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INVESTIGATION OF INVOLVEMENT OF SEROTONIN IN ANTI-OBESITY ACTION BY FAT BURNER CAPSULE

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Abstract
The objective of the present investigation was to evaluate the possible role of serotonin in the anti-obesity action of Nutrigrow fat burner capsules. The total ash value of the capsule powder was 8.15 % with 2.65 % acid insoluble ash and 4.55 % water soluble ash. The water soluble and alcohol soluble extractives were 1.58 % and 1.54 % respectively. The results of preliminary qualitative phytochemical screening revealed that all the classes of phytochemicals (alkaloids, glycosides, saponins, tannins, phenolics, flavonoids, proteins & amino acids, sterols and triterpenoids) were present in NFB. The anti-obesity effect of the NFB weight loss capsules was assessed by observing the effect of the capsule content on the food intake habit of experimental mice. It is visible from the results that NFB was immediate in exhibiting anorexic effect (50.000 % inhibition in the first 30 min) while its anorexic potential started to lower by the 4th hour (26.011 %). Both NFB and fluoxetine were effective in reducing the food intake significantly.

Keywords
Obesity, food intake, fluoxetine, fat burner, standardization

Introduction

Obesity is a severe chronic metabolic disorder, characterized by the accumulation of excess fat in adipose tissue, as a result of imbalance between energy intake and energy expenditure. The common index used to assess overweight and obesity in adults is body mass index (BMI)[1,3]. According to the World Health Organization (WHO) reports, about 2.8 million deaths occurring throughout the world due to complications arising from obesity and overweight[4]. More than two-third of adults and almost one-third of children and adolescents in the United States are overweight or have obesity. Forty-five percent of Americans who are overweight and 67% of those with obesity are trying to lose weight. Health experts agree that making lifestyle changes—including following a healthy dietary pattern, reducing caloric intake, and engaging in physical activity—is the basis for achieving long-term weight loss. But because making diet and lifestyle changes can be difficult, many people turn to dietary supplements promoted for weight loss in the hope that these products will help them more easily achieve their weight-loss goals.

Dietary supplements promoted for weight loss encompass a wide variety of products and come in a variety of forms, including capsules, tablets, liquids, powders, and bars. Manufacturers market

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these products with various claims, including that these products reduce macronutrient absorption, appetite, body fat, and weight and increase metabolism and thermogenesis. Weight-loss products can contain dozens of ingredients, and some contain more than 90. Common ingredients in these supplements include botanicals (herbs and other plant components), dietary fiber, caffeine, and minerals. Serotonin plays an important role in maintaining appetite and hence weight gain. Several marketed formulations claiming to be able to reduce obesity have mushroomed in the market. The objective of the present investigation to investigate the role of serotonin in the antiobesity action of marketed preparation Fat burner capsules.

Material and methods

Nutrigrow fat burner capsules were purchased from the online store Flipkart India. All chemicals and reagents used were for AR grade and purchased from were chemical suppliers of the city. Experimental animal were procured from approved local breeders.

Collection of marketed product

Nutrigrow fat burner capsules were purchased from the online store Flipkart India. The material was received in discrete packaging containing 120 capsules of the formulation. The formulation was abbreviated as NFB for the study.

Organoleptic Study of NFB

Organoleptic properties are the aspects of food or other substances as experienced by the senses, including taste, sight, smell, and touch, in cases where dryness, moisture, and stale-fresh factors are to be considered[5].

Physicochemical properties of NFB

Physiochemical studies such as water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash, were carried out as per the WHO guide lines. The capsules were emptied in butter paper and the powder was used for determination of the ash values[6] and extractive values[7].

Preliminary Phytochemical Screening of NFB[8, 9]

NFB was qualitatively evaluated for alkaloids (Mayer's test), glycosides (Keller-Killiani's test), saponins, tannins and phenolics (ferric chloride and alkaline reagent tests), flavonoids (zinc hydrochloride reduction test), proteins and amino acid (ninhydrin test), sterols and terpenes (Liberman-Burchard and Salkowski tests).

