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Research Article

Effect of alcoholic root extract of Withania somnifera on experimentally induced anorexia in rats

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Abstract:

Anorexia nervosa is a behavioral disorder characterized by ego-syntonic self-starvation, denial of illness and ambivalence towards treatment. Treatment refusal and drop-out rates are high also relapsing is common. Physical stress and LPS administration significantly affect the feeding behavior in rodents. In previous studies reported *Rhodiola rosea* L. have shown anti anorectic effect due to its antistress and adaptogenic property. However there is no report on effect of *Withania somnifera* root extract on feeding behavior in experimentally induced models of anorexia in rats.

Objective of our study was to evaluate effect of *Withania somnifera* root extract on feeding behavior in animal model of anorexia. Wistar rats of either sex were used in present study. Vehicle or W.S (100 and 300mg/kg, p.o.) was administered for 21 days in a) freely feeding rats b) 20 hr food deprived rats c) stress induced anorexic rats and d) LPS induced anorexic rats. In freely feeding rats after administration of vehicle or W. S. food was withdrawn for one hr and offer again. In 20 hr food deprived rats, vehicle or W.S. was administered in 20 hrs fasted animals and food was kept for 4 hrs. In stress induced anorexic rats, vehicle or W.S. was administered one hour later stress were applied daily for one hr and then food was kept. In LPS induced anorexic rats were administered with LPS (100µg/kg, i.p.), the food was withdrawn for four hour, later vehicle or W.S. was administered, one hr later food was kept. All above procedures was continued for 21 days. At the end of study food consumption, number of attempts for food consumption and body weight were measured. Results of present study shows that alcoholic root extract of *Withania somnifera(*100 and 300 mg/kg*)* dose dependently increased food consumption, number of attempts for food consumption and body weight in stress induced anorexic rats and LPS induced anorexic rats but it fails to show any effect on freely feeding and 20 h food deprived rats. In conclusion, the present study provides original evidence that oral administration of alcoholic root extract of WS have shown anti anorexic effect in stress and LPS induced anorexia.

Keywords: Adaptogen, anorexia, LPS, restrain stress, *Withania somnifera*

INTRODUCTION

Anorexia Nervosa is a serious, debilitating condition that causes significant physical, emotional, and functional impairment [1] . It is a very complex behavior, which results from the interaction between a wide range of external and internal factors[2]. The condition is characterized by destructive weight loss, behavior and a refusal to maintain body weight at or above a minimally normal weight for age and height.

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A critical challenge for neurosciences is to understand the regulation of food intake in mammals [3]

There are different factor that induces anorexia, such as stress, pro-inflammatory cytokines, food deprivation etc. Previous studies indicate that food consumption is reduced under stressful conditions, and that corticotropin-releasing hormone may play an important role in mediating this behavioural response to stress. Where as several studies showed that both peripherally administered LPS and interleukins activate central serotonergic activity, and that the changes in serotonergic activity occur in a temporal manner that is consistent with its potential involvement in anorexia [4] . Besides intraperitoneal injection of fluoxetine, a selective 5-HT reuptake inhibitor, produces a suppression of eating by reportedly increasing extracellular levels of endogenous 5-HT in forebrain nerve terminal regions via the blockade of the 5-HT transporter [5]

Medical, nutritional and psychological interventions are typically used in the treatment of Anorexia Nervosa. Medicinal treatment involves use of antidepressants, hormones, etc., but the literature on the effectiveness of these drugs in a pediatric population is very limited. Unfortunately, there is no effective treatment for anorexia nervosa, and recovery can take many years. Even after some weight gain, many people with anorexia remain quite thin and risk of relapse is very high [6] . Based on human and animal research, it appears a variety of nutritional and botanical substances, such as adaptogenic herbs, specific vitamins including ascorbic acid, vitamins B_1 and B_6, coenzyme forms of vitamin B_5 and B_{12}, amino acid tyrosine, and other nutrients such as lipoic acid, phosphatidylserine, and plant sterol/sterolin combinations may allow individuals to sustain an adaptive response and minimize some of the systemic effects of stress[7] . Also substantial evidences indicate that, the intracellular utilization of metabolic fuels generates signals that are used by the central nervous system to control feeding behavior [8]

