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Effect of Liv.52 on Fenfluramine-induced Anorexia

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ABSTRACT

Cyproheptadine is known to counteract the anorexia induced by fenfluramine, a well-known anorectic agent. Liv.52, an indigenous herbal formulation, is a proven appetite stimulant effective in liver disorders, anorexia and malnutrition. This study evaluated the appetite-stimulating action of Liv.52 in fenfluramine-induced anorexia and compared it with cyproheptadine.

The study was divided into two phases. In the first, the effect of pre-treatment with Liv.52 in counteracting fenfluramine-induced anorexia for 14 days was studied in 24 Wistar preconditioned rats, divided into four groups. In the second phase, a comparison was made between Liv.52 and cyproheptadine pretreatment followed by fenfluramine challenge in 18 preconditioned rats divided into three groups.

The parameters adopted were the afternoon food intake and the overnight food intake. Liv.52 was found to stimulate the appetite resulting in significant improvement in both the parameters by day 14, whereas only the afternoon food intake improved with cyproheptadine by day 6.

Food intake is influenced by the biorhythms of hunger and satiety. The balance between satiety and appetite is lost during anorexia. Liv.52 possibly normalizes the hunger-satiety cycle and stimulates the appetite.

INTRODUCTION

Fenfluramine induces anorexia through a serotoninergic mechanism[1], which is counteracted by cyproheptadine, a known 5-HT antagonist[2]. Cyproheptadine is often clinically advocated as an appetite stimulant[3,4].

Liv.52, a herbal preparation, has been widely prescribed in liver disorders[5-8]. It is also known to be effective in anorexia and malnutrition[9-13].

The mechanism of action of Liv.52 in appetite stimulation is not well defined. The present study was designed to elucidate the mechanism of action of Liv.52 in stimulation of appetite by counteracting fenfluramine-induced anorexia.

MATERIALS AND METHODS

Animals

Forty two laboratory inbred female rats of original Wistar strain, weighing between 200-300 gm (3-4 months old), were housed individually and acclimatized to a constant room temperature of 20-22^{o}C, and 12-hour light and 12-hour dark cycles. These animals were pre-conditioned to a certain dietary pattern 2 weeks prior to the commencement of the treatment.

Drugs used in the study

1. Liv.52 powder (The Himalaya Drug Company), 3 gm/kg orally
2. Fenfluramine HCl (Serdia), 10 mg/kg orally
3. Cyproheptadine HCl (Merind), 6 mg/kg.

Pre-conditioning of animals to a dietary pattern:

The animals were acclimatized to a dietary pattern for 2 weeks. Between 16.30 hours to 09.30 hours (next morning) the rats were given 20 gm of standard rat feed pellets (Lipton) in pre-weighed containers. The leftover food was withdrawn at 9.30 hours next morning and weighed again to accurately quantify the overall food consumption. This was termed as Overnight Food Intake. The rats were fasted between 09.30 hours to 14.30 hours during the day. At 14.30 hours, 15 gm of a semisolid, palatable diet was offered for 30 minutes in pre-weighed containers. The leftover food was removed at 15.00 hours and weighed. This was designated as the Afternoon Food Intake. The semisolid palatable diet consisted of condensed milk as a sweetening agent, water and powdered food pellets in 1:3:4 proportions. Daily record of afternoon as well as overnight food intake was maintained.

Liv.52 pre-treatment followed by fenfluramine challenge

The effect of pre-treatment with Liv.52 in counteracting fenfluramine-induced anorexia for 14 days was studied in 24 pre-conditioned animals.

The animals were divided into four groups: (1) Group I was given tap water orally at 10.30 hours from day 1 to day 14, (2) 6 rats in Group II were given Liv.52 (3 gm/kg) orally as uniform suspension in tap water at 10.30 hours from day 1 to 14. This treatment preceded the afternoon food intake by 4 hours, (3) Rats in Group III were administered fenfluramine (10 mg/kg) orally, suspended in water, at 12.30 hours on day 7 and day 14 in a single dose. This treatment preceded the afternoon food intake by 2 hours and (4) Group IV animals received Liv.52 (3 gm/kg) as suspension in tap water orally at 10.30 hours from day 1 to 14. On day 7 and day 14, in addition, they also received fenfluramine (10 mg/kg) orally as a single dose, at 12.30 hours, i.e. 2 hours after Liv.52 treatment.

