats to survive in the physiological limits. This situation creates the photoperiodic memory of rats. It was shown that natural bright period increases rat welfare and survival rate, however, long dark period is resulted with a low heart rate in animals (Azar et al., 2008). Besides natural lightening period of rats (12h Light: 12h Dark), long and short lightening periods have been researched for determine the animal model for human (Boon et al., 1997; Ebling 1994). Although the decrease in growth and metabolic rate of day were reported in short lightening period studies (Boon et al., 1997), it was determined that the long lightening period has the positive effect on weight (Ebling, 1994). It was also shown that increasing or decreasing lightening treatments are associated with feed consumption and body weight of rats (Shöamker and Heideman, 2002; Markova et al., 2003). Shöamker and Heideman (2002) reported that there is a decrease in body weight within the weights of heart muscle, adrenal gland and liver in rats, which had been fed with melatonin, compared to control group, under normal lightening period. Insight of literatures, to evaluate the some morphological and physiological parameters, in the present study, it was trying to modelize a stress model for future human studies. For this purpose crowded environment and hosting alone stresses were studied under long lightening period.

Exogenous vitamins such as vitamin E and vitamin C, some minerals and natural additives are considered as protective against stress. (Botsoglu et al., 2002; Sengezer and Gungor, 2008; Altiner et al., 2017). It is known that feed additives improve the digestive system of animals and enable them to capture their genetic potential in growth performance. In recent years Spirulina has a considerable place among these natural additives. Spirulina is a planktonic, spiral, blue-green algae which is also a traditional food of Mexican and African societies. *S. platensis* is widely used as a natural supplement to regulate the effects of stress in

organism. It's known as an important herbal supplement due to its immunomodulator, antioxidant and protective effects. *S. platensis* has important contents such as high protein, polyphenols, phycocyanin, minerals and vitamin C (Khan et al., 2005; Seyidoglu et al., 2017). It can be digested easily due to its non cellulose structure on its cell wall, and thereby it enhances growth (Moreira et al., 2011; Seyidoglu and Galip, 2014; Seyidoglu et al., 2017a). Several studies reported the effects of Spirulina on haematological parameters and growth performances in rats (Araujo et al., 2003; Simsek et al., 2007; Promya and Chitmanat, 2011). Araujo et al. (2003) determined increased body weight gain in rats which had been added 10% Spirulina to feed. On the other hand, Simsek et al. (2007) identified that 300 mg/kg *S. platensis* increase the erythrocyte count and haemoglobin concentration in rats. In another study which have done with fishes (*Silurus glanis*), the Erythrocyte and Leukocyte counts were increased by 3% and 5% Spirulina additive (Promya and Chitmanat, 2011). Researchers specified that these effects are correlated with the stimulating effect of Spirulina on the stem cell activity of bone marrow.

In this study, we examined the effects of *S. platensis*, which is called as an alternative super food by the World Health Organization, on weight, body mass index and hematological parameters in rats exposed to a mix stress model which includes crowded environment and hosting alone, under long lightening period.

Materials and methods

Animals: The experimental protocols were approved by the Animal Care and Use Committee of Bursa Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of University Animal Experimentation Local Ethics Committee (Approval No: 2018-07/02). Thirty six adult, healthy, male Sprague-Dawley rats (age about 10 -12 weeks and average body weight 200-250 g) were included in this study and the animals were allocated into 4 experimental groups. The groups were Control (C -basal diet), Stress (S-basal diet), *S. platensis* (1500 mg/kg/day) (Sp), *S. platensis* (1500 mg/kg/day) and Stress (SpS).

Experiment Set: Each cage, with four transparent sides, had 3 rats for 5 trial weeks. The first week was the adaptation period to trial. The second and third weeks are the application periods of *S. platensis* to rats.

S. platensis (Egert, Izmir-Turkey) were given 1500 mg/kg/daily by oral gavage. In the study, 3 different stress applications were mixed and applied during 4th and 5th weeks with supplementation of *S. platensis* as follow. **Light : Dark Cycle Stress (Long Lightening):** In normal condition, the rats live in 12h light and 12h dark cycle in one day period. This stress was exposed to all rats with 18h light and 6h dark cycle during the 4th and 5th weeks of trial. **Hosting Alone Stress:** This stress was applied in a separate cage (50x50) of which 4 sides and ground covered with white paper. The rat was left alone for 30 min and was given neither food nor water during the stress application period. This stress was applied on Monday, Wednesday, Friday and Sunday of the 4th week of the trail. **Crowded Environment Stress:** This stress was applied by placing 6 rats in a cage, which is designed for 3 rats, for 30 min. Neither food nor water was given to the animals during the stress application period. This stress was applied on Tuesday, Thursday and Saturday of the 5th week of the trial.