Pharmacological evaluation of anti-obesity potential

Animals Used

Female Swiss albino mice weighing 20–25 g, were housed six per cage with free access to food and water at laboratory conditions. The animals were used following at least a 2-day period of adaptation to the laboratory conditions.

Drugs Used and Grouping of Animals

NFB capsules marketed by Nutrigrow was suspended in distilled water and administered at a dose of 0.5 g/kg, p. o. Fluoxetine HCl and (±)-8-Hydroxy-2-(dipropylamino)tetralin

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hydrobromide (± 8-OH-DPAT) were dissolved in distilled water. Both these drugs were administered intraperitoneally at constant volume of 1 ml/100 g body weight.

Group-1 (Vehicle control): Normal mice which developed in normal condition and give normal diet.

Group-2 (NFB treatment): Mice administered with 0.5g/kg, p.o NFB suspension

Group -3 (Fluoxetine treatment): This group served as standard and administered with fluoxetine solution (10 mg/kg, intraperitoneally)

Group -4 (± 8-OH-DPAT pretreatment): Mice administered with ± 8-OH-DPAT (0.1 mg/kg, intraperitoneally)

Group -5 (± 8-OH-DPAT pretreatment): Mice treated with ± 8-OH-DPAT 30 minutes prior to administration of NFB

Group -6 (± 8-OH-DPAT pretreatment): Mice treated with ± 8-OH-DPAT 30 minutes prior to administration of fluoxetine

Study of effect of NFB on food intake[10]

Mice were kept in groups of six in test cages and included a vehicle-treated control group and the various drug treatment groups. The food and water were withheld 1 h before the experimentation. NFB was administered orally and after 15 min preweighed sweetened chow (5 g) was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was weighed after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

Study of effect of fluoxetine on food intake

As done with NFB, mice were kept in groups of six in test cages and included a vehicle-treated control group and the various drug treatment groups. The food and water were withheld 1 h before the experimentation. Fluoxetine hydrochloride was administered intraperitoneally and after 15 min preweighed sweetened chow (5 g) was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was weighed after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

Study of effect of ± 8-OH-DPAT pretreatment food intake

The food and water were withheld 1 h before the experimentation. 8-OH-DPAT (0.1 mg/kg, i. p.) was administered intraperitoneally to the animals and after 15 min preweighed sweetened chow (5 g) was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was weighed after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

Study of effect of ± 8-OH-DPAT pretreatment food intake ability in treatment groups

The food and water were withheld 1 h before the experimentation. 8-OH-DPAT (0.1 mg/kg, i. p.) e was administered intraperitoneally to the animals. After 30 min, NFB or fluoxetine was administered to these animals and after 15 min preweighed sweetened chow (5 g) was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

weighed after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

Results and Discussion

Organoleptic Standardization of NFB

The procured NFB capsules were evaluated for texture, color, taste and odor. The results are shown in table 1.

Table 1 Organoleptic features of contents of NFB capsule

Color	Odor	Taste	Texture
Off white/Cream	Odorless	Tasteless	Powder

Physicochemical features of NFB

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. Total ash value of is an indication of the amount of minerals and earthy materials present in the formulations. NFB exhibited 8.15 % total ash with 2.65 % acid insoluble ash and 4.55 % water soluble ash. The water soluble and alcohol soluble extractives were 1.58 % and 1.54 % respectively suggesting the formulation to be suitable for human use.

Qualitative phytochemical screening

The powder of NFB was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The result is presented in table 2.