Comparison between Liv.52 and cyproheptadine pre-treatment followed by fenfluramine challenge

The appetite-stimulating effect of Liv.52 was compared with that of cyproheptadine. Eighteen pre-conditioned rats were divided into 3 groups. As in the previous study, animals in Group I received water orally at 10.30 hours from day 1 to day 14. Group II animals received cyproheptadine, 10 mg/kg orally, at 10.30 hours from day 1 to day 14. Cyproheptadine was suspended in water. Rats in Group III received Liv.52, 3 gm/kg, in suspension at the same time for 14 days. On day 14, all the animals were challenged by a single dose of fenfluramine, 10 mg/kg at 12.30 hours.

RESULTS

Liv.52 pre-treatment followed by fenfluramine challenge

Table 1 indicates that the difference in the overnight food intake of different groups was statistically not significant indicating low variability between the groups before starting the treatment.

Table 1: Overnight food intake (out of 20 gm) of the different groups on day 0 (Mean ± SE)				
Day	Group I Tap water	Group III Liv.52	Group III Fenfluramine	Group IV Liv.52 + Fenfluramine
0	8.00 ± 0.59	8.50 ± 1.88	7.50 ± 1.38	8.83 ± 1.33

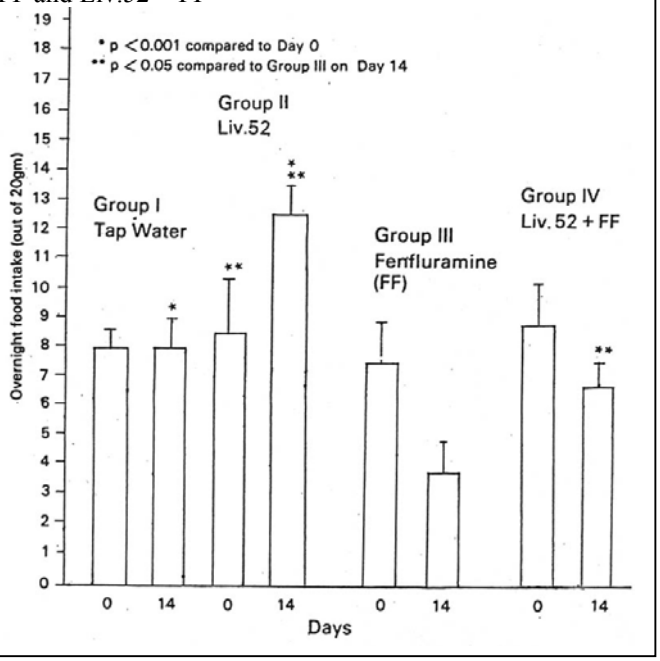
Following the single dose of fenfluramine challenge on day 7 and day 14 to Group III animals, there was significant reduction in both the afternoon and overnight food consumption. The reduction in food consumption was more at night (Table 2).

Table 2: Reduction in the food intake (in gm) due to fenfluramine (Group III) (Mean ± SE)		
Days	Afternoon food intake	Overnight food intake
0	10.33 ± 1.54	7.50 ± 1.38
7	$4.00 \pm 0.73^{+}$	$1.17 \pm 0.31^{++}$
14	5.67 ± 1.17*	3.83 ± 1.01**

$^{+}p<0.01$ compared to day 0
$^{++}p<0.001$ compared to day 0
*,** $p<0.05$ compared to day 0

Figure 1 depicts the change in overnight food intake with different treatments. There was no change in overnight food intake of Group I animals receiving water. Animals treated with only Liv.52 in Group II showed increase in the food intake. This increase was significant on day 14, as compared to the food intake in Group I animals receiving tap water and also compared to day 0 of the same group.

Figure 1: Change in the overnight food intake with Liv.52, FF and Liv.52 + FF



In animals of Group IV pre-treated with Liv.52, fenfluramine caused significant reduction in food intake on day 14. However, the extent of this reduction in food intake was less with Liv.52 pre-treatment for 14 days, as compared to that due to fenfluramine alone. It was observed that Liv.52 partly counteracted the effect of fenfluramine.

