

# Neurochemical and behavioral effects of *Cinnamomi cassiae* (Lauraceae) bark aqueous extract in obese rats

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**Abstract:** Obesity is a risk factor leading to a number of chronic and metabolic disorders. Obesity is the fifth leading cause of global deaths. At least 2.8 million adults are dying each year as being overweight or obese. *Cinnamomi cassiae* is widely used traditional medicinal plant, used indigenously, to decrease glucose and cholesterol. 5-Hydroxy tryptamine (5-HT; Serotonin) is an important neurotransmitter reported to be involved in the pathophysiology of anorexia. Present study was designed to investigate the neurochemical and behavioral effects of cinnamon bark aqueous extract (CBAE) in obese rats and to find the possible involvement of 5-HT in reducing the body weight in these experimental animals. CBAE was repeatedly administered orally in the test animals for 5 weeks. A decrease in the food intake along with a concomitant increase in brain 5-HT level was observed in rats administered with CBAE. Findings may help in extending therapeutics in the pathophysiology of obesity and related eating disorders. Decrease activities in behavioral models were also monitored in CBAE treated animals.

**Keywords:** cinnamon bark aqueous extract (CBAE), Serotonin (5-HT), 5-hydroxy Indol acetic Acid (5-HIAA), Tryptophan (TRP)

## INTRODUCTION

Cinnamic aldehyde, cinnamic acid and methylhydroxy-chalcone polymer (MHCP) are the main components obtained from *Cinnamomi cassiae* (Lauraceae). The animal and human based studies had suggested potential benefit of cinnamon for the treatment of hyperlipidemia (Qin *et al.*, 2003), stomach ulcer (Akira, 1986), bacterial infections (Prabuseenivasan *et al.*, 2006) and diabetes (Sung Hee *et al.*, 2006).

Appetite is controlled by a major interaction between central nervous system and many organs of body. Evidences suggested that hypothalamus controls appetite and obesity. Brain lesioning and stimulation studies have identified ventromedial hypothalamus as the satiety center and lateral hypothalamus area as the hunger center (Stellar, 1954; Bray *et al.*, 1990). 5-HT in this context is hypothesized to play an important role (Blundell, 1992). Since 5-HT plays an important role in the regulation of feeding behavior, effects of cinnamon bark aqueous extract (CBAE) on 5-HT should also be evaluated. Present study was designed to monitor the neurochemical and behavioral effects of repeated administration of CBAE. Findings would be beneficial in understanding the mechanism of action of cinnamon bark aqueous extract as well as its implication in the treatment of conditions involving altered 5-HT metabolism.

## MATERIALS AND METHODS

### *Preparation of Extract and Doses*

Cinnamon barks were purchased from local market. Identification was provided by experienced botanist from the Department of Agriculture, University of Karachi. 50 grams of bark were crushed in blender. The powder was soaked in 200 ml of water and left for 24 hour at 4°C. The mixture was filtered and stored. Extract was administered to animals in volumes of 2ml/day. While control animals were administered with water in equal amount (2ml/day) (Bano *et al.*, 2012).

### *Experimental Protocol*

Albino Wistar male rats weighing 280-320g were purchased from H.E.J. Research Institute, University of Karachi. Animals were placed individually in the home cages under 12 h light-dark cycle and controlled room temperature (23±2°C) with free access to standard rodent diet and tap water for one week, prior to starting the experiment so that rats could become adopted to laboratory environment, as reported elsewhere (Bano *et al.*, 2013; Bano *et al.*, 2011).

Experimental protocol was approved by the Local Animal Care Committee. Twenty four rats were randomly divided into two equal groups each containing 12 animals: (i) Test and (ii) Control. Test group received aqueous extract of herb. The control group received normal water equivalent to that of aqueous extract of herb. Extracts were given daily by feeding tube at 10:00hrs. When 15-19% body

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weight reduction was observed in herbs treated rats, the behavioral activities of the animals were monitored and rats were decapitated 24hr after monitoring behavior. Untreated rats were also decapitated at the same time. Plasma and brain samples were preserved at -70°C for neurochemical and biochemical estimations.

### Behavioral parameters

#### Food intakes and body weights

Cumulative food intakes (g) were determined by taking the difference of food given /week and simultaneously body weights were also monitored as reported previously (Bano *et al.*, 2009; Bano *et al.*, 2012).

#### Light dark box activity

Apparatus used in this investigation consisted of two compartments. One was dark and second was made up of transparent plastic. The compartments were of equal size (26×26×26cm) and had shuttle access (12×12cm) between them that allowed animals to pass through it freely. Rats were placed in dark compartment. Numbers of entries in light compartment as well as time spent in the same were measured for five minutes. Procedure was same as described elsewhere (Maribel *et al.*, 2006).