Measurement: The effects of *S. platensis* on height, waist circumference (WC), body mass index (BMI) and waist circum/height ratio were determined at the beginning and the end of the study. Also, body weights were measured weekly. Blood samples were obtained by the puncture of the heart under isoflurane anesthesia at the end of the 5th week from overnight-fasted rats. The blood hematocrit, haemoglobin, counts of erythrocyte and leukocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and types of leukocyte (neutrophil, lymphocyte and monocyte) were determined by using automatic blood counter device (VetScan HM5, ABAXIS) in laboratory of Veterinary Medicine Faculty Animal Hospital, Bursa Uludag University.

Statistical analysis: Statistical analyses were performed with SPSS (Version 17.0). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at $P < 0.05$. For hematological and morphological parameters, if the differences between groups were provided to be significant ($P < 0.05$), differences evaluated by Tukey's test (Dowdy and Wearden, 1981). On the other hand, in non-homogenous groups, differences between means were

analyzed by Kruskal Wallis and following Mann Whitney U test between groups one by one. Also, the variance analysis for repetitive measurements were analyzed using a repeated measures ANOVA for weekly body weight (Dawson and Trapp, 2001).

Results

The important blood parameters which measured for stress condition such as leukocytes, neutrophils and neutrophil : lymphocyte (N/L) ratio obtained from the study are given in Figure 1-2-3. Leukocytes were decreased in S group compared to C group statistically ($p:0,026$). In SpS group, it was increased significantly compared to S ($p:0,014$). On the other hand, leukocytes were increased in Sp group compared to C ($p:0,022$). Neutrophils and N/L ratio were increased in S group compared to C ($p:0,009$; $p: 0,002$ neutrophils, N/L ratio respectively). Although there were no statistically differences, neutrophils and N/L ratio were decreased in SpS group compared to S ($p > 0.05$). Also, N/L ratio were decreased in group Sp compared to C ($p:0,030$). On the other hand, there were no significant changes in other some blood parameters (Table 1; $p > 0.05$).

In the study, there was no statistically difference among all groups for body weight weekly shown in Figure 4 ($p > 0.05$). Nevertheless, no significant differences were found among all groups in some morphological parameters such as BMI, height, WC and waist circum./height ratio (Table 2, $p > 0.05$).

Table 1: Some hematological parameters in control and experimental groups. (The values represent mean± standard error from n=9).

Blood Parameters	Experimental groups			
	C	S	Sp	SpS
RBC ($10^6/\text{mm}^3$)	7.43±0.14	6.99±0.06	7.29±0.17	7.08±0.20
Hb (g/dl)	12.87±0.17	13.38±0.27	13.37±0.06	12.90±0.13
HCT (%)	41.88±0.75	39.85±0.55	41.21±0.92	39.98±0.39
Lym (%)	74.33±1.27	71.61±2.16	78.05±2.88	72.06±1.86
Mon (%)	3.25±0.38	4.15±0.54	3.38±0.28	3.98±0.31
MCV (fl)	56.00±0.63	58.40±0.40	56.00±0.55	55.80±0.20
MCH (pg)	17.85±0.20	18.40±0.11	17.86±0.23	18.32±0.14
MCHC (g/d)	31.98±0.16	32.40±0.32	31.90±0.26	32.20±0.21

C = Control, S = Stress, Sp = *S. platensis*, SpS = Stress + *S. platensis*, RBC = Erythrocyte, Hb = Hemoglobin, HCT = Hematocrit, Lym = Lymphocyte, Mon = Monocyte, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration.

Table 2. Some morphological parameters in control and experimental groups. (The values represent mean \pm standard error from n = 9)

Morphological parameters	Experimental Groups			
	C	S	Sp	SpS
Initial body mass index (kg/m ²)	0.17 \pm 0.01	0.17 \pm 0.01	0.16 \pm 0.00	0.17 \pm 0.01
Final body mass index (kg/m ²)	0.19 \pm 0.00	0.18 \pm 0.00	0.18 \pm 0.01	0.18 \pm 0.01
Initial body length (cm)	38.67 \pm 0.88	37.89 \pm 1.08	38.78 \pm 0.60	38.00 \pm 0.99
Final body length (cm)	40.39 \pm 0.65	41.33 \pm 0.55	41.75 \pm 0.33	41.17 \pm 0.56
Initial waist circumference (cm)	14.00 \pm 0.22	14.17 \pm 0.47	13.89 \pm 0.22	13.61 \pm 0,47
Final waist circumference (cm)	14.39 \pm 0.23	14.83 \pm 0.25	15.00 \pm 0.19	14.78 \pm 0.32
Initial waist circum/length	0.36 \pm 0.00	0.37 \pm 0.00	0.36 \pm 0.01	0.36 \pm 0.01
Final waist circum/length	0.36 \pm 0.00	0.36 \pm 0.00	0.36 \pm 0.01	0.36 \pm 0.01

