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Role of ayurvedic formulation in the treatment of anorexia: An experimental study

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Abstract
Ayurveda system of medicine is well known to treat loss of appetite; clinically known anorexia. Intuppu Churna is a commonly used Ayurvedic formulation for the treatment of *Agnimandya* i.e anorexia. The medicine consist three types of herbs: *Terminalia chebula* (Combretaceae), *Piper longum* (Piperaceae), *Trachyspermum roxburghianum* (Umbelliferae) and one mineral - rock salt. The objective of the study was to determine the antianorectic effects of these drugs individually and to compare these effects with the effect of Intuppu. The activity of the drugs was studied after anorexia was induced in rats by (1) physical stress arising from immobilization for 60min; (2) intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS, 100 µg/kg body weight); and (3) intraperitoneal administration of fluoxetine (8 mg/kg body weight). Similar doses of the extracts were tested on freely feeding rats and on rats that had been food deprived for 20 h. Corticotrophin releasing factor (CRF, 0.3 µg/rat) can induce anxiogenic-like behavior and reduced food intake. This model was also studied, and the results were compared. The present study shows that Intuppu churna effectively reduces the marked anorexia induced in rats by exposure to different stressful conditions. IF 200 and 400 mg/kg significantly reversed restraint stress, LPS, Fluoxetine and CRF induced anorexia. While the drug in the used dose range does not affect food intake of freely feeding and non-stressed food deprived rats. IF shows the anti-anorectic effect in restraint stress, LPS, Fluoxetine and CRF induced anorexia models which indicates that the action of IF is nonspecific.

Keywords: Ayurveda, intuppu, anorexia, LPS, CRF, fluoxetine

Introduction
Occasional fasting is good for maintaining health. When it becomes an obsession, it becomes a disorder. Anorexia is such an eating disorder with reduced appetite or total aversion to food. Anorexia describes any loss of appetite and concomitant reduction in food intake that occurs in the presence of readily accessible food sources. Generally speaking, anorexia occurs in two broad sets of conditions (Figure 1).

In the first set, anorexia is a symptom that accompanies a number of pathologies. In turn, these pathologies can be subdivided into two groups; (1) the anorexia that is widely believed to originate psychologically, of which Anorexia Nervosa is a most prominent. (2) Anorexia that is associated with disease states, of which the cachexia (Disease associated wasting) that accompanies AIDS, cancer and other conditions is widely known. In the second set Anorexia is an apparent as an adaptive behavioral response to certain homeostatic challenges. Some of these may originate externally, as is the case of anorexia that accompanies some types of stress, or it may be to a physiological challenge such as dehydration. The psychological stress can affect the food intake. Part of the adaptive response to certain types of stress anorexia may impart temporarily [1]. Restraint or immobilizations are the stressors used most widely to investigate this type of anorexia [2].

Temporary stress might affect mechanisms in the body that regulate energy expenditure and food intake [3]. The stress is responsible for the release of corticotrophin releasing factor (CRF) in hypothalamic region which in turn inhibit the feeding. If CRF is administered centrally, it can induce anxiogenic like behavior and reduce food intake [4] and if administered by intra-venous route (IV) or intraperitonealy (IP), it is found to inhibit the gastric emptying [5].

Furthermore, complex neuronal mechanisms are involved in the feeding inhibition. Proinflammatory cytokines play an important role in the feeding inhibition by stimulation of Tumor necrosis factor-α (TNF- α) and Interleukin-1 (IL- 1). This release of cytokines can be stimulated by ingestion of Lipopolysaccharide (LPS) of bacterial cell wall [6]. The available data suggests that serotonergic system also plays a role in mediation of cytokine induced inhibition of feeding. Fluoxetine which is a selective 5-Hydroxy tryptamine (5-HT) reuptake inhibitor can induce the anorexia [7]. To overcome this problem many promising pharmacological agents have been clinically tested but, unfortunately, failed to demonstrate

