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EVALUATION OF ANTI-OBESITY EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF *CRINUM ASIATICUM* LEAVES

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ABSTRACT
The objective of the present investigation was to evaluate the anti-obesity effect of *Crinum asiaticum* leaf extract (hydro-alcoholic). The hydro-alcoholic extraction was carried out using ethanol-water (80:20) as the extracting solvent. The extract obtained was dark brown in color, sticky solid. The yield of the extract was found to be 2.19 g (2.92% w/w) of the dry crude leaf powder. The results of phytochemical screening of the extract suggests the presence of alkaloids, saponins, tannins, phenolics, flavonoids, terpenes and protein in the leaf extract. The total phenolic content of hydro-alcoholic extract of *C. asiaticum* was calculated by Folin-Ciocalteau reagent method and was found to be 83.14 GAE mg/g. The anti-obesity effect of the extract was assessed by observing the effect of the extract on the food intake habit of experimental mice. It was observed that fluoxetine and the hydro-alcoholic extract were hypophagic and restrict the intake of food by the mice. On the other hand (±) - 8- OH-DPAT is a known agonist of 5-hydroxytryptamine and exhibits hyperphagic action. On co-administration of (±) - 8- OH-DPAT with the extract, it was found that the hyperphagic effect of (±) - 8- OH-DPAT was antagonized by the extract.

KEYWORDS: Hypophagic, hyperphagic, antiobesity, *Crinum asiaticum*, phenolics.

INTRODUCTION
Obesity is a term applied to excess body weight with an abnormally high proportion of body fat.[1] 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.[2] Globally 650 million adult population, 42 million children under the age of 5years are overweight or obese; interestingly the prevalence of obesity is higher in women (about 297 million) when compared with men (205 million).[3]

Crinum asiaticum is a perennial bulbous herb bearing feathery green leaves from the Amaryllidaceae family of the Plantae kingdom. In ethnomedicine, it is employed to relieve anguish from a plethora of ailment conditions such as boils, contusions, earache, edema, fever, fractures, gastrointestinal complaints, hernia, mumps, rheumatism, tonsillitis, urinary difficulties and vomiting, amongst others.[4] The essential oil extracted from the leaf of C. asiaticum was identified to possess a number of compounds representing 93.14% of the sample, namely 3-methylbutanal, 3-methyl-2-buten-1-ol, hexanal, furfural, benzaldehyde, phenol, citronellol, (-)-cis-carveol, methyl eugenol, a-gurjunene, b-eudesmol, 1-hexadecanol, methyl octadecenoate, oleic acid and ethyl linoleate. However, the major components were reported to be 1-hexadecanol (27.69%), oleic acid (23.32%) and methyl octadecenoate (12.45%).[5] The plant has been recently explored for its anticancer[6-8], anti-Alzheimer[9], anti-inflammatory[10], anti-oxidant and antibacterial[11] actions.

From the literature, it was clear that the *in vivo* efficacy of *C. asiaticum* leaf extracts has not been reported till against obesity despite the presence of various flavonoids that have been previously reported as anti-obesity agents in various reports. Hence it was decided upon to evaluate the anti-obesity effect of *Crinum asiaticum* leaf extract (hydro-alcoholic).

MATERIAL AND METHODS
Preparation and extraction of the plant material
The leaves of the plant were collected from local nursery of Gwalior, washed with distilled water, dried under shade and powdered using a blender. 78 g of powder was evenly packed in the extractor of the soxhlet apparatus and subjected to defatting with petroleum ether followed by extraction with ethanol-water (80-20) using hot continuous extraction for 9 h. The extract were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation

to reduce the volume to one-tenth. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators for further processing.[12]

Preliminary phytochemical screening

The hyroalcoholic extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation during the testing was used as analytical responses to these tests.[13]

Total Phenolic Content

For total phenolic content determination, 200 µL of test sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at 3 min, 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.[14]

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. The experiments were carried out between 10.00 and 17.00 h at room temperature (22 ± 2 °C). All the experimental protocols have been approved by the Institutional Animals Ethics Committee and conducted according to the Indian National Science Academy Guidelines on the Use and Care of Experimental Animals.

Drugs Used and Grouping of Animals

The extract was suspended in distilled water and administered at a dose of 0.1 g/kg, p.o. and 0.2g/kg, p.o. Fluoxetine HCl and (±)-8-Hydroxy-2-(dipropylamino) tetralin 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 (Extract treatment): Mice administered with 0.1g/kg, p.o extract suspension; Group-3 (Extract treatment): Mice administered with 0.2g/kg, p.o exctract suspension; Group-4 (Fluoxetine treatment): This group served as standard and administered with fluoxetine solution (10 mg/kg, intraperitoneally); Group-5 (± 8-OH-DPAT pretreatment): Mice administered with ± 8-OH-DPAT (0.1 mg/kg, intraperitoneally); Group-6 (± 8-OH-DPAT pretreatment): Mice treated with ± 8-OH-DPAT 30 minutes prior to administration of extract 0.2g/Kg.

Study of effect of extract on food intake

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. Extract 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.[15] Similarly the effect of flouxetine was studied on food intake.

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

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.

To study the effect to extract, the extract was administered to these animals after 30 min of pretreatment 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.

RESULTS AND DISCUSSION

Extraction and phytochemical screening

The plant sample was collected from the local surroundings of Bhopal and authenticated. The leaves of the plant are long lance shaped having smooth margin (Figure 1).

Figure 1: *Crinum asiaticum* plant.

The hydro-alcoholic extraction was carried out using ethanol-water (80:20) as the extracting solvent. The extract obtained was dark brown in color, sticky solid. The yield of the extract was found to be 2.19 g (2.92% w/w) of the dry crude leaf powder. The results of phytochemical screening of the extract suggested the presence of alkaloids, saponins, tannins, phenolics, flavonoids, terpenes and protein in the leaf extract.

