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ORAL ADMINISTRATION OF *PHYTOLACCA BACCIS* (POKE ROOT) EXTRACT INCREASES BRAIN SEROTONIN METABOLISM AND DECREASES FOOD INTAKE IN RATS

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Abstract

Central 5-hydroxytryptamine (5-HT; serotonin) system plays a key role in the regulation of food intake and body weight. Evidence also shows that causes of obesity are also linked to the brain serotonin levels. Most of the medications for the treatment of obesity work by increasing the availability of anorexiogenic neurotransmitter in the central nervous system (CNS). In the present work *Phytolacca baccis*, a weight reducing homeopathic medication was used to study its effects on food intake and brain serotonin metabolism in rats. After 4 weeks of daily administration of drug, the rats exhibited a decrease in food intake and body weight. Brain serotonin metabolism on the other hand increased. The drug also exhibited anxiogenic effects in rats when tested in an elevated plus maze (EPM). The results of the present study suggest that *Phytolacca baccis* induced increases of brain 5-HT levels may be involved in the weight reducing effects of the drug.

Introduction

Body weight is regulated by complex interrelationship between central and peripheral factors. Recent findings suggest that these central and peripheral components of weight control are mediated by several key neurotransmitters. Increasing evidence shows that CNS is involved in the regulation of body weight (Huang *et al*., 2001) and causes of obesity are linked to the brain serotonin concentrations (Blundell, 1992; Bickerdike, 2003). It is generally accepted that the central 5-hydroxytryptamine (5-HT; serotonin) system plays a major role in the regulation of food intake and body weight (Blundell, 1984, 1991). Drugs increasing extracellular 5-HT by releasing or inhibiting the uptake of 5-HT have been shown to reduce food intake (Rowland & Carlton, 1986; Haleem, 1994). Also direct 5-HT infusion into the brain reduces food intake in rats (Shor-Posner *et al*., 1986). On the other hand drugs inhibiting the firing rate of 5-HT neurons have been shown to increase food intake under certain conditions (Breisch *et al*., 1976; Stallone & Nicolaides, 1989). A number of medications are available for decreasing body weight and the treatment of obesity. These medications include drugs, which reduce appetite and consequently food intake by increasing the availability of serotonin in the CNS (Borsini *et al*., 1982). Various herbal and homeopathic preparations are also available for the treatment of obesity. *Phytolacca baccis* (Poke root) (Family; Phytolaccaceae) is a medicinal plant used for the treatment of various diseases including obesity. Alcoholic extract of the plant berries is marketed as an anti-fat remedy. It is said to be an excellent remedy for obesity and has a powerful effect on fibrous tissues.

Since most of the anti-obesity drugs act by altering the brain 5-HT concentrations, the present work was undertaken to study the effects of *Phytolacca baccis* ∅ (Homeopathic; weight reducing drug) on brain 5-HT metabolism and food intake in rats.

The aim was to investigate the possible neurochemical mechanism behind the weight reducing action of the drug.

Materials and Methods

Locally bred male Wistar rats weighing 200-250g (purchased from Agha Khan University Hospital, Pakistan) were housed individually under a 12:12h light:dark cycle (lights on at 06:00h) with access to cubes of standard rodent diet and tap water for atleast 3 days before experimentation. *Phytolacca baccis* ∅ (70% alcohol) (Wilmar Schwabae; Germany) was purchased locally. The drug was fed orally at a dose of 1ml/kg body weight daily for 4 weeks. Animals were divided into control (6 rats) and test (6 rats) groups. The drug was administered orally to rats with the help of a feeding tube. An equal amount of vehicle (70% alcohol) was given orally to the control rats for the same period of time. After 4 weeks of treatment the rats were decapitated between 10:00 and 11:00h to collect plasma and brain samples. Whole brains were stored at –70^{o}C until analysis of indoleamines by HPLC-EC as described earlier (Haleem *et al*., 1990; Haleem & Perveen, 1994). Plasma and brain tryptophan levels were also determined by HPLC-EC. . A 5 II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), Octyl Sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9 at an operating pressure of 2000-3000 psi on Schimadzu HPLC LEC 6A detector at an operating potential of 0.8 volts for biogenic amines and 1.0 for TRP.