therapeutic efficacy against anorexia or cachexia. Among other examples, endocannabinoids showed limited effects in AIDS patients [8] and no greater effect over placebo in advanced cancer patients [9]. Pharmacological agents currently in use to combat anorexia are thalidomide, eicosapentaenoic acid (A long chain ω-3 polyunsaturated fatty acid), corticosteroids and progestrogens derivatives [10]. Despite the fact they exert well documented anti-inflammatory actions, these agents show only temporary benefits on appetite, numerous side effects and their mechanism of actions on feeding remain unclear. Various models have been developed for the investigation of anorexia for the synthetic drugs. However the claims of traditional drugs regarding its antianorectic role remains unexplored. Herbal formulas are favored in Ayurveda because the founders of Ayurveda recognized the possible synergistic and counterbalancing effects of herbs [11]. Sometimes none of the herbs in a formula exhibit therapeutic effects individually; but the formula could be effective [12]. Literature survey revealed that there is no work reported on antianorectic activity of Intppukana churna. So the study was designed to support the claim.

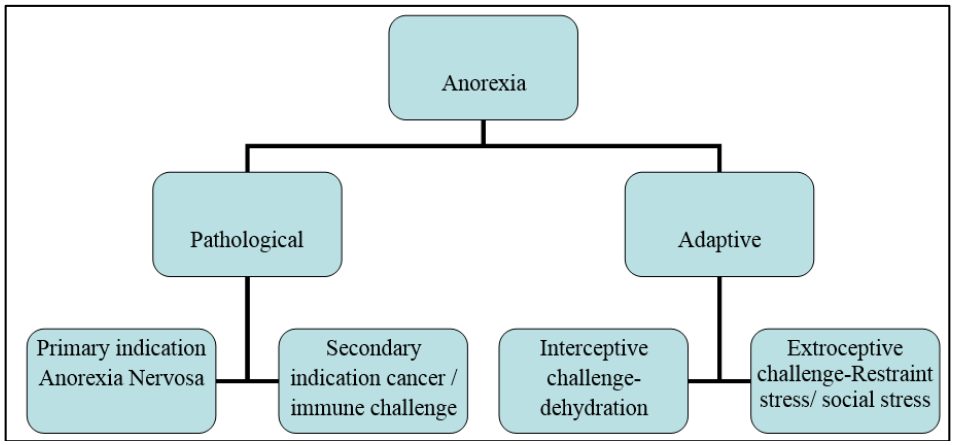

Fig 1: Types of anorexia

Material and Methods

Preparation of formulation Intuppukana churna

One part of Rock salt, two parts Fruits of *T. roxburghianum* (*Apiaceae*), 4 parts fruits of *P. longum* (Piperaceae) and six parts of *T. chebula* (Combretaceae) powdered previously and passed through sieve no. 80 were mixed uniformly [13].

The anti-anorectic activity of *T. roxburghianum* (TR), *T. chebula* (TC), *P. longum* (TC), Rock salt (RS) and IF was tested and compared with the activity of Intuppukana churna. According to OECD guideline 423 the doses are selected 100,200 and 400 mg/kg for the study [14]. The Dose at which, *T. roxburghianum* (TR), *T. chebula* (TC), *P. longum* (TC) and Rock salt (RS) showed the activity was taken for comparison with IF. Similarly in case of absence of response, the highest dose level *i.e.* 400 mg/kg was selected for the comparative study with IF.

Animals

Male Wistar rats weighing 250–300 gm were selected for anti-anorectic tests. The animals were individually housed in a cage, maintained at 22 ± 2 °C in a room with a 12 h light/dark cycle (Lights on at 6 am) and had free access to feed and water *ad libitum* during quarantine period. The study is conducted with current ethical regulations on animal research and related rules of our Institute and all animals used in the experiment received human care. All the pharmacological experimental protocols were approved by Institutional animal ethics committee.

Material

All extracts were dissolved in absolute ethanol and diluted with tap water in order to maintain the final ethanol concentration of 1% v/v in all treatment conditions. Each extract was administered by intragastric administration at dose of 100, 200, 400 mg/kg. The same vehicle was administered to the control group.

CRF (Rat; Sigma Aldrich Germany) was dissolved in sterile isotonic saline prior to use given by Intravenous route (IV) at the dose of 0.3µg/rat.

Lipopolysaccharide from *E. coli* (Sigma-Aldrich Germany; LPS) was dissolved in pyrogen free isotonic saline and given by intraperitoneal injection (IP) at the dose of 100µg/kg. Fluoxetine Hydrochloride (Sigma-Aldrich Germany; FLU) was dissolved in sterile physiological saline and administered IP at the dose of 8 mg/kg/ml.