#### Elevated plus maze activity

The apparatus used in the present studies consisted of two open arms (50×10cm) and two closed arms (50×10×25cm). The maze was elevated from the floor at a height of 60cm. Rats were entered in the middle of apparatus. Time spent in the open and numbers of entries in open arm were monitored in a period of 5 minutes (Rodgers *et al.*, 1998).

#### Decapitation of rat brain

Immediately after decapitation, the skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for the estimation of biogenic amines and metabolites using High performance liquid chromatography with electrochemical detection (HPLC-EC) (Bano *et al.*, 2012).

#### Extraction of brain samples

Biogenic amines and metabolites were extracted with perchloric acid (HClO<sub>4</sub>; 70%) from brain tissue. 5 times volume of the extraction medium was added to the brain tissues. Samples were homogenized by using electrical homogenizer and subjected to ultracentrifugation at 6000 rpm for 20min at 4°C. The supernatant was transferred to eppendorf tubes and injected to HPLC-EC for neurochemical assay. HPLC-EC determination was carried out as described earlier (Ikram *et al.*, 2012; Bano *et al.*, 2012).

### Neurochemical estimations by HPLC-EC

HPLC-EC determination was carried out as described earlier (Bano *et al.*, 2012; Mirza *et al.*, 2013). A 5μ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating potential of

### Plasma tryptophan analysis by HPLC

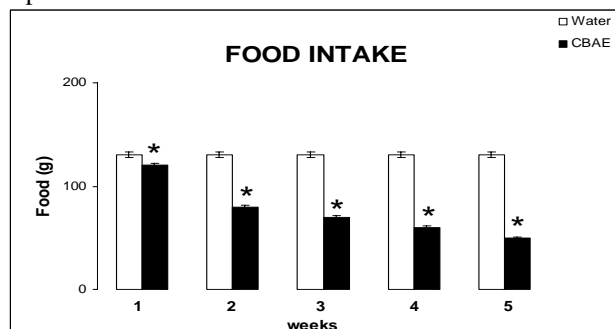
Estimation of monoamines and their metabolites was made by HPLC with visible detector. Method was essentially same as reported by Bano *et al.*, (2012).

### STATISTICAL ANALYSIS

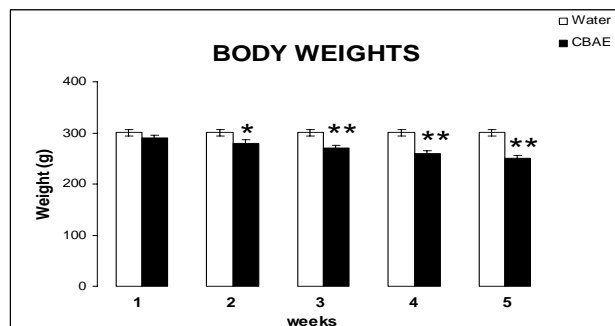
Data were analyzed by Student's *t*-test. Values of  $p < 0.05$  were considered as significant. Data expressed in fig. as mean  $\pm$  standard deviation (SD).

### RESULTS

Fig. 1 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on food intake. Analysis of data by Student's *t*-test showed a decrease ( $p < 0.01$ ) in food intake by the test animals upon repeated administration of CBAE.



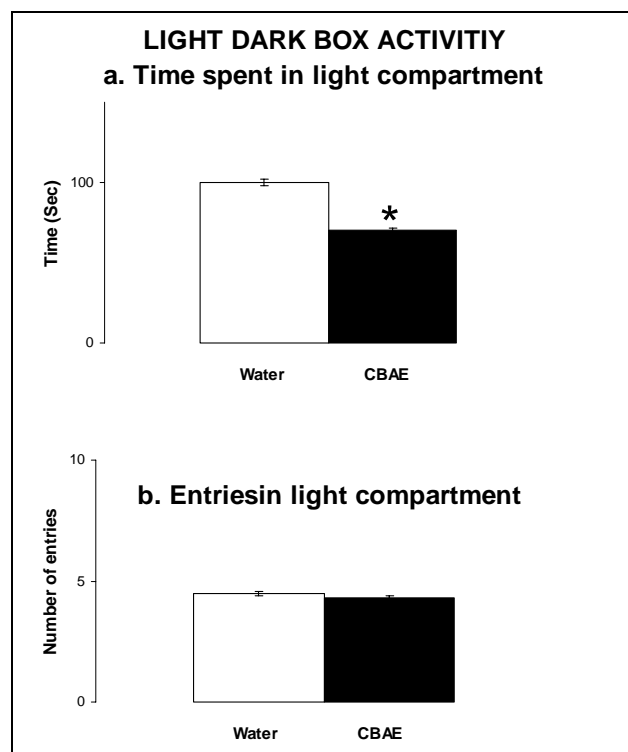
**Fig. 1:** Effects of repeated administration of CBAE on food intake values (for 5 weeks). Values are represented as means  $\pm$  SD (n=12). Significant differences following Student's *t*-test: \* $p < 0.01$ , as compared to water treated controls.



