Anethum graveolens seeds aqueous extract stimulates whole brain 5-hydroxytryptamine metabolism and reduces feeding behavior and body weight in obese rats

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Abstract: The percentage of overweight and obese person has increased markedly since several decays. Obesity is associated with increased risked factor for many diseases such as, diabetes, heart complications, arthritis and certain types of cancer. Feeding behavior is in controlled by a major interaction between central nervous system and many organs of the body. The role of serotonin (5-HT) in feeding behavior is well recognized. The aim of present study was to evaluate the effect of *Anethum graveolens* seeds aqueous extract (AGAE) on food intake, body weight and serotonin metabolism in over weight rats. Five weeks oral administration of AGAE shows significant decrease in body weight, food intake and significant increase in whole brain 5-HT, 5-HIAA and tryptophan level in brain and plasma of experimental animals. Increased level of 5-HT induced satiety and suppressed food intake and result is the reduction in body weight.

Keywords: *Anethum graveolens* seeds aqueous extract (AGAE), 5-hydroxytryptamine (5-HT), 5-hydroxy Indol Acetic Acid (5-HIAA), High Performance Liquid Chromatography(HPLC), Tryptophen (TRP).

INTRODUCTION

Hypothalamus in the brain controls appetite has been proven by many evidences whereas obesity mediates due to lesion of the hypothalamus. Brain lesioning and stimulation studies suggested ventromedial hypothalamus as the satiety center and the lateral hypothalamus area as hunger center (Stellar, 1996; Bray *et al.*, 1990). Several studies proved that the cause of obesity is linked to the serotonin level in brain (Blundell, 1992; Fruhbeck *et al*., 1998).

Serotonin plays an important role in the central nervous system and participates in a range of cognitive, physiological and behavioral function such as memory, mood, emotion, wakefulness sleep, appetite and temperature regulation (Rapport *et al*., 1948).

Extensive researches conducted on animal linked to brain serotonergic system for the regulation of appetite and eating behavior (Fernstrom *et al*., 1971; Fernstrom *et al*., 1971; Gurzon *et al*., 1972; Blundell *et al*., 1986).

Excessive level of serotonin is also implicated in appetite inhibition (Blundell *et al*., 1984; Leibowitz, 1984). In animals including humans, Serotonin (5HT, 5-hydroxytryptamine) synthesized from essential amino acid L-tryptophan by a short metabolic pathway in serotonergic neuron of central nervous system and in the enterochromaffin cells of gastrointestinal tract (Fuller *et al.*, 1990; Berger *et al.*, 2009). Conventionally treatments and procedures that increase activity of 5-HT in synaptic cleft or directly activate 5-HT receptor have been reported to reduce food consumption. For example, Fenfluramine (Guy *et al., 1989;* Weintraub *et al.*, 1992; Scalfi *et al.*, 1993) stimulates serotonin release and inhibits its reuptake; Sibutramine (Heal *et al.*1998; Feldman, 1998; Simansky & Vaidya, 1990; Heisler *et al.*, 1997; Heisler *et al.*, 1999) and Fluxetine (Haddock *et al.*, 2002; Li *et al.*, 2005) are serotonin reuptake inhibitor and monoamine oxidase inhibitor; m-Chlorophenylpiperazine (Vickers *et al.*, 2003;Schreiber & De, 2002) decreases food intake by increasing, brain serotonin level and reduces body weight. Selective depletion directly or indirectly decreases 5-HT level in brain, which develops hyperphagia and obesity (Daniel & Lora 2007).

The uses of herbs and herbal drugs have increased in the daily life for last three decades because herbal products are safe, effective and freely available to everyone at economical as compared to synthetic drugs (Abad *et al.*, 2010).

Herbs are the basic prerequisite for all traditional medicine systems such as Unani, Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, African and Native American (Hassan *et al.*, 2010).

The present work has been designed to study the effect of *Anethum graveolens* seeds aqueous extract on behavioral

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(food intake and body weight), biochemical (plasma tryptophan) and neurochemical (5-HT, 5-HIAA and tryptophan) in overweight rats.

MATERIAL AND METHOD

Animals

24 Albino Wistar male rats weight between 280-320 grams were used. All animals were placed in separate cages under 12 h light-dark cycle and control room temperature (23±2^{o}C) with free access to specially prepared diet and water for one week, prior to starting the experiment so that rats could adopt themselves to new conditions.

Preparation of extract

Anethum graveolens seeds purchased from local market. Identification was provided by experienced Botanist from the scientific institute of Agriculture Department, University of Karachi. The 50gm seeds were crushed in blender. The powder seed was soaked in 200 ml of water and left for 24 hour at 37^{o}C. The mixture was filtered and the filtrate was properly stored until ready to use.

Experimental protocol

The animals were divided into two experimental groups. The test group received 2ml *Anethum graveolens* aqueous extract contain eight grams seeds. The control group received water, amount equivalent to that of aqueous extract of herb. Weighed amount of food was placed in the hopper of all cages. Body weight and food intake of each group was monitored weekly.