Table 2 Phytochemical screening NFB

Phytochemical Tested	Observation	Inference
Alkaloid	Cream precipitate formation in Mayer's Test	Present
Glycoside	Greenish color in acetic acid layer in Keller-Killiani Test	Present
Saponin	Frothing Formation	Present
Tannins	Yellow color precipitate in Alkaline Reagent Test	Present
Phenolics	Bluish green color in Ferric chloride Test	Present
Flavonoids	Red color formation in Zinc reduction Test	Present
Proteins and Amino acids	No color formation in Ninhydrin Test	Present
Sterols	Green Color in Burchard Test	Present
Triterpenoids	Grey color in Salkowski Test	Present

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Anti-obesity action

The ability of rodent to consume sweetened chow or high fat diet is a highly effective model for study of anti-obesity action of drugs. A standard chow died provides 12.98% fat energy, 58.62% carbohydrates and 28.40% proteins, contributing to total of 1243.6 kcal/100g. On the contrary, a high fat diet (sweetened chow) usually contains 50.52% fat, 31.50% carbohydrates, and 17.98% proteins, contributing to 1765.8 kcal/100g.

The anti-obesity effect of the NFB weight loss capsules was assessed by observing the effect of the capsule content on the food intake habit of experimental mice. The amount of food remained unconsumed post 4 hours of treatment in each group was weighed and the food intake in grams was calculated per 20 g body weight (Table 3 & 4).

Table 3 Weight of food unconsumed by mice

		Weight of HFD remaining (g)				
	Treatment	**0.5 h**	**1h**	**2h**	**3h**	**4h**
Group I	Vehicle	4.69	4.32	3.91	3.63	3.44
Group II	NFB	4.73	4.46	4.21	4.08	3.86
Group III	Fluoxetine	4.81	4.72	4.56	4.24	4.08
Group IV	8-OH-DPAT	4.36	3.74	3.53	3.41	3.27
Group V	8-OH-DPAT + NFB	4.68	4.34	4.09	3.89	3.72
Group VI	8-OH-DPAT + Fluoxetine	4.74	4.46	4.29	4.05	3.97

Table 4 Weight of food consumed by mice

		Cumulative Food intake/mouse (g/20g body weight)				
	Treatment	**0.5 h**	**1h**	**2h**	**3h**	**4h**
Group I	Vehicle	0.31	0.68	1.09	1.37	1.56
Group II	NFB	0.27	0.54	0.79	0.92	1.14
Group III	Fluoxetine	0.19	0.28	0.44	0.76	0.92
Group IV	8-OH-DPAT	0.64	1.26	1.47	1.59	1.73
Group V	8-OH-DPAT + NFB	0.32	0.66	0.91	1.11	1.28
Group VI	8-OH-DPAT + Fluoxetine	0.26	0.54	0.71	0.95	1.03