Experimental procedure

All the experiments were carried out at 10.30 am during the light phase of the cycle

1. Effects of IF and its ingredients on food intake in freely feeding rats

To evaluate the general effect of TR, TC, PL, RS and IF on food intake, all extracts effects were examined in freely feeding rats. The animals were divided into thirteen groups involving eight animals in each group. All animals received intragastric administration of vehicle, TR, TC, PL, RS and IF in dose of 100, 200 and 400 mg/kg. Their food was removed temporarily for 1 h and offered again later. Food consumption was determined at 30, 60, 90, and 120 min and 4, 6, and 24 h after test extract administration, by weighing the food cups and by subtracting the spillage from total food intake.

2. Effects of IF and its ingredients on restraint stress induced anorexia

The rats (n =117) were subjected to 20 h food deprivation to evaluate the effects of all extracts on food consumption under restraint stress conditions and then given intragastric administration of vehicle, TR, TC, PL, RS and IF (100, 200 and 400 mg/kg). One hour later restraint stress was induced in the rats by restraining in cylindrical Plexiglas tubes for 60 minutes [15]. After the restraint of 60 min the rats were

returned to their home cages and offered food ad libitum. The food consumption was recorded 30, 60, 90 and 120 min, and 4, 6, and 24 h later. Control group animals (n= 10) were food deprived but not subjected to restraint received intragastric administration with vehicle and returned to their home cage.

3. Effects of IF and its ingredients on LPS induced anorexia

LPS is a pathogenic agent which induces a moderate infection which is associated with reduction in food consumption [16]. The rats (n=100) were food deprived for 20 h and then injected with 100µg/kg LPS. 4 h later they received intra gastric administration of vehicle, TR, TC, PL RS and IF (100,200 and 400mg/kg). Control group rats (n =10) were food deprived and received vehicle. Sixty minutes after test extracts administration, rats were provided food and food consumption of individual rat was determined 30, 60, 90 and 120 min, and 4, 6, and 24 h later.

4. Effects of IF on Fluoxetine induced anorexia

Fluoxetine is a selective 5- HT reuptake inhibitor which is responsible for the reduction in food intake. It can be beneficial to evaluate the selectivity of the antianorectic effect of IF. Rats (n=104) were food deprived for 20 h. They received intragastric administration of vehicle, TR, TC, PL, RS and IF (100,200 and 400 mg/kg), and after 60 min injected intraperitonealy with 8mg/kg FLU [17]. 30 min later all rats were given free access to food and their food consumption was recorded 30, 60, 90 and 120 min, and 4, 6, and 24 h later. Control group animals were food deprived and received IG administration of IF vehicle and followed by FLU vehicle injection IP to observe the effect of FLU on food consumptions.

5. Effects of IF on food intake of food deprived rats

To correlate the hypophagic effect of stress with orexigenic action of IF and its individual ingredient, extracts were evaluated for food consumption in food deprived rats which were not subjected to stress conditions. In this experiment food deprived rats (n=104) received IG administration of vehicle, TR, TC, PL, RS and IF (100, 200 and 400 mg/kg) and control group (n= 10) is of non –deprived rats. Food was offered ad libitum 1 h after administration, and food intake was recorded at 30, 60, 90 and 120 min, and 4, 6, and 24 h later.

6. Effects of IF on CRF induced anorexia

IV CRF (0.15-10µg) inhibited gastric emptying in rats similar to partial restraint stress resulting reduction in food intake [18]. To evaluate this effect, 20 h food deprived rats (n= 104) received intragastric administration of vehicle, TR, TC, PL, RS and IF (100,200 and 400 mg/kg), and 60 min later received intravenous injection of CRF (0.3µg/rat/ ml). Rats were given free access to food 20 min after the CRF injection and their food intake was determined 30, 60, 90 and 120 min, and 4, 6, and 24 h later. The control group received (n= 8) IG and IV administration of IF and CRF vehicle respectively to observe the effects of CRF.

Statistical analysis

The data obtained in aforesaid experiments was analyzed by two way ANOVA followed by a post hoc Bonferroni test. Values are represented as mean ± SEM for each group of animals at indicated numbers (n). The *P* values less than 0.05 were considered significant.