Comparison between Liv.52 and cyproheptadine pre-treatment, both followed by fenfluramine challenge

After conditioning to the specific food pattern, there was no variability between the animals in the different groups on day 0. As indicated in Table 3, Fig. 2, cyproheptadine and Liv.52 treatment to Groups II and III respectively from day 1 to 13 improved the afternoon food intake of the animals. The average food intake of the animals treated with Liv.52 for 13 days was significantly

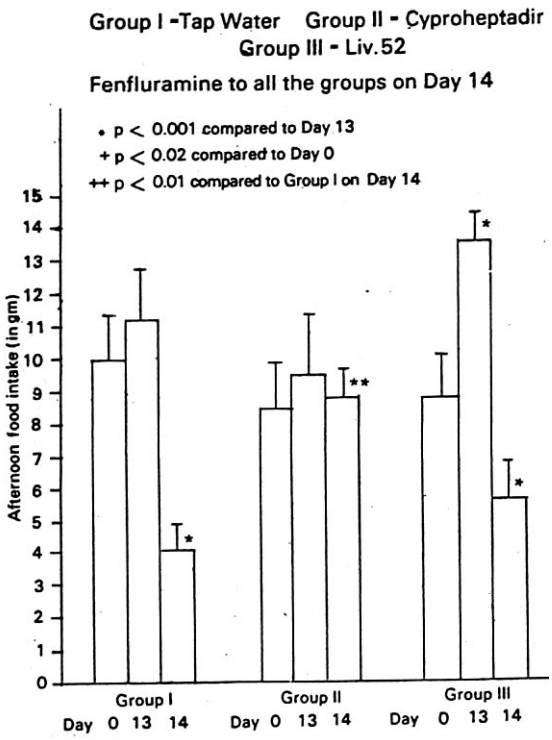
Table 3: Effect of fenfluramine on the afternoon food intake (in gm) in the different animal groups (treated with Liv.52 and cyproheptadine) on day 14 (Mean ± SE)		
Groups	Food intake on day 13	Food intake on day 14 (after fenfluramine)
Group I (Tap water)	11.20 ± 1.59 (74.67%)	4.40 ± 0.93* (29.33%)
Group II (Cyproheptadine)	9.50 ± 1.85 (63.33%)	6.75 ± 0.95 (58.33%)
Group III (Liv.52)	13.60 ± 0.87 (90.67%)	5.60 ± 1.21** (37.33%)

* $p<0.001$ as compared to day 13 and average of day 1 to 13
** $p<0.001$ as compared to day 13 and average to day 1 to 13

higher compared to day 0. Cyproheptadine increased the food intake of Group II animals significantly by day 6 and thereafter there was marginal decline.

On day 14, following fenfluramine challenge, there was significant reduction in the afternoon food intake of tap water-treated animals in Group I (Table 3). The cyproheptadine-treated group did not show any decreased in the food intake. Also, food intake in the cyproheptadine-treated group was significantly higher as compared to that in the water-treated animals on day 14 after fenfluramine challenge (Fig. 2). Significant reduction in the food intake of Liv.52-treated animals was also observed as compared to their average intake with fenfluramine challenge.

Figure 2: Comparison between Cyproheptadine and Liv.2 pretreatment followed by Fenfluramine challenge



DISCUSSION

Fenfluramine is a well-known anorectic agent. It acts via the release and prevention of uptake of serotonin in the presynaptic neurones[14]. Brain serotonin and carbohydrate intake are linked. In this study, a carbohydrate-rich diet was offered to the animals in the afternoon. Fenfluramine, 10 mg/kg, brought about significant reduction in food intake, both in the afternoon as well as at night. This observation confirmed the suitability and validity of the animal model used for inducing anorexia, irrespective of a bland standard diet or a carbohydrate-rich diet.

Liv.52 stimulated the appetite, improving the food intake both in the afternoon and at night significantly by day 14. Cyproheptadine increased the afternoon food intake significantly by day 6. However, on day 13, there was marginal reduction, probably due to development of tolerance.

On day 14, significant reduction in food intake due to fenfluramine was observed in the group of animals receiving water thus reconfirming the suitability of this animal model for food intake and anorexia studies.

The cyproheptadine-treated group showed no change in the afternoon food intake in response to fenfluramine on day 14, indicating antagonism by cyproheptadine to the fenfluramine effect. Fenfluramine to the fenfluramine effect. Fenfluramine did not induce anorexia in the groups pre-treated with cyproheptadine. The degree of antagonism on day 14 was significant when compared to fenfluramine-induced anorexia in the water-treated animals.