Withania somnifera, also known as Ashwagandha, Indian ginseng, or winter cherry, has been an important herb in the Ayurveda and indigenous medical systems for over 3000 years. Studies indicate Ashwagandha possesses antistress [9] adaptogenic [10], antioxidant [11] , anti-inflammatory[12], antitumor[13] , immunomodulatory [14] , antiulcer[15] and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary and central nervous systems. Toxicity studies reveal that Ashwagandha appears to be a safe compound[16] . However no studies have been reported effect of *Withania somnifera* root extract on feeding behavior in animal model of anorexia.

MATERIALS AND METHODS

Test drug: A dry ethanolic root extract of *Withania somnifera* (WS), provided by Shanbhag Ayur Products, Yallapur, Karnataka was used.WS was suspended in 1% CMC which then administered to rats orally at an optimum dosage of 100 mg/kg (WS 1) and 300 mg/kg (WS 2).Lipopolysaccharide (No L-2630) purchased from Sigma Aldrich, USA and

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dissolved in pyrogen-free isotonic saline which is administered by intraperitoneal route at the dose of 100µg/kg.

Animals:

Wistar rats of either sex (150-200 g) from our breeding stock were used for the study. They were housed in clean and transparent poly propylene cages with six animals in each cage and maintained at 27ºC with 12: 12 h light-dark cycle for a period of 7 days prior to the study. They were fed standard rat chow and water ad libitum.

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines.

Experimental animal models and study design:

To investigate the effect of *Withania somnifera* root extract on Feeding Behavior in

1. Freely feeding rats.
2. 20 h Food-deprived rats
3. Restraint stress induce anorexic rats.
4. Lippolysaccharide induce anorexic rats.

Effects of WS on freely-feeding rats:

To evaluate the general effects of WS on food intake, attempts for food intake and body weight in freely-feeding rats. The animals (n=24) were divided into four groups that received oral administration of vehicle or WS at 100 and 300 mg/kg. Their food was temporarily removed and offered again 1 h later. This procedure was continued for 21 days. After last dose, food consumption, no of attempts for food consumption was determined at 120th min. food intake was measure by weighing of the food cups, with subtraction of the spillage from the total food intake. Experiments took place at 10:00 a.m., during the light phase of the cycle; the rats were not accustomed to eating at this time of the day, as shown by the low food intake in controls. At the end of study body weight were measured.

Effects of WS on food intake in food-deprived rats

To evaluate the effects of WS on 20 h food-deprived rats that had not been subjected to the stress conditions. For this purpose, 20 h food-deprived rats (*n*=18) received an oral administration of vehicle or WS (100 and 300 mg/kg). Another group of non-deprived rats (*n=6*) served as the control. Food was given 1 h after the drug or vehicle administration. This procedure continued for 21 days, after last dose consumption, no of attempts for food consumption was determined at 120th min. Body weight was measure at the end of study.

Effects of WS on restraint stress-induced anorexia.

To evaluate the effects of WS on food consumption under restraint-stress conditions, rats (*n=24*) were subjected to 20h food deprivation, and then received oral administration of vehicle or WS (100 and 300mg/kg). One hour afterwards, restraint stress was induced in the rats by being restrained in cylindrical Plexiglas tubes for 60min, which has been reported to produce a marked inhibition of food intake. After the 60min restraint, the rats were returned to their home cage, where food was made available. This procedure continued for 21 days, after last dose their food consumption,

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attempts for food consumption was determined at 120th min. Body weight were measure at the end of study. Another group of deprived but non stressed rats (*n=6*) served as a control for the effects of the restraint stress; the rats of this group were oral administered with vehicle and returned to their own cages without being subjected to restraint.