Food intake was monitored weekly by giving rats weighed amount of food and weighing the remaining food in the hopper of the cages.

Body weights of the rats were also monitored weekly during the 4 weeks of the treatment. Growth rate was calculated in terms of percentage of initial body weight.

Plus maze apparatus used in the present study consisted of four equal sized arms in which two were open and two were closed. Each arm was of 50 cm length and 10 cm width. Arms were joined by central area of 5 cm^2. The length of the wall of closed arm was 40 cm. The maze was elevated from ground at a height of 60 cm. To determine activity a rat was placed in the center of the plus maze and the time spent in the open arm was monitored for 5 min. The test is based on the principal that an animal placed in an elevated plus shaped maze passes very little time in open arm. This is because of the fear of elevation of the maze and thinness of the open arm. For the type of apparatus used in the present study, 5 min are thought to be sufficient for the animal to explore the maze. Plus maze activity of control and test (drug treated) rats were monitored in a balanced design to avoid order effect.

Statistical Analysis were performed by Student's *t*-test. P values < 0.05 were taken as significant.

Results

The effect of drug on cumulative food intake and growth rate during the treatment showed significant decrease in food intake (16%; $p<0.05$) and growth rate (3%; $p<0.05$) of rats after 4 weeks of drug administration (Fig. 1). The drug significantly increased brain tryptophan ($p<0.05$), 5-HT ($p<0.01$) and 5-HIAA ($p<0.01$) levels. Plasma tryptophan levels were comparable in control and drug treated rats (Fig. 2). Analysis by

student's *t*-test showed a significant ($p<0.05$) decrease in open arm ambulatory activity in drug treated rats (Fig. 3).

Discussion

A variety of herbal preparations for weight loss are available. These herbal preparations and drugs act either by a central mechanism that reduces food intake or by a peripheral mechanism that increases thermogenesis in adipose tissue. In the present study a frequently prescribed anti-obesity drug *Phytolacca baccis* was investigated for its effect on brain serotonin metabolism and food intake in rats. The experimental results indicate a decrease in food intake and body weight and an increase in brain 5-HT metabolism. The present results therefore provide evidence that appetite reducing effects of *Phytolacca baccis* are produced by an increase in brain serotonin metabolism. The observed increases of brain 5-HT were associated with an increase in the levels of its precursor tryptophan. Tryptophan hydroxylase, the rate-limiting enzyme of the biosynthesis exists unsaturated with its substrate (Aschroft *et al*., 1965; Moir & Eccleston, 1968). Therefore, the rate of 5-HT synthesis is primarily dependent upon the brain concentration of its precursor *In vivo* (Fernstrom & Wurtman, 1971; Leathwood & Fernstrom, 1990, Hutson *et al*., 1985).

The levels of free tryptophan in plasma have been shown to have a role in determining the brain levels of tryptophan in different physiological and pharmacological conditions (Knott & Curzon, 1972; Curzon & Fernando, 1976). An increase in brain tryptophan concentration as observed in the present study may not be explained in terms of increased concentration of plasma total tryptophan. It is however possible that factor such as an increase in free tryptophan concentration in plasma (Knott *et al*., 1977) or a decrease in circulating levels of large neutral amino acids (Leathwood, 1987) is involved in the greater availability of tryptophan from the circulation to the brain.

Increased levels of brain 5-hydroxy-indoleacetic acid (5-HIAA) are often taken as a measure of increased 5-HT release. Drug treated rats in the present study exhibited an increased level of brain 5-HIAA which indicates an increased release of brain serotonin in the synaptic cleft. Greater availability of serotonin towards hypophagic 5-HT receptors may well be involved in the observed decreases of food intake. Previous research specifically implicates 5-HT2C receptors as playing a key role in the regulation of appetite (Grignaschi & Samanin, 1992, Halford *et al*., 2005; Kennett *et al*., 1995, 1997). Serotonin is also known to play a role in anxiety (Kahn *et al*., 1988) and involvement of 5-HT2C receptors in anxiety is also reported (Wallis & Lal, 1998). The results of elevated plus maze test in the present study revealed that the drug has an anxiogenic effect.