The degree of anorexia was significantly reduced in the overnight food intake following fenfluramine challenge in animals pre-treated with Liv.52. A similar trend was observed in the afternoon. Possibly Liv.52 antagonizes partially the effect of fenfluramine through the serotoninergic mechanism.

Food intake is influenced by the biorhythms of hunger and satiety. The balance between satiety and appetite is lost during anorexia. Kulkarni *et al.*, have shown that Liv.52 normalised the appetitic-satiety rhythm in children, while cyproheptadine did not[15]. Liv.52 possibly corrects the physiological balance. It is possible that the action of Liv.52 is more physiological than pharmacological and it acts possibly through correcting the physiological imbalance.

Rats are nocturnal animals and in this study the increase in the overnight food intake was apparently more with Liv.52 treatment, compared to the increased food intake in the afternoon. This supports the contention that Liv.52 possibly normalizes the hunger-satiety cycle and stimulates the appetite.

REFERENCES

1. Duhault, J., Boulanger, M., Voisin, C., Malen, Ch. and Schmitt, H., "Fenfluramine and 5-hydroxytryptamine, Part 2. Involvement of brain 5-hydroxytryptamine in the anorectic activity of fenfluramine". *Arzneim. Fosch.* (Drug Research) (1975) : 25, Nr. 11, 1758-1762.

2. Douglas, William, W., "Histamine and 5-hydroxytryptamine (serotonin) and their antagonists". In: Louis S. Goodman and Alfred Gilman (Eds). *The Pharmacological Basis of Therapeutics* (1985), 7th Edition, 634, Macmillan Publishing Co., New York.

3. Stone, C.A., Wenger, H.C., Ludden, C.T., Stavonski, I.M. and Ross, C.A. "Antiserotonin, antihistaminic properties of cyproheptadine". *J. Pharmac. Exp. Ther.* (1961) : 131, 73-84.

4. Andronic, A. and DiMascio, A., "Appetite stimulating and weight gain properties of cyproheptadine (Periactin) in geriatric subjects". *Curr. Ther. Res.* (1971) : 13, 40-41.

5. Dayal, R.S., Kalra, K. and Mital, U.P. "Liv.52 therapy in cases of malnutrition, infective hepatitis, infantile cirrhosis and anorexia". *The Antiseptic* (1974): 71, No. 2, 89.

6. Habibullah, C.M., Chandra, Vinod, Padmanabhan, C.G. and Ram Krishna, R., "Liv.52 in acute viral hepatitis : results of a double blind study". *The Antiseptic* (1978) : 8, 491.

7. Mehrotra, M.P. and Tandon, S., "Liv.52 – a clinico-biochemical trial in hepatic cirrhosis." *Curr. Med. Pract.* (1973): 17, 4, 185.

8. Kulkarni, R.D., "Effect of ethanol on liver function and its relation to hangover : protective effect of Liv.52." *Ind. Drugs* (1980) : 11, 363.

9. Indira Bai, Rao, W.P.R. and Subba, K.V., "Therapy of anorexia with Liv.52". *The Antiseptic* (1970) : 8, 615.

10. Pai, V.R., Borgave, M.A., Sule, C.R., Kale, S.S. and Kale, S., "Liv.52 therapy in hypoproteinaemia". *J. Ind. Med. Prof.* (1972) : 6, 8447.

11. Saxena, S., "Liv.52 in anorexia in paediatric practice". *Curr. Med. Pract.* (1971) : 1, 580.

12. Reddy, Y.R., Sulochana, K.S. and Mathur, Y.C., "A clinical study of the effect of Liv.52 on the growth pattern of normal and malnourished children in rural areas (310 cases)". *Paed. Clin. India* (1975) : 3, 157.

13. Khetarpal, S.K., Ram Kumar, L. and Lubhaya, R., “Malnutrition, hepatic function and Liv.52 therapy”. *Curr. Med. Pract.* (1972) : 11, 481.

14. Welsh, J.H., “Distribution of 5-HT in the nervous system of animals”. *Adv. Pharmac.* (1968) : 6A, 171.

15. Kulkarni, R.D., “Biological rhythms and medicine”. *Curr. Med. Pract.* (1980) : 8, 305.