Sample collection

Rats were decapitated when 15-19% body weight reduction was observed in AGAE treated rats. Plasma and brain samples were collected and stored at -70°C for the estimation of Plasma Tryptophan, Brain Tryptophan, 5HT and 5HIAA.

Estimation of neurochemical

Estimation of monoamines (neurotransmitters) and their metabolites in the whole brain samples of rats was made by HPLC-EC method as reported by Haleem *et al.*, (2004).

All biogenic amines can be detected in a single sample by reversed phase HPLC with electrochemical detector. This technique separates biogenic amines and their metabolite on the basis of their hydrophobicity. The stationary phase used for the analysis of biogenic amines was 5µm Octadecyle saline (ODS) column. The mobile phase was passed through this column under high pressure by using high pressure. A measured amount of sample (15ul or more) was applied (Via injection) through injector on top of the column. Biogenic amines were determined by using electrochemical detector (Schimadzu LEC 6A detector) at working potential of $\pm$0.8 to 1.0 V.

After detection, message from the detector may be recorded by an integrator. Each sample was identified and comparing with the retention time of standard (Haleem *et al*., 2004; Haleem and Khan, 2003).

Method of sample extraction

Frozen brain samples were homogenized in 5-10 volume of extraction medium by glass homogenizer with Teflon pestle at 1000 revolution /minute. This homogenated were allowed to stand for 10-15 minutes to help precipitation. Supernatant transfer in Eppendorf tubes was centrifuged at 12,000 rpm for 20-25 minutes at 4^{o}C. Supernatants were used for the estimation of indolamines.

Extraction of tryptophan from plasma

To 0.01ml of plasma, 0.2ml of 0.4% perchlorate having 0.1% sodium *meta*-bisulphate, 0.01% EDTA and 0.1% cysteine was added and thoroughly mixed .The solution was then centrifuged at 12,000 rpm for 10-15 minutes at 4^{o}C in Eppendorf tubes. Supernatants were then used for the determination of TRP by HPLC.

STATISTICAL ANALYSIS

The significance differences between the mean of the treated and untreated groups were analyzed by student's *t*-test. Values of $p<.05$ were considered as significant. Data expressed in fig. as mean ±standard deviation (SD).

RESULTS

Five weeks oral administration of AGAE significantly ($P<0.01$) decrease weekly food intake and body weight (fig. 1a, 1b) and increase 5-HT, 5-HIAA (figs. 2a, 2b) level in brain and plasma tryptophan level (fig. 3a, 3b) as compare to their respective control.

DISCUSSION

Six weeks oral administration of AGAE indicates its potential usefulness as an anti-weight gaining herb. AGAE act as a potent agonist of 5-HT metabolism and increases 5-HT turn over in whole brain of rats (fig. 2a, 2b) as well as increases tryptophan concentrations in blood and brain (fig. 3a, 3b).

Reduction in food intake (fig. 1a) observed after the one-week oral administration of AGAE in over weight rats. Continuous treatment with this herbal extract gradually decreases body weight (fig. 1b). Due to reduction in food intake ultimate result is the weight reduction in over weight rats in the treated group.

5-HT neurotransmitter plays a vital role in feeding behavior. Many researches provide valuable information about the concentration of 5-HT in brain. Decrease 5-HT levels in brain develop hyperphagia and obesity (Breisch *et al.*, 1976; Saller *et al.*, 1976), while increase level of

this neurotransmitter produce hypophagic effect and reduce body weight (Vickers *et al.,* 2003; Heal *et al.,* 1998; Guy *et al.,* 1989; Saida *et al.,* 2006).

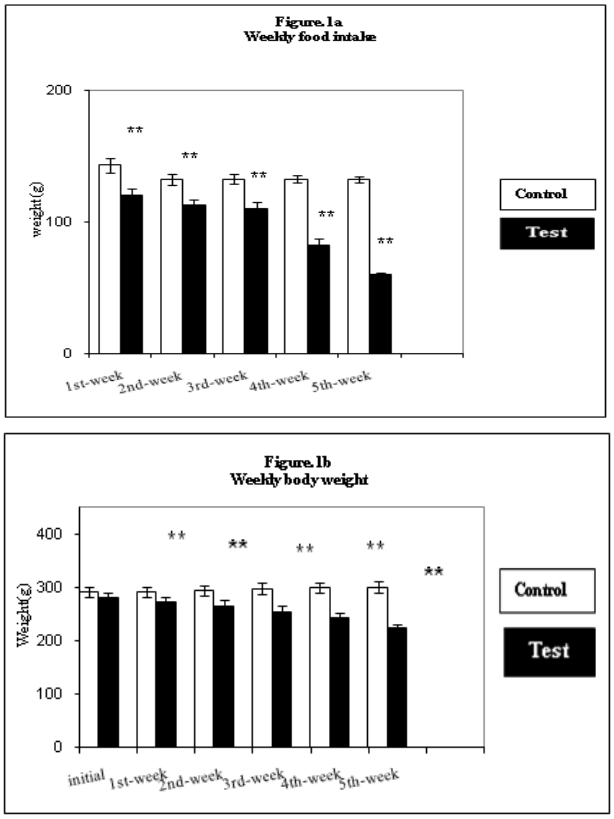

Fig. 1a-1b: Effect of repeated administration of AGAE on food Intake and body weight of rats. Values are mean ± SD (n=12) significant difference by Student *t*-test **p<0.01 from respective controls.