C = Control, S = Stress, Sp = *S. Platensis*, SpS = Stress + *S. platensis*,

Discussion

The impact of light and photoperiodicity on physiology of mammalian species is well documented. Photoperiodically sensitive animals respond to altered light regimen with changes in growth, food intake, reproductive status and behaviour. The optimal photoperiod is unknown for most species but a 12 h light: 12 h dark cycle is used for most of the laboratory animals (Harper and Lawrence, 2011). Limited studies have found the effects of the lighting period and life cycle on hematological parameters for Sprague Dawley rats. Nelson et al. (1994) reported that many rat species are sensitive to photoperiodic phase. It was also reported that although weight gains were decreased by 8 hour light stress in Harlan Sprague Dawley rats, in Brown Norway rats it was increased (Francisco et al., 2004). Poyraz (2000) reported that the growth parameters were effected by light duration in rodents. Although Warner et al. (2010) found that the shortened light for hamster is associated with low growth, Moffatt et al. (1991) determined that growth is stimulated by short light time. The other stress factor, crowded environment, is defined as animal density in a cage that stimulates the physiological, behavioral and molecular changes in organism (Benyo et al., 2007). Besides that, animals have limited physical activity, feed intake and growth in crowded environments (Armario et al., 1984). Some researchers found that crowded environment stress causes a decrease in body weight and food intake and thereby body mass index (Marin et al., 2007; Eid et al., 2010). On the other hand, it was reviewed that hosting alone is an important stress factor which has negative influences on organism such as depression, anxiety, irritability or hostility (Ernst and Cacioppo,

1999). All these instances are correlated with growth and physiology (Miller, 1998; Dantzer et al., 1999; Kiecolt-Glaser and Glaser, 2002). Some researchers reported that hosting alone stress is more stressful than other stress factors, whereas some of them observed no differences (Giralt and Armario, 1989; Gambardella et al., 1994; Sharp et al., 2002). However, in the present study, no differences were occurred on morphological parameters and weights for the stress conditions.

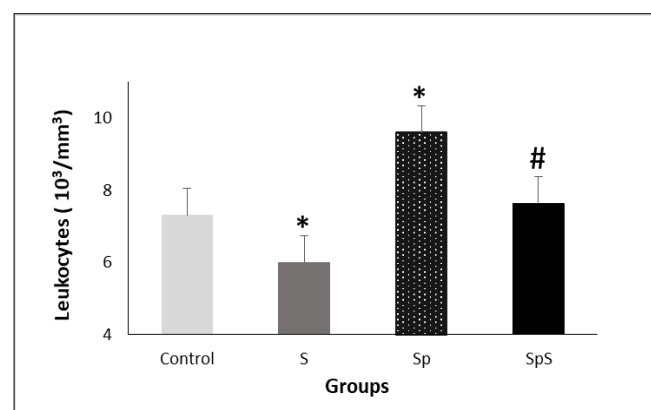


Figure 1. Leukocytes number in control and experimental groups.

S = Stress, Sp = *Spirulina platensis* SpS= Stress + *S. platensis*. All data are expressed as means \pm SE. * p < 0.05, S versus C group ; Sp versus C group, # p < 0.05, SpS versus S group.

Importantly, in this study, WBC was decreased in S group although it was increased in SpS (Stress and *S. platensis*). In contrast, significant increase in WBC was observed in *S. platensis* group (Sp) when compared with control group (C). There is no significant difference on WBC between the groups Sp and *S. platensis* is called as a super food which has several

effects on growth, antioxidant mechanism, health and life quality (Gorber et al., 2007; Park et al., 2008; Nasirian et al., 2017; Seyidoglu et al., 2017). It's also important for growth and cell regeneration. It was reported that spirulina has an inhibitory effect on development of leucopenia and anemia induced by lead and cadmium in rats (Simsek et al., 2009).

2013). It was observed that *S. platensis* has positive impact on interleukin and tumor necrosis factor which are responsible to cellular response in carps, and also helps to produce red and white blood cell and interferons in rats (Lisheng et al., 1991; Watanuki et al., 2006).