**Fig. 2:** Effects of repeated administration of CBAE on weekly body weights (for 5 weeks). Values are represented as means  $\pm$  SD (n=12). Significant differences following Student's *t*-test: \* $p < 0.05$ , \*\* $p < 0.01$ , as compared to water treated controls.

Fig. 2 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on body weights. Analysis of data by Student's *t*-test showed a reduction in body weights of test animals ( $p < 0.01$ ) upon repeated administration of CBAE.

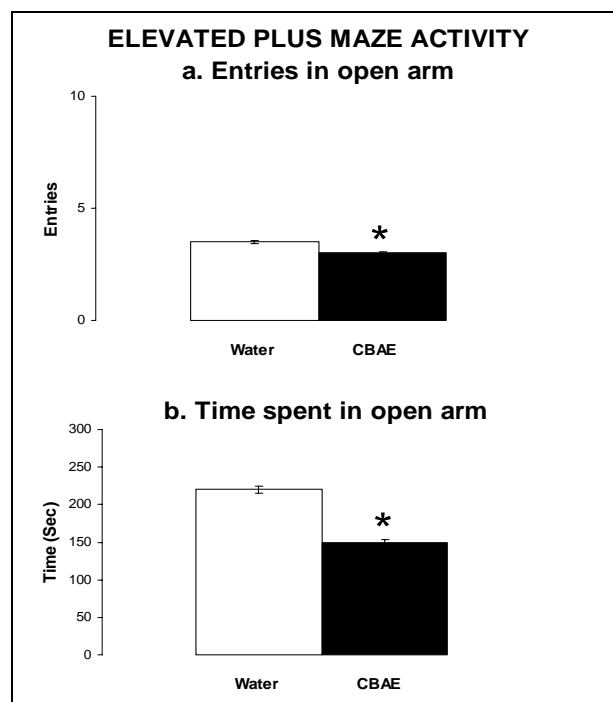
Fig. 3 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on light dark box activity. Analysis of data by Student's *t*-test showed that repeated administration of CBAE decreased ( $p < 0.01$ ) time spent in light box (fig. 3a). However, number of entries in the light compartment (fig. 3b) was not affected much.



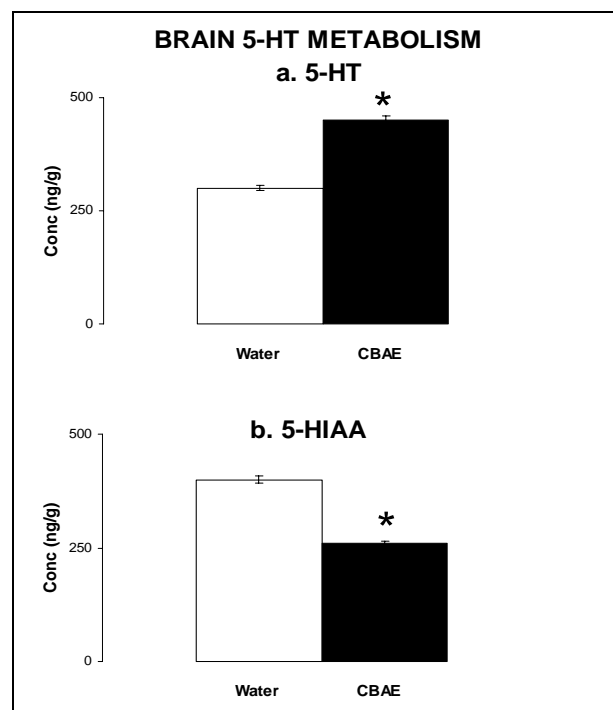
**Fig. 3:** Effects of repeated administration of CBAE after week 5. Values are represented as means  $\pm$  SD ( $n=12$ ). Significant differences following Student's *t*-test: \* $p < 0.01$ , as compared to water treated controls.

Fig. 4 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on elevated plus maze activity. Analysis of data by Student's *t*-test showed that repeated administration of CBAE decreased entries ( $p < 0.01$ ) in the open arm (fig. 4a) as well time spent in the very same (fig. 4b).

Fig. 5 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on 5-HT metabolism. Analysis of data by Student's *t*-test showed that repeated administration of CBAE increased ( $p < 0.01$ ) the levels of 5-HT (fig. 5a), while significant decrease in 5-HIAA level (fig. 5b) in the brain.