Effects of WS on LPS-induced anorexia

To determine the selectivity of the anti-anorectic effect of WS, a model of anorexia induced by LPS injection was used. Administration of low doses of LPS, a pathogenic agent, induces a moderate infection that is associated with a reduction in food consumption. Food-deprived rats (*n*=18) were injected intraperitoneally with 100µg/kg LPS, and 4 h later they received oral administration of 100 and 300 mg/kg WS or its vehicle. Another group of food-deprived rats (*n*=6) received the respective vehicles and served as controls. Sixty minutes after WS administration, the rats were their food offered access to food pellets. This procedure continued for 21 days and their food consumption and attempts for food consumption was determined after last dose at 120th min. Body weight were measure at the end of study.

RESULTS

Effects of WS on freely-feeding rats:

As shown in figure 1 oral administration of vehicle or WS (100 and 300 mg/kg) did not shows any significant changes in food consumption, number of attempts for food consumption and body weight when compared with control and freely feeding group respectively. (Table 1)

Effects of WS on food-deprived rats

As shown in figure 2 food consumption, number of attempts for food consumption and body weight was significantly increased in 20 h food-deprived rats, as compared with non- deprived rats. Treatment with WS (100 and 300 mg/kg) had not shown any significant effects on food consumption, body weight and number of attempts for food consumption as compare with 20 h food-deprived rats (Table 2).

Effects of WS on restraint stress-induced anorexia:

As shown in figure 3 it revealed a highly significant treatment effect for restraint-stress-induced anorexia. The stress markedly reduced food consumption, number of attempts for food consumption and body weight, as compared with the control group that was not subjected to these stress conditions. Treatment with WS dose dependently increase food consumption, number of attempts for food consumption and body weight. This shows significantly reversed the anorectic effects of the restraint stress (Table 3).

Effects of WS on LPS-induced anorexia:

As shown in figure 4 and statistical evaluation shows that LPS treated animals shows decrease in food consumption, number of attempts for food consumption and body weight when compare with control group. Animals treated with WS (100 and 300mg/kg) shows significant increase in amount of food consumption. Also similar result was observed for number attempts for food consumption and gain in body weight (Table 4).

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Table 1. Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg, p.o., for 21 days) on food consumption, number of attempts for food consumption and change in body weight in freely feeding rats.

Animal no.	Food Consumption (Grams)				Number of attempts for Food consumption				Body Weight (Grams)			
	Control	FF	FF + W.S 100mg/kg	FF + W.S 300mg/kg	Control	FF	FF + W.S 100mg/kg	FF + W.S 300mg/kg	Control	FF	FF + W.S 100mg/kg	FF + W.S 300mg/kg
1	10.97	8.00	7.13	8.23	22.	22.	22.	18.	22.5	22.0	24.5	25.5
2	7.63	7.33	10.80	7.25	24.	24.	24.	19.	24.0	25.5	26.5	20.5
3	5.83	7.20	8.66	7.21	20.	26.	27.	27.	24.0	25.5	23.0	25.5
4	7.14	8.88	7.21	9.25	22.	24.	26.	26.	22.0	21.5	22.0	21.0
5	9.60	7.20	6.20	8.00	24.	22.	20.	22.	25.0	29.0	26.5	22.0
6	6.88	10.20	9.14	9.25	23.	20.	19.	29.	20.5	21.0	18.0	24.0
Mean ± SEM	**8.008± 0.7793**	**8.135± 0.4911**	**8.190± 0.6826**	**8.198± 0.3710**	**22.50± 0.6191**	**23.00± 0.8563**	**23.00± 1.317**	**23.50± 1.839**	**22.83± 0.7491**	**23.83± 1.276**	**23.17± 1.222**	**22.83± 0.8724**
P- Value	--	--	--	--	--	--	--	--	--	--	--	--

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Table 2. Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg, p.o., for 21 days) on food consumption, number of attempts for food consumption and change in body