Results and Discussion

The crude drugs were purchased and authenticated at Agharkar research institute with following voucher specimen numbers *Piper longum* (Piperaceae)- F-145; *Trachyspermum roxburghianum* (Apiaceae)- F-147; *Terminalia chebula* (Combretaceae)- F-146.

Effect of IF and its constituent on freely feeding rats

The extract of IF and its constituents (TR, TC, PL, RS) did not modify the food intake of freely feeding rats at any of the doses tested As shown in Figure 2 the statistical analysis revealed no significant effect on the food consumption of freely feeding rats up to 24 h.

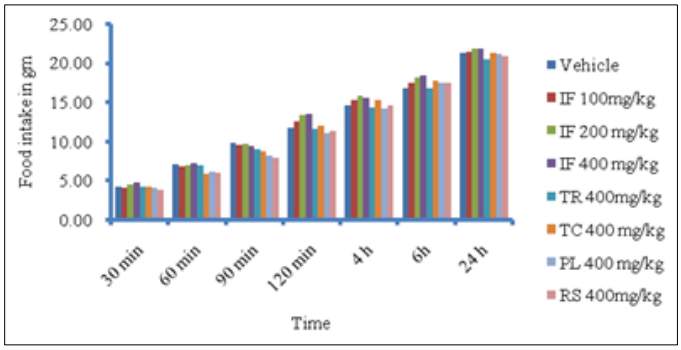

Fig 2: Comparison of effects of IF and its constituents on freely feeding rats

Figure 2 Effects of IG administration of vehicle and IF (100, 200 and 400 mg/kg) and TR, TC, PL and RS (400 mg/kg) on cumulative food intake in freely feeding rats. Data represent mean food intake (± SEM) of 10 rats. **P* < 0.05, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant.

Effects of IF and its constituents on restraint stress-induced anorexia

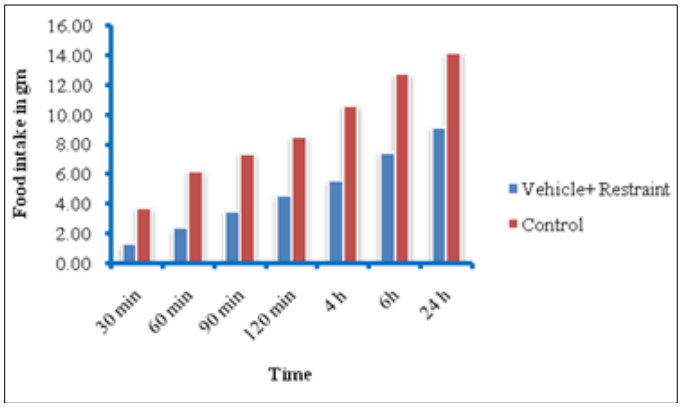

Fig 3A: Effect of restraint stress on food intake of rats

As shown in 3 (A); restraint stress significantly reduced the food intake in rats as compared with non-stressed rats. Pre-treatment with IF (400 mg/kg) significantly reversed the anorectic effect of the restraint stress. The effect started 120 min after drug administration *(p<0.05)* and lasted up to 24 h (p<0.0001). The constituents, viz.TC, PL and RS, also showed statistically significant results *p<0.05, p < 0.01, p<0.01* respectively; individually at doses 400 mg/kg; after 4 h. The effect of IF (100 and 200 mg/kg) was not statistically significant (*p>0.05*) (Figure 3 B).

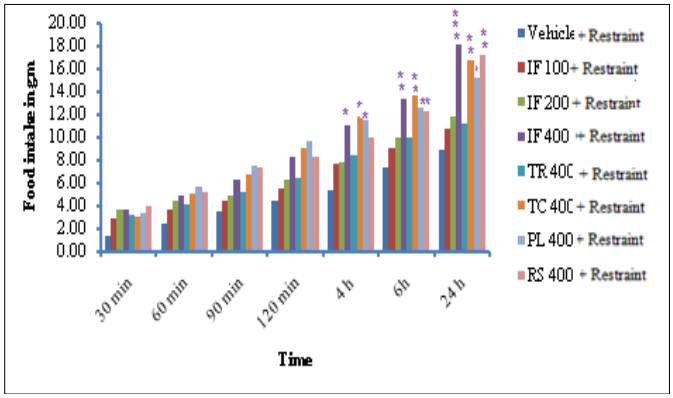

Figure 3B Effects of IG administration of IF (100, 200 and 400 mg/kg) and TR, TC, PL and RS (400 mg/kg) on restraint stress-induced anorexia. Data represent mean food intake (±SEM) of 10 rats. *p<0.05, **p<0.01, ***p<0.001, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant

Fig 3B: Comparison of IF and its constituents on restraint stress induced anorexia

Effect of IF and its ingredient on LPS induced anorexia

As shown in Figure 4 A, IP administration of LPS induced a marked reduction in the food intake of the rats. Pretreatment with IF (100,200, and 400 mg/kg) significantly reversed the LPS induced anorexia (*p< 0.01* after 4 h and lasted up to 24 h). Constituents of IF *viz.* TR, PL and RS at 400 mg/kg directly affects the food intake after 4 h (*p<0.05).* (Fig 4B)

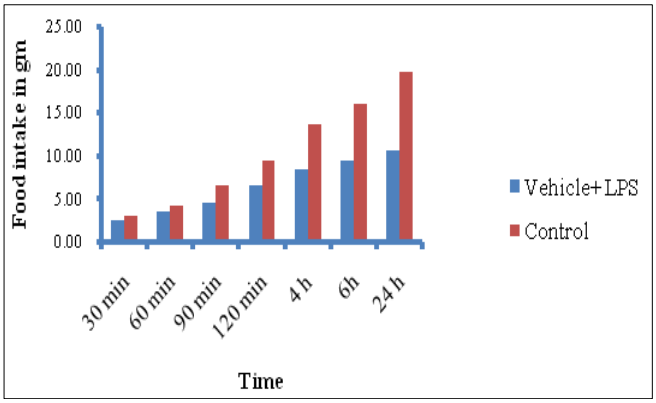

Fig 4 A: Effect of LPS on food intake of rats

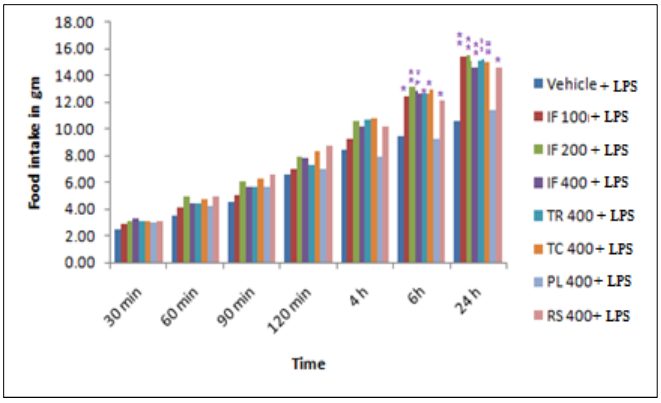

Fig 4 B: Comparison of effects of IF and ingredients on LPS induced anorexia

Figure 4B Effects of i.g. administration of IF (100, 200 and 400 mg/kg) and TR, TC, PL and RS (400 mg/kg) on LPS induced anorexia. Data represent mean food intake (±SEM) of 10 rats. *p<0.05, **p<0.01, ***p<0.001, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant

Effects of IF and its constituents on Fluoxetine-induced anorexia

As shown in Figure 5 A, IP administration of Fluoxetine produced a marked inhibition in feeding. Pre-treatment with IF and its ingredients significantly reversed the effect of Fluoxetine. Pre-treatment with IF 400 mg/kg resulted in a significant increase in the food (*p>0.05*) after 90 min. and the significance increases up to 24 h (*p> 0.001*). TC, PL and RS 400 mg/kg also showed the significant increase in the food intake (*p>0.05)* after 90 min. and the significance increased up to 24 h (*p>0.01*) (Figure 5B).