The drug in general increases brain serotonin metabolism and decreases food intake in rats and hence possesses both anorexiogenic and anxiogenic effects. The results tend to suggest that these effects of drug are possibly mediated *via* an increased serotonin availability towards respective receptors. The increased availability of tryptophan to brain increases brain 5-HT levels which in turn send a neurochemical signal for the inhibition of appetite and hence food intake. This decreased food intake ultimately leads to a gradual decrease in body weight.

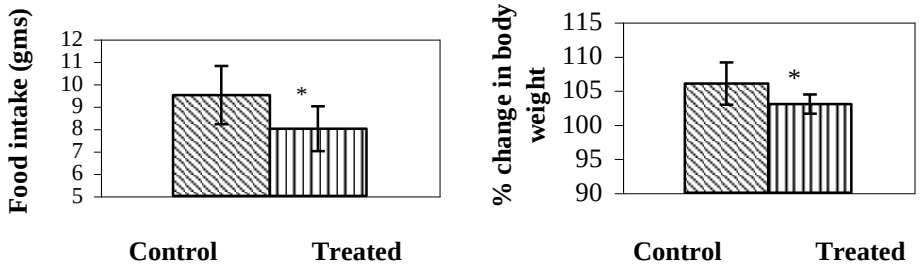

Fig.1. Effect of *Phytolacca baccis* on cumulative (24h daily) food intake and growth rate in rats. Values are means + SD (n = 6). Significant differences by Student's *t-test*, * p<0.05 from respective controls.

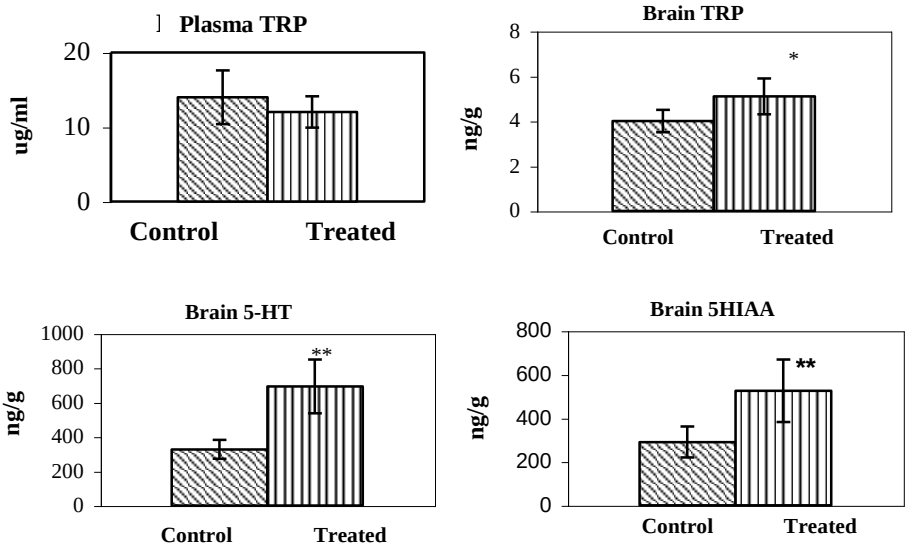

Fig. 2. Effect of *Phytolacca baccis* on plasma tryptophan, brain tryptophan, 5-HT and 5-HIAA concentrations in rats. Values are means + SD (n = 6). Significant differences by student's *t-test*, *p<0.05, **p < 0.01 from respective controls.

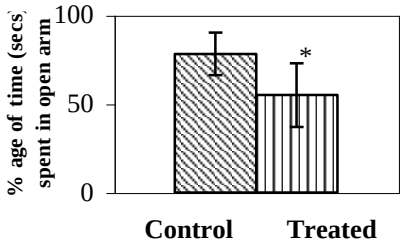

Fig. 3. Effect of *Phytolacca baccis* on plus maze activity of rats. Values are means + SD (n = 6). Significant differences by Student's *t-test*, *p < 0.05 from respective controls.

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