Animal no.	Food Consumption (Grams)				Number of attempts for Food consumption				Body Weight (Grams)			
	Control	FD	FD + W.S 100mg/kg	FD + W.S 300mg/kg	Control	FD	FD + W.S 100mg/kg	FD + W.S 300mg/kg	Control	FD	FD + W.S 100mg/kg	FD + W.S 300mg/kg
1	7.11	9.32	10.86	8.14	22.	29.	30.	28.	22.5	22.0	26.0	26.0
2	7.63	7.04	5.27	7.32	24.	22.	27.	24.	24.0	27.0	24.5	28.0
3	5.83	7.54	9.80	8.25	26.	24.	24.	28.	24.0	29.0	25.5	24.0
4	7.14	7.82	9.24	9.35	26.	32.	26.	30.	22.0	22.5	24.5	26.5
5	9.60	8.72	6.25	8.21	24.	21.	28.	26.	25.5	25.5	25.0	24.0
6	6.88	6.99	6.22	6.61	28.	34.	28.	27.	20.5	29.5	27.0	24.0
Mean ± SEM	**7.365± 0.5090**	**7.905± 0.3826#**	**7.940± 0.9420**	**7.980± 0.3804**	**25.00± 0.8563**	**27.00± 2.221#**	**27.17± 0.8333**	**27.17± 0.8333**	**22.83± 0.7491**	**25.67±1. 308#**	**25.17± 0.4773**	**25.33± 0.6667**
P- Value	--	P<0.05	--	--	--	P<0.05	--	--	--	P<0.05	--	--

#P<0.05 when compare with control group.

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Table 3. Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg, p.o., for 21 days) on food consumption, number of attempts for food consumption and change in body weight in stress induce anorexic rats.

Animal no.	Food Consumption (Grams)				Number of attempts for Food consumption				Body Weight (Grams)			
	Control	Stress	Stress + W.S 100mg/kg	Stress + W.S 300mg/kg	Control	Stress	Stress + W.S 100mg/kg	Stress + W.S 300mg/kg	Control	Stress	Stress + W.S 100mg/kg	Stress + W.S 300mg/kg
1	10.97	2.00	10.86	15.20	22	17	22	30	22.5	11.5	25.0	33.0
2	7.63	2.38	9.17	18.00	24	14	24	34	24.0	12.5	30.5	35.5
3	5.83	1.20	9.80	16.78	26	11	21	32	245	9.0	28.0	34.0
4	7.14	2.71	12.00	15.80	22	10	25	28	22.0	8.0	30.5	40.0
5	9.60	2.30	13.86	13.60	24	8	24	24	25.0	7.5	28.0	28.5
6	6.88	2.38	13.73	15.80	28	7	23	31	20.5	7.5	31.5	34.5
Mean ± SEM	**8.008± 0.779**	**2.162± 0.213$^{\#\#}$**	**11.57± 0.806****	**15.86± 0.604****	**24.33± 0.954**	**11.17± 1.53$^{\#\#}$**	**23.17± 0.600****	**29.83± 1.424****	**22.83± 0.749**	**9.000± 0.856$^{\#\#}$**	**28.67± 0.881****	**34.00± 1.571****
P- alue	--	P<0.001	P<0.001	P<0.001	--	P<0.001	P<0.001	P<0.001	--	P<0.001	P<0.001	P<0.001

$^{\#\#}$P<0.001 when compare with control group and **P<0.001 when compare with vehicle treated group.

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Table 4. Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg, p.o., for 21 days) on food consumption, number of attempts for food consumption and change in body weight in LPS induce