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

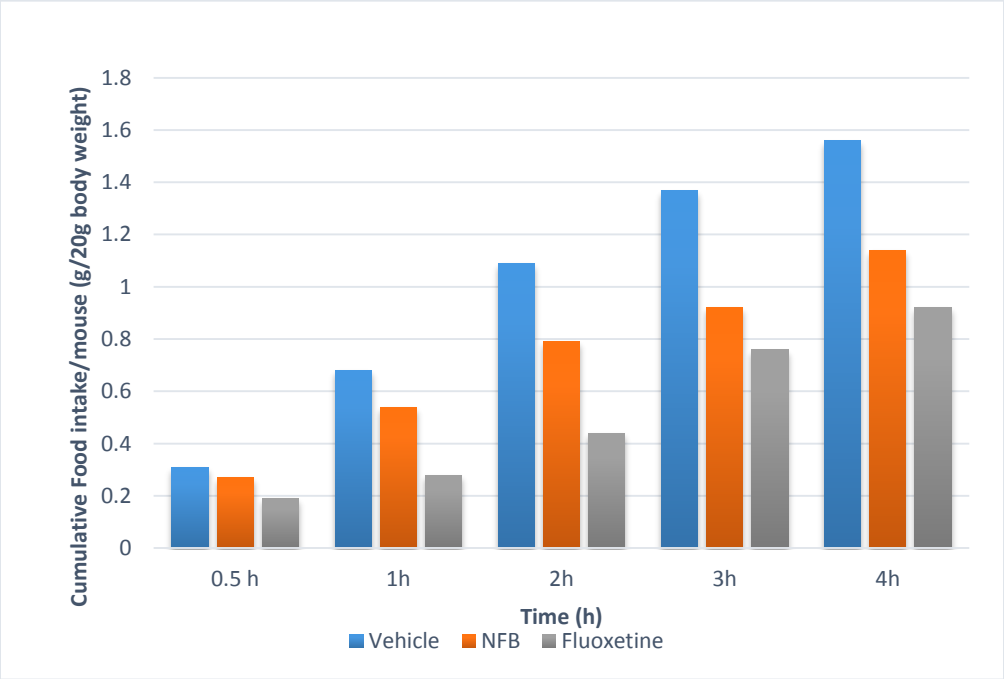

Figure 1 Effect of NFB (0.5 g/Kg, p.o) and fluoxetine (10 mg/Kg, i.p) on food intake in mice