Total Phenolic Content

The extract was evaluated for quantifying the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. The total phenolic content in extract is expressed as gallic acid equivalents. The total phenolic content of hydro-alcoholic extract of *C. asiaticum* was 83.14 GAE mg/g.

Acute Toxicity Study

The acute toxicity test was performed by using the dried extract at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of antiobesity studies.

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 extract was assessed by observing the effect of the extract 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.

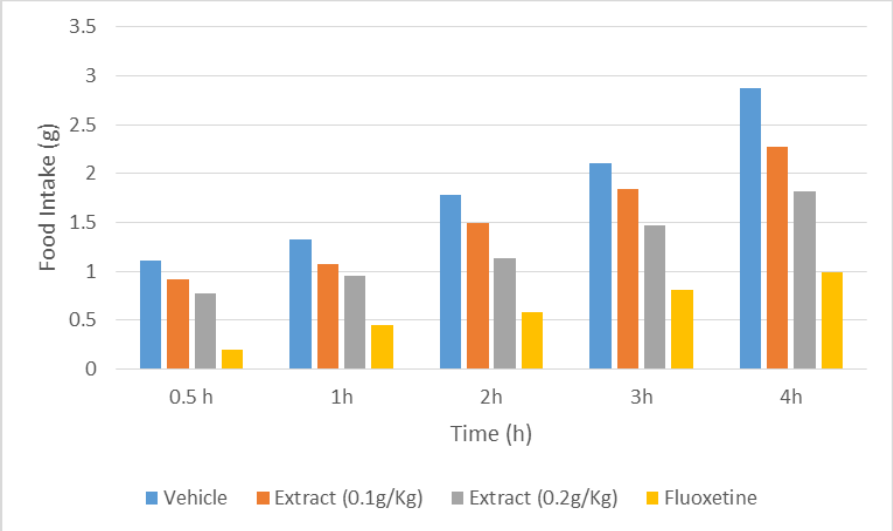

Figure 2: Effect of extract and fluoxetine on food intake in mice.

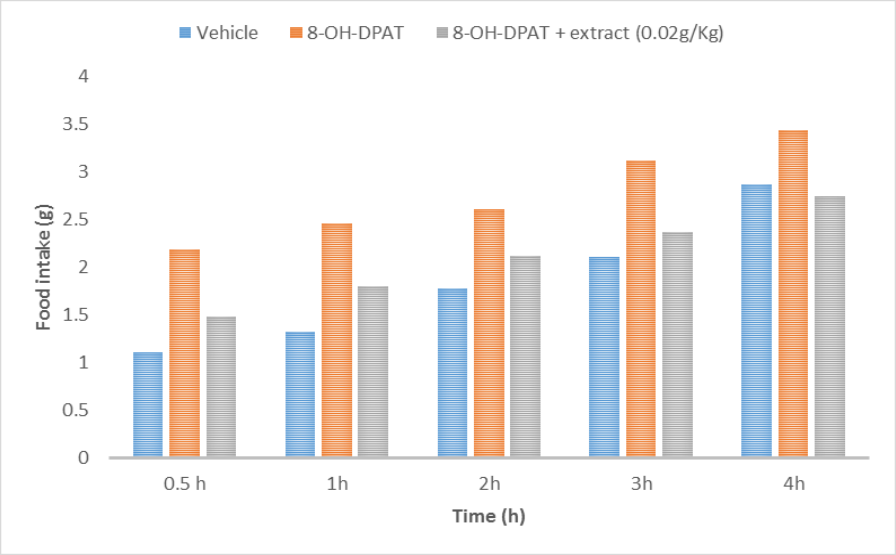

Figure 3: Effect of co-administration of (±) - 8- OH-DPAT + extract on food intake in mice.

As clearly visible from the figures above, fluoxetine and extract are hypophagic and restrict the intake of food by the mice (Figure 2). On the other hand (±) - 8- OH-DPAT is a known agonist of 5-hydroxytryptamine and exhibits hyperphagic action (Figure 3). On co-administration of (±) - 8- OH-DPAT with the extract, it was found that the hyperphagic effect of (±) - 8- OH-DPAT was antagonized by the extract (Figure 4).

The reduction in food intake was calculated with reference to the food intake exhibited by hyperphagic animals (Table 1).

Table 1: Percent reduction in food intake by treated animals.

Treatment	Percent reduction in food intake as compared to 8-OH-DPAT				
	0.5 h	1h	2h	3h	4h
8-OH-DPAT + extract	31.96	26.72	18.39	24.28	20.05

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 the extract of *Crinum asiaticum* was immediate in exhibiting anorexic effect while its anorexic potential started to lower by the 4th hour.

The result of the study showed that 8-OH-DPAT, a 5-HT1A 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-HT1A autoreceptors in the raphe nuclei.[16] The antagonism of 8-OH-DPAT-induced hyperphagia by fluoxetine is also consistent with the earlier findings. Thus, it is likely that the hydroalcoholic extract of *Crinum asiaticum* may also mediate its effect on food intake through 5- HT1A receptors as well since it significantly antagonized 8-OH-DPAT-induced hyperphagia.

CONCLUSION

About 2.8 million deaths occurring throughout the world due to complications arising from obesity and overweight. In this study, we investigated the anti-obesity action of ornamental plant *Crinum asiaticum* by assessing the effect of the hydro-alcoholic extract of the leaves of this plant on food intake habit in rat. From the results it was likely that the hydroalcoholic extract of *Crinum asiaticum* may also mediate its effect on food intake through 5- HT1A receptors as well since it significantly antagonized 8-OH-DPAT-induced hyperphagia.

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