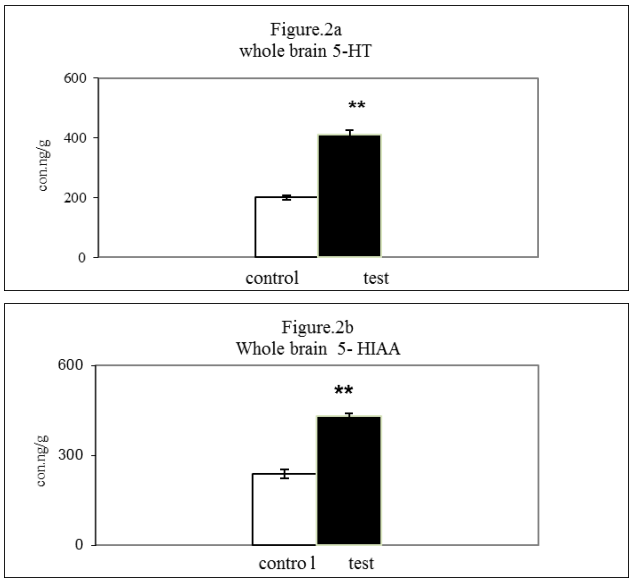

Fig. 2a-2b: Effect of repeated administration of AGAE on 5-HT and 5-HIAA level of rat's brain. Values are mean ± SD (n=12) significant difference by Student *t*-test **p<0.01 from respective controls

Present research show increased level of serotonin metabolisms and its precursor tryptophan. Tryptophan is essential amino acid and precursor of 5-HT. Its high concentration in plasma-increased availability of TRP to brain that leads to increased synthesis of 5-HT in brain (Saida *et al.,* 2006; Ferstrom, 1983).

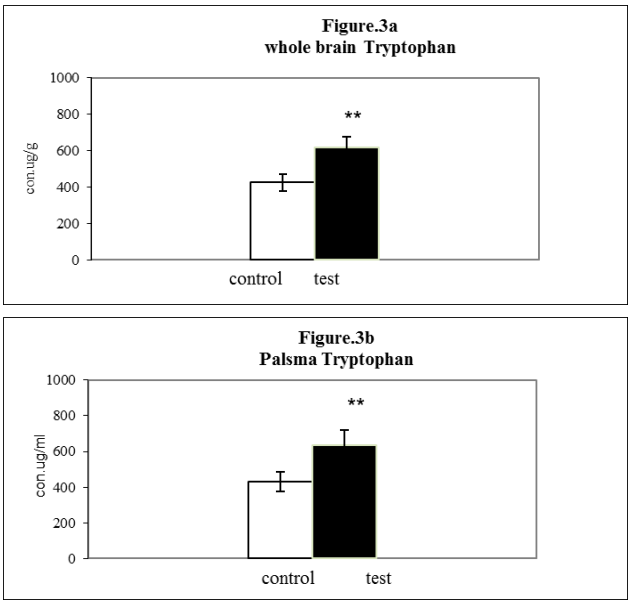

Fig. 3a-3b: Effect of repeated administration of AGAE on brain and plasma Tryptophan of rats. Values are mean ± SD (n=12) significant difference by Student *t*-test **p<0.01 from respective controls.

Decreased in body weight could possibly be related to increase in TRP. This study showed increased 5-HT levels decreased body weight could be compensatory response to higher tryptophan level in brain. This is in agreement with previous studies (Ferstrom, 1983) that increased availability of tryptophan to brain stimulates 5-HT synthesis and metabolism in whole brain.

This study shows the direct correlation between tryptophan concentration and 5-HT metabolism.

CONCLUSION

AGAE enhances 5-HT levels and decreases feeding behavior and in turn it produces hypophagic effects. The present findings tend to demonstrate the hypophagic effect of AGAE, which are possibly mediated via increase in serotonin (5-HT) availability towards respective receptor. Increase brain 5-HT levels, which in turn generate a neurochemical signal for the inhibition of appetite and decreases body weight.

An increase in 5-HT concentration reported here represents both intracellular as well extra cellular. Although, the extra cellular 5-HT concentration has not determined in the present study. It is expected that an

increase in 5-HT levels is indication of increase in extra cellular 5-HT concentration and hence an increase in 5-HT at receptor sites, which producing hypophgia.

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