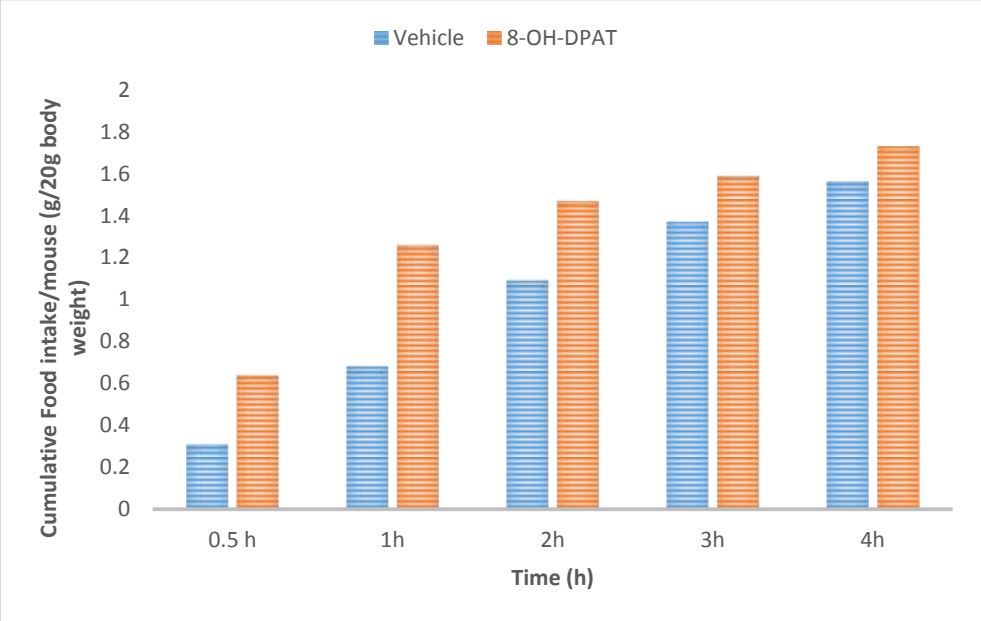

Figure 2 Effect of (±) - 8- OH-DPAT on food intake in mice

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

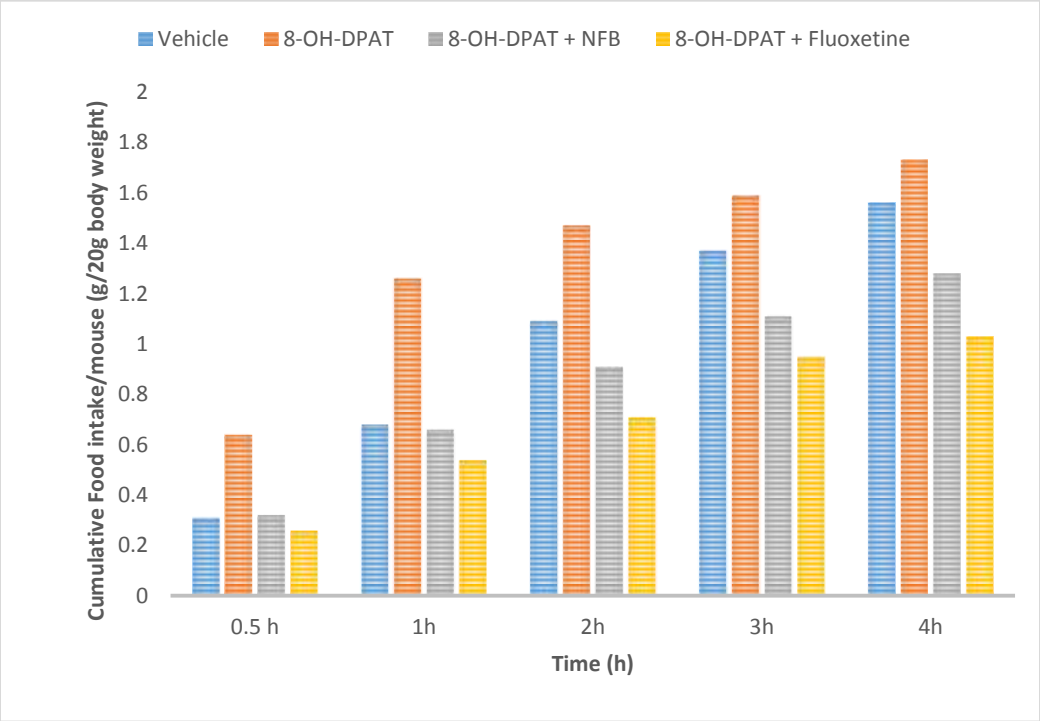

Figure 3 Effect of co-administration of (±) - 8- OH-DPAT + NFB and (±) - 8- OH-DPAT + fluoxetine on food intake in mice

As clearly visible from the results above, fluoxetine and NFB are hypophagic and restrict the intake of food by the mice (Figure 1). On the other hand (±) - 8- OH-DPAT is a known agonist of 5-hydroxytryptamine and exhibits hyperphagic action (Figure 2). On co-administration of (±) - 8- OH-DPAT with either NFB or fluoxetine, it was found that the hyperphagic effect of (±) - 8-OH-DPAT was antagonized by both NFB and fluoxetine (Figure 3).

The reduction in food intake was calculated with reference to the food intake exhibited by hyperphagic animals (Table 5).

Table 5 Percent reduction in food intake by treated animals

	Percent reduction in food intake as compared to 8-OH-DPAT				
Treatment	**0.5 h**	**1h**	**2h**	**3h**	**4h**
8-OH-DPAT + NFB	50.000	47.619	38.0958	30.188	26.011
8-OH-DPAT + Fluoxetine	59.375	57.142	51.700	40.251	40.623

The reduction in food intake was converted to percentage reduction and the plot of reduction with time was obtained. It is visible from the plot that NFB was immediate in exhibiting anorexic

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effect while its anorexic potential started to lower by the 4th hour. Both NFB and fluoxetine were effective in reducing the food intake by about 50% by the 4th hour.

The result of the study showed that 8-OH-DPAT, a 5-HT_{1A} agonist, causes inhibition of the endogenous satiety system and increases food intake at low doses. Additionally, studies have demonstrated that the hyperphagic effects of 8-OH-DPAT were a consequence of the reduced 5-HT synthesis and release caused by the agonistic action of the drug at somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei[11]. The antagonism of 8-OH-DPAT-induced hyperphagia by fluoxetine is also consistent with the earlier findings. Thus, it is likely that NFB may also mediate its effect on food intake through 5- HT_{1A} receptors as well since it significantly antagonized 8-OH-DPAT-induced hyperphagia.

The study helped in elucidating the probable involvement of serotonin in the anti-obesity action of Nutrigrow fat burner capsules.

Conclusion

In the present study, an attempt was made to elucidate the possible mechanism for anti-obesity action of online marketed nutraceutical product Nutrigrow fat burner capsules. The capsules were able to reduce the food intake in mice and thus can be attributed to be hypophagic. The food intake in mice was not reverted by co-administration of hyperphagic agent 8-OH-DPAT, a potent full agonist of 5-HT_{1A} receptor. This led to the conclusion that the action so Nutrigrow fat burner capsules can be attributed to antagonism of serotonin.

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