Animal no.	Food Consumption (Grams)				Number of attempts for Food consumption				Body Weight (Grams)			
	Control	LPS	LPS + W.S 100mg/kg	LPS + W.S 300mg/kg	Control	LPS	LPS + W.S 100mg/kg	LPS + W.S 300mg/kg	Control	LPS	LPS + W.S 100mg/kg	LPS + W.S 300mg/kg
1	10.97	1.8	13.3	17.9	22.	10.	21.	30.	22..5	8.0	26.0	37.0
2	7.63	1.9	16.8	15.6	24.	13.	24.	32.	24..0	9.0	30.5	35.0
3	5.83	5.9	14.6	18.6	26.	8.	28.	26.	24..0	5.0	22.0	40.0
4	7.14	4.6	14.8	19.5	22.	7.	18.	34.	22..5	10.5	25.0	33.0
5	9.60	1.6	15.9	16.5	24.	5.	20.	34.	25.5	8.5	32.0	41.5
6	6.88	0.9	15.8	18.2	28.	12.	16.	30.	20.0	9.0	20.0	37.5
Mean ± SEM	**8.008 ±0.7793**	**2.783 ±0.8105$^{\#\#}$**	**15.20 ±0.5013****	**17.72 ±0.5828****	**24.33 ±0.9545**	**9.167 ±1.249$^{\#\#}$**	**21.17 ±1.759****	**31.00 ±1.238****	**22.83 ±0.7491**	**8.167 ±0.7032$^{\#\#}$**	**25.83 ±1.869****	**37.17 ±1.222****
P-alue	--	P<0.001	P<0.001	P<0.001	--	P<0.001	P<0.001	P<0.001	--	P<0.001	P<0.001	P<0.001

$^{\#\#}$P<0.001 when compare with control group and **P<0.001 when compare with vehicle treated group.

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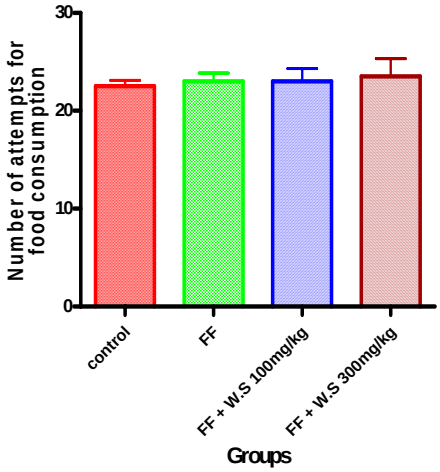

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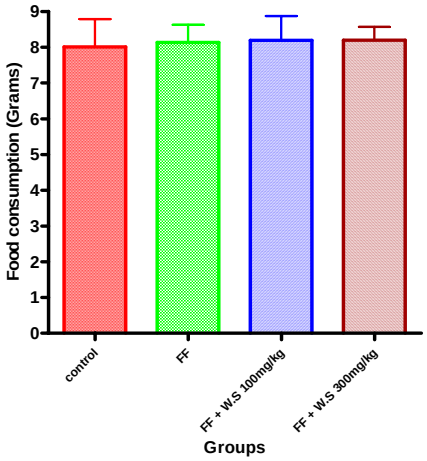

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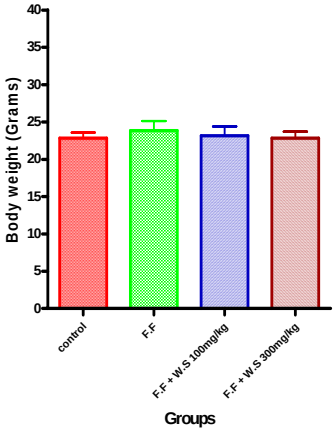

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Figure 1: Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg p.o., for 21 days) in freely feeding rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight. Data represent mean ± SEM of rats.

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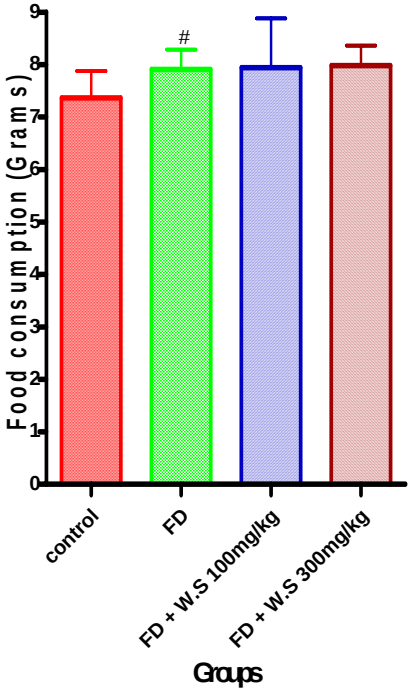