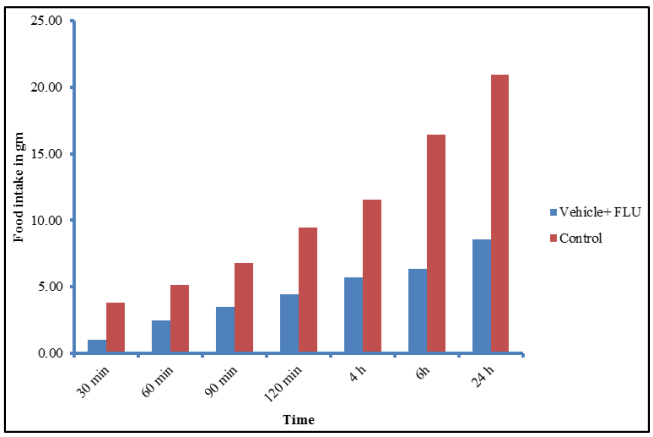

Fig 5 A: Effect of Fluoxetine on food intake of rats

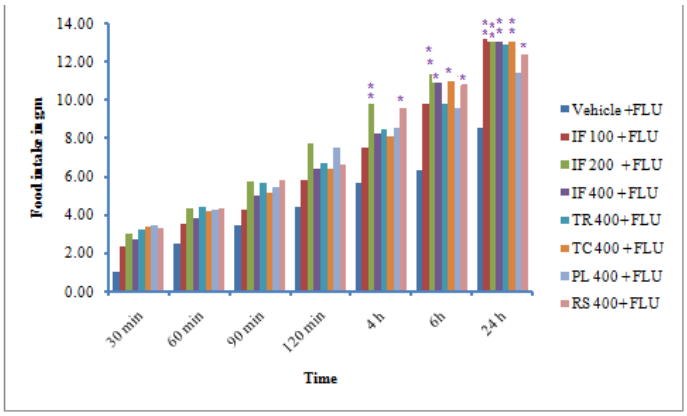

Figure 5 B Effects of IG administration of IF (100, 200 and 400mg/kg) and TR, TC, PL and RS (400mg/kg) on Fluoxetine induced anorexia. Data represent mean food intake (±SEM) of 10 rats. *p<0.05, **p<0.01, ***p<0.001, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant

Fig 5B: Comparison of effects of IF and ingredients on Fluoxetine induced anorexia

Effects of IF and its constituents on food intake of food-deprived rats

Deprivation of food did not increase the food intake significantly compared with non deprived rats. Pretreatment with IF (100, 200 and 400 mg/kg) and its constituents, TR, TC, PL and RS (400 mg/kg), had no significant effect on the feeding of the rats (*P* > 0.05) (Figure 6).

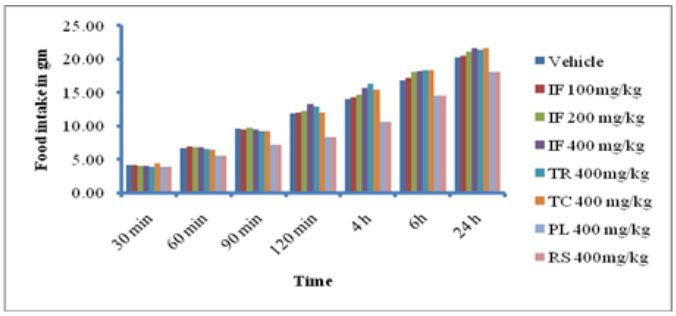

Figure 6 Effects of IG administration of IF (100, 200 and 400 mg/kg) and TR, TC, PL and RS (400 mg/kg) on food deprived rats. Data represent mean food intake (±SEM) of 10 rats. *p<0.05, **p<0.01, ***p<0.001, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant.

Fig 6: Comparison of effects of IF and ingredients on food deprived rats

Effects of IF and its constituents on CRF-induced anorexia

Intravenous injections of CRF induced a marked inhibition in the feeding of rats (Figure 7A). Statistical analysis revealed that pretreatment with IF at a dose of 400 mg/kg had a significant anti anorectic action ($P < 0.05$) from 60 min onward and the significance increased at 24 h ($P < 0.001$). TC and PL also showed the significant difference in food intake after 90 min and 12 min respectively ($p< 0.05$) and continued upto 24 h. IF 100 and 200 mg/kg also reversed the anorectic effect of CRF significantly after 90 min ($p< 0.05$). The significance of IF 200mg/kg increases after 6 h ($p< 0.01$) (Figure 7B).