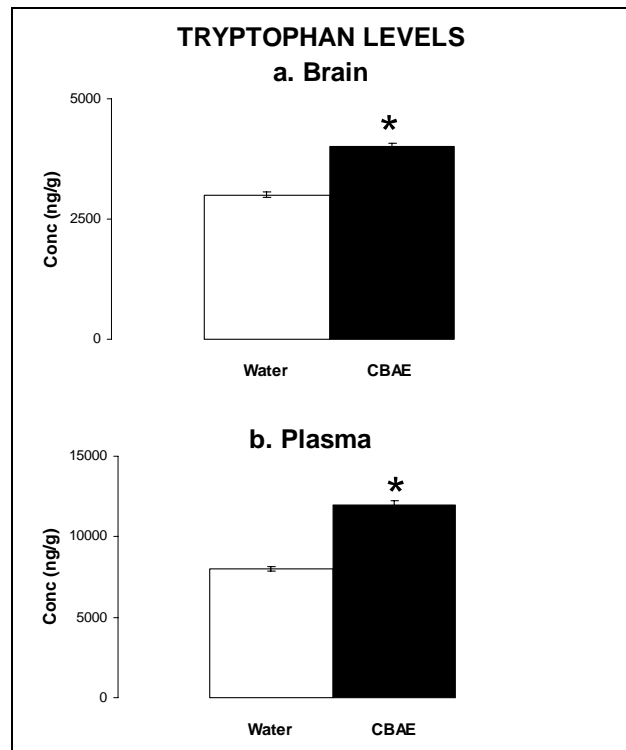


**Fig. 4:** Effects of repeated administration of CBAE on elevated plus maze activity after week 5. Values are represented as means  $\pm$  SD ( $n=12$ ). Significant differences following Student's *t*-test: \* $p < 0.01$ , as compared to water treated controls.



**Fig. 5:** Effects of repeated administration of CBAE on whole brain 5-HT metabolism after week 5. Values are represented as means  $\pm$  SD ( $n=12$ ). Significant differences following Student's *t*-test: \* $p < 0.01$ , as compared to water treated controls.

Fig. 6 shows effects of repeated administration of Cinnamon Bark Aqueous Extract (CBAE) (2 ml/day) on brain and plasma tryptophan. Analysis of data by Student's *t*-test showed that repeated administration of CBAE increased ( $p < 0.01$ ) the levels of tryptophan in brain (fig. 6a) as well as in plasma (fig. 6b).



**Fig. 6:** Effects of repeated administration of CBAE on whole brain & plasma TRP levels. Values are represented as means  $\pm$  SD ( $n=12$ ). Significant differences following Student's *t*-test: \* $p < 0.05$ , as compared to water treated controls.

## DISCUSSION

Body weight could possibly be related to increase in 5-HT. Many evidence suggests an involvement of 5-HT in feeding behavior. CBAE decrease food intake and body weights in the present study (fig. 1-2). Others have reported involvement of 5-HT in anorexia (Haleem *et al.*, 1993). Results from present study suggest that increased serotonin might be responsible of hypophagic effect of CBAE. Since increased availability of 5-HT at 5-HT<sub>2C</sub> receptor could induce hypophagia (Baxter *et al.*, 1995).

Behavior in elevated plus maze (EPM) and light dark box is well established animal model for evaluating anxiolytic/anxiogenic properties of compounds (Salemet *et al.*, 2005; Altaf, 2008). CBAE showed anxiogenic behavior as monitored in light dark box (fig. 3) as well as elevated plus maze (fig. 4).

CBAE increased the 5-HT metabolism (fig. 5). Role of 5-

HT in feeding behavior is well documented (Halford *et al.*, 2007; Ferstrom and Wurtman, 1971). Numbers of researchers have demonstrated that increase in 5-HT function in brain suppressed food intake (Curzon, 1990). CBAE increase serotonin level in whole brain which results in increased satiety and effects resemble to the hypophagic effects of m-chlorophenyl piperazine (Vickers *et al.*, 2003; Schreiber and Dev Vry, 2002).

Increase 5-HT level may be responsible for anxiogenic effect in experiment animals. Decrease in 5-HIAA level in present study show that CBAE decrease 5-HT metabolism in whole brain of obese rats.

The hypophagic effects of CBAE observed in present study may possibly be related to the high concentration of 5-HT precursor tryptophan in plasma and brain (fig. 6) resulting in increased availability of tryptophan to brain which in turn results in increased brain 5-HT levels (Leathwood & Fernstrom, 1990) who reported that increases of brain 5-HT were correlate with an increase in the level of its precursor tryptophan.

## CONCLUSION

In conclusion, present study validates the folk medicinal use of these herbs as weight reducing agent. Result could be implicated for the treatment of eating/related disorders involving decreased serotonin metabolism.

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