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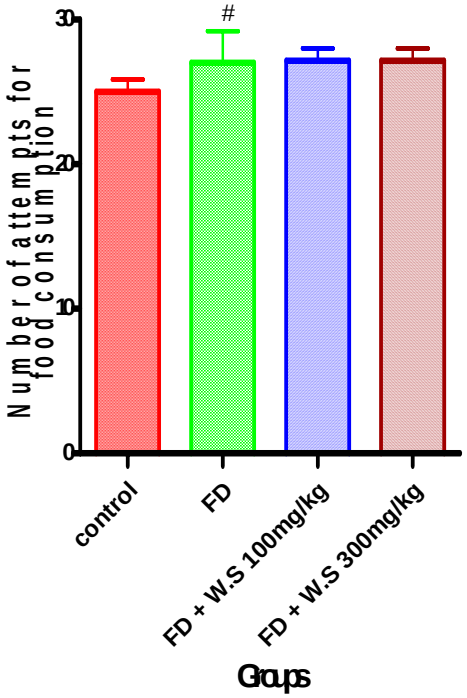

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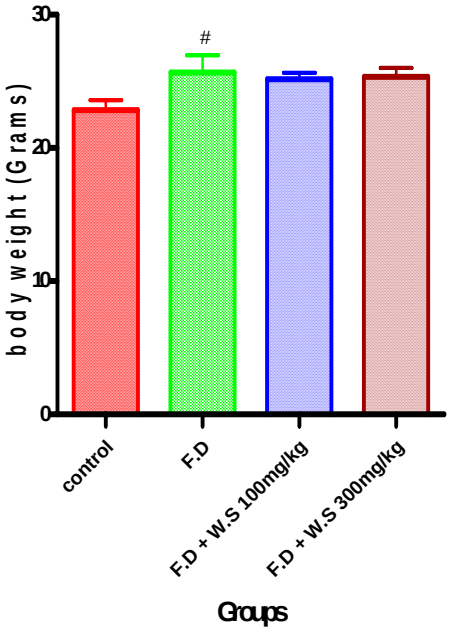

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Figure 2: Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg p.o., for 21 days) in 20 h food deprived rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight. Data represent mean ± SEM of rats. $^{\#}P<0.05$, significant when compare with control group.

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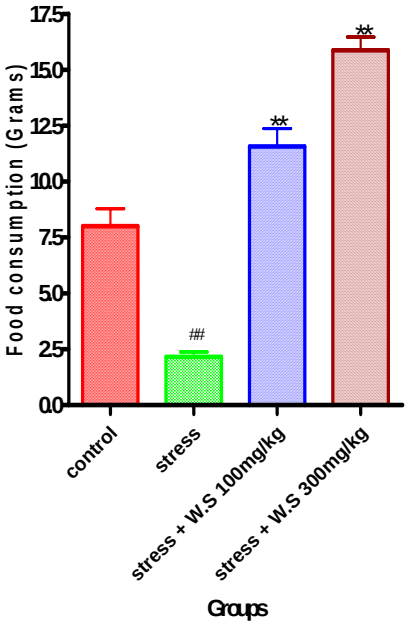