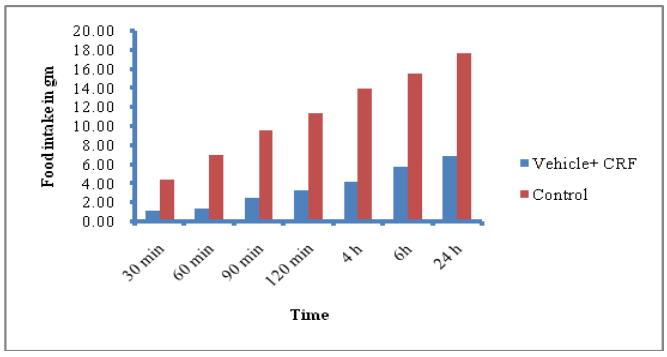

Fig 7A: Effect of CRF on food intake of rats

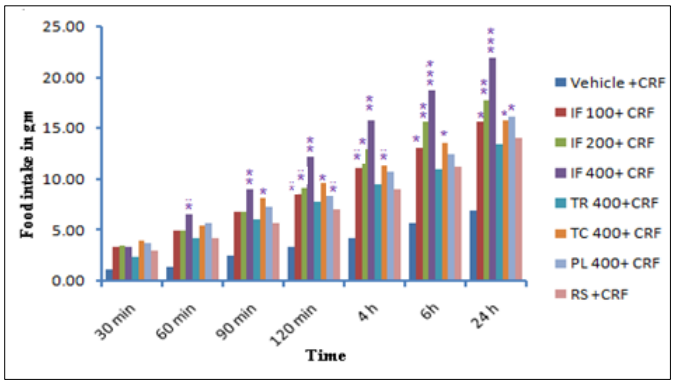

Figure 7 B Effects of IG administration of IF (100, 200 and 400 mg/kg) and TR, TC, PL and RS (400 mg/kg) on CRF induced anorexia. Data represent mean food intake (±SEM) of 10 rats. *p<0.05, **p<0.01, ***p<0.001, significant differences from the vehicle-treated rats; where not indicated, the differences are not statistically significant

Fig 7B: Comparison of effects of IF and ingredients on CRF induced anorexia

Discussion

The present study shows that Intuppukana churna effectively reduces the marked anorexia induced in rats by exposure to different stressful conditions. IF 200 and 400 mg/kg significantly reversed restraint stress, LPS, Fluoxetine and CRF induced anorexia. While the drug in the used dose range does not affect food intake of freely feeding and non-stressed food deprived rats. This suggests that the inhibitory effect of Intuppukana churna on different stress induced anorexia is not related to direct orexigenic action. IF shows the anti-anorectic effect in restraint stress, LPS, Fluoxetine and CRF induced anorexia models which indicates that the action of IF is nonspecific.

Acute stress like restraining rats induces activation of brain monoamine system as well as CRF system [18]. Therefore the anti-anorectic effect of IF could be attributed to its ability to modulate the activation of several components of stress response system such as the sympatho- adrenal system, which mainly controls the rapid response to an acute stressor.

LPS mediates anorexia through stimulating synthesis of different pro- inflammatory cytokines and cytokines act on brain mechanisms involved in the control of feeding behavior directly (via neuronal mechanism) or indirectly (via modulation of brain chemistry). Cytokine induced feeding inhibition involves multiple mechanisms that involve interactions among multiple chemical and neuronal systems [19]. IF acts as anti-inflammatory agent by acting on the pro inflammatory cytokines so it can be concluded IF may affect any one or both the chemical or neuronal mechanism of feeding via inhibiting the release of pro inflammatory cytokines.

Intravenous CRF directly affects the gastric emptying and inhibits the feeding. IF reversed this condition and this effect can be attributed to direct action on gastric emptying. Similarly the action of IF on Fluoxetine induced feeding inhibition may be due to initiation of 5- HT reuptake.

The study shows that the various neuronal and chemical reactions are involved in the anti-anorectic activity. The extract used is a combination of herbal drugs which contains variety of chemical constituents; it is very difficult to attribute the activity to any one drug. Piperine, the major content of *P. longum* may enhance the bioavailability of all other drugs in the formulations or it may act as an anti-anorectic. Phenolic constituents from *T. chebula* posses strong anti-oxidant activity and are responsible to scavenge free radicals released during stress conditions. All crude drug content together shows synergistic/ additive effect and support the Ayurveda concept of combined drug therapy instead of a single drug/ drug constituent treatment.

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