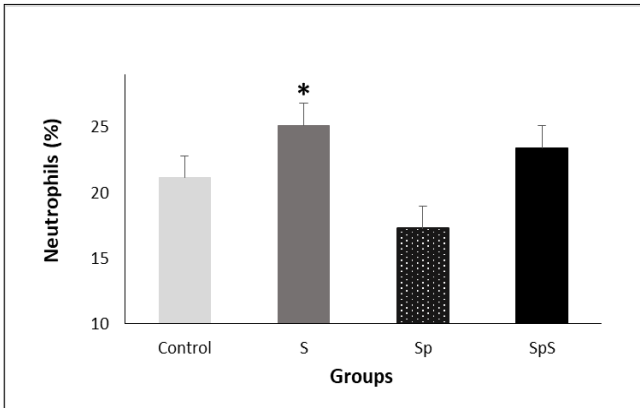


Figure 2. Neutrophil percentage in control and experimental groups.

S = Stress, **Sp** = *Spirulina platensis*, **SpS** = Stress + *S. platensis*. All data are expressed as means ± SE. * p < 0.05, S versus C group ; Sp versus C group, # p < 0.05, SpS versus S group.

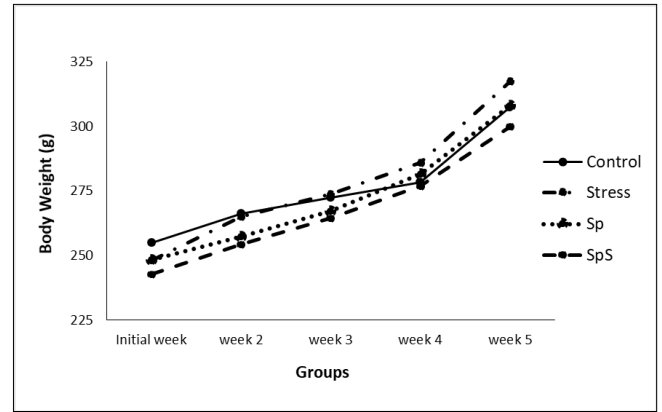


Figure 4. Weekly body weight in control and experimental groups.

Sp = *Spirulina platensis*, **SpS** = Stress + *S. platensis*. All data are expressed as means ± SE. There is no statistically difference among all groups for body weight (p > 0.05).

Conclusion

Stress changes the natural homeostasis of organism either growth or physiological condition. In the present study, there were no differences found in weights and hematological parameters except leukocytes, neutrophils and N/L ratio in group S compared to C. Also all parameters were in their normal reference values. This may be explained with the environmental condition, feeding procedure, the antioxidant dose and rat species. Nevertheless, N/L ratio is one of the physiological stress marker which also used to determine the stress indicator. In the present study, N/L ratio was found higher in S group compared to C, and also it was decreased with *S. platensis* feeding in group Sp, statistically. It can be said that *S. platensis* would exhibit a higher level of welfare and is effective for stress conditions.

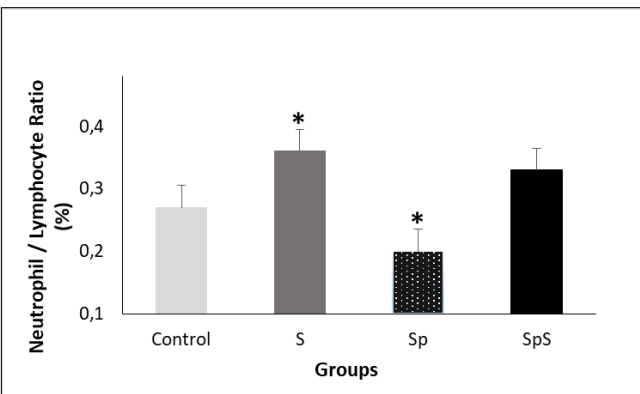


Figure 3. Neutrophil : Lymphocyte ratio in control and experimental groups.

S = Stress, **Sp** = *Spirulina platensis*, **SpS** = Stress + *S. platensis*. All data are expressed as means ± SE. * p < 0.05, S versus C group ; Sp versus C group, # p < 0.05, SpS versus S group.

Sixabela et al. (2011) determined decreased hematocrit in rats fed Spirulina due to its effect on hydration status and plasma volume. *S. platensis* known as a powerful antioxidant in herbal supplements. Its contents phycocyanin, tocopherols, beta carotene and vitamin C are in progress of growth and health (Karkos et al 2011; Abdel-Daim et al.,

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