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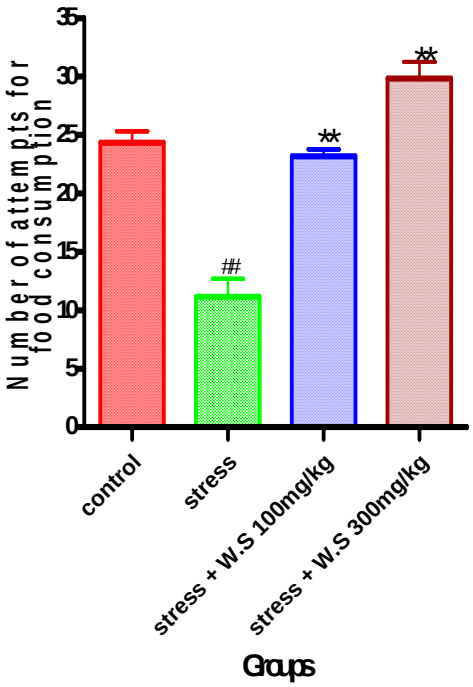

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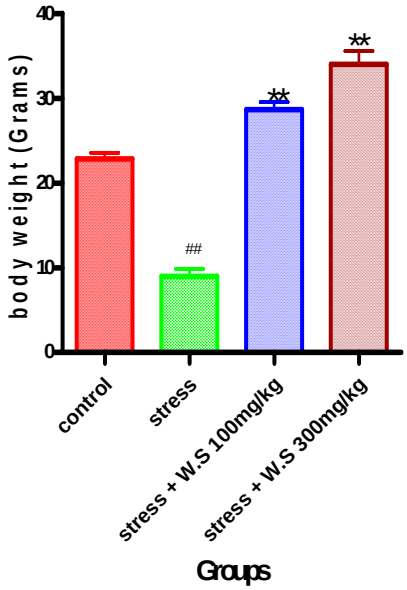

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Figure 3 Effects of oral administration of vehicle or *Withania somnifera* root extract (100 and 300 mg/kg p.o., for 21 days) in stress induce anorexic rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight. Data represent mean ± SEM of rats. $^{\#\#}P<0.001$ significant when compare with control group and $^{**}P<0.001$ significant when compare with vehicle treated group

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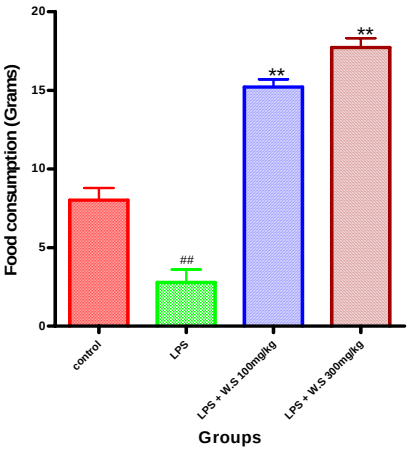

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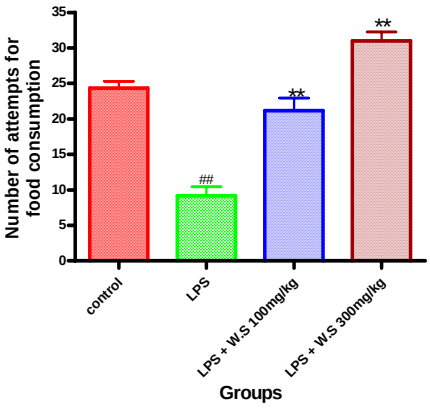

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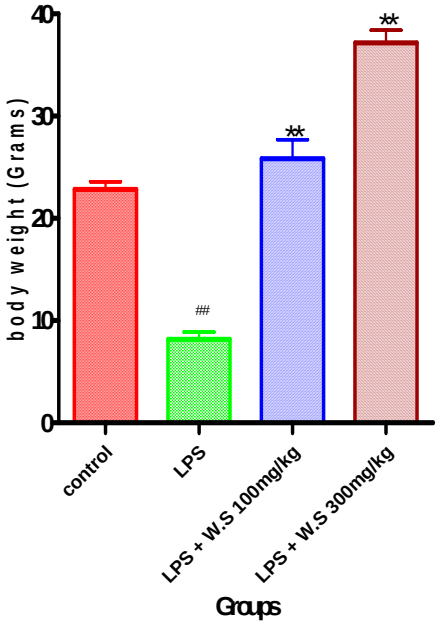

(c)

Figure 3 Effects of oral administration of vehicle or *Withania somnifera* root (100 and 300 mg/kg p.o., for 21 days) in LPS induce anorexic rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight. Data represent mean ± SEM of rats. $^{\#\#}P<0.001$ significant when compare with control group and $^{**}P<0.001$ significant when compare with vehicle treated group

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DISCUSSION

In the present study treatment with alcoholic root extract of WS prevented the physical stress & LPS induced anorexia. As evidenced by increase in food consumption, number of attempts for food consumption and change in body weight. Where as this changes was not observed in freely feeding and 20 hour food deprived rats. These finding suggest that preventive effect of WS on stress and LPS induced anorexia is not related to non specific orexigenic action of WS.

Previous preclinical studies suggest that application of chronic stress cause marked reduction in food consumption[17]. Also it suggests that central mechanisms involved in the stress-induced inhibition of food consumption, certain peptides and neurotransmitters are thought to be involved in the response[18] . It is well established that monoamines and corticotropin-releasing factor influence feeding behavior and mediate behavioral and physiological responses to stress. Several investigators have attributed stress-induced anorexia to activation of corticotropin-releasing factor and/or serotonin (5-hydroxytryptamine, 5-HT) pathways [19]. Both of these transmitters are elevated in response to stress in a number of brain areas, including those that are involved in the regulation of feeding behavior[17].

In consistent with these reports, in the present study application of physical stress significantly decreased the food consumption, number of attempts for food consumption and body weight[20]. [17]. Treatment with WS dose dependently reversed the anorectic effect followed by application of physical stress. Similar effect was demonstrated in previous studies treatment with *Rhodiola rosea* L. reverse the anorectic effect followed by physical stress, by virtue of their antistress and adaptogenic properties[17]. Similarly alcoholic root extract of W.S shows antistress, adaptogenic and antioxidant effect in foot shock and forced swim induce stress[4]. This gives an idea that in present study anti anorectic effect of alcoholic root extract of W.S treatment on stress and LPS induce anorexia may be due to its antistress, adaptogenic and antioxidant property.

Lipopolysaccharide (LPS) a endotoxin derived from the cell walls of dead and disintegrating gram-negative bacteria trigger many of the host's responses to bacterial infection [21].Doses of LPS mimicking the clinical aspects of bacterial infections reduce food consumption after parenteral administration in a variety of animal. The food consumption reduction during bacterial infection is result to complex neural, neurohumoral and endocrine interactions between bacterial products and endogenous mediators in the periphery as well as in the brain[18]. . It's well known that cytokines that they release such as interleukins and tumor necrosis factors, prostaglandins, interleukin, leptin and interferon are responsible for a number of pathological features like fever that shows decrease in food consumption, number of attempts for food consumption and body weight[22].

In agreement with previous reports, in the present study administration of LPS decreased food consumption, number of attempts for food consumption and body weight [17].. Treatment with alcoholic root extract of WS dose dependently reversed LPS induce anorexia. Lugarini et al has suggested that treatment with NS-398 reverses the anorectic effect of LPS by inhibition of inflammatory mediators[17]. Similarly alcoholic root extract of W.S. have shown potent anti inflammatory activity in various animal

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models and they suggested that this effect would be due to inhibition of inflammatory mediators[23]. In View of these reports, in the present study we suggest that anti anorectic effect of alcoholic root extract of WS in LPS induce anorexia may be due to inhibition of inflamatory mediators.

SUMMARY AND CONCLUSION

In summary, our results demonstrated that treatment with alcoholic root extract of WS has shown anti anorectic effect in physical stress and lipopolysaccharide (LPS) induce anorexia.

In conclusion, the present study provides original evidence that oral administration of WS alcoholic root extract results in a potent inhibition of the anorectic effects induced by stress and LPS, and provides functional evidence of claimed anti-stress, adaptogenic and anti inflamatory properties of the plant. However, further comparative studies using the main active components of this extract are necessary.

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