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# Oral administration of O-2 lean, an anti-obesity herbal composition increased 5-HT metabolism, decreased food intake and body weight in overweight rats

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**Abstract**: Feeding behavior is complex processes controlled by the neruroendocrine system.5-HT play an important role in regulation of energy balance by suppressing food intake .Depletion of brain serotonin increase feeding behavior and develop obesity. Many serotoninergic compounds are available in market for the management of body weight. O2-Lean is an anti-obesity herbal formulation prepared by combination of different herbs. Oral administration of aqueous suspension of O2-Lean caused a significant decrease in body weight, food intake, and increase in whole brain 5-HT 5HIAA, tryptophan and plasma tryptophan in over weight rats treated with 0.096g/2ml O2-Lean in comparison to control group.

**Keywords**: O-2 lean suspension (O2LS), 5-hydroxyindol acetic acid (5-HIAA), 5-Hydroxytryptamine (5-HT).
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## INTRODUCTION

According to (WHO) world health organization, one billion adults that are approximately overweight currently exist in the world, of which three hundred million are obese[32].Herbal medicines have been used in the treatment weight management for a long time. A number of herbal preparations are available in the market for weight reduction.

O-2Lean is an herbal formulation prepared from Chromium polynicotinate (25µg), citrus extract 6% (15.635grm). Ginger root powder(6.25grm), green tea extract95%(20grm), guarana 22% (250mg), hydroxy citric acid (250mg), L-tyrosine(100mg), N. Acetyl-L-carntine (12.5mg), vitamin B5(5mg), white willow bark fine powder (25grm).chromium enhance insulin activity[1] and green tea is used for weight reduction[2].

Many pathophysiological mechanisms are involved in developing obesity. Feeding behavior is in control by a major interaction between central nervous system and many organs of body. Many evidences proved that hypothalamus control appetite. Obesity mediates with lesion of the hypothalamus. Brain lesioning and stimulation studies suggested ventromedial hypothalamus as the satiety center and to the lateral hypothalamus area as the hunger center [1-2]. Several studies prove that cause of obesity is linked to the serotonin level in brain[3]. 5-HT and its agonists suppress food intake when administered either peripherally or centrally in both freely feeding and food-deprived animals[4-9].

Therapeutic agents that act targeting the serotonergic system effect on feeding behavior. Increases activity of 5-HT in synaptic cleft or directly activate 5-HT receptor have been reported to reduce food consumption. For example Fenfluramine[10-12] stimulates serotonin release and inhibits its reuptake, Sibutramine[7,13-16] and Fluxetine [17-18] are serotonin reuptake inhibitor and MAO inhibitor chlorophenylpiperazine[19-20]. Increase concentration of this monoamine decrease body weight and increase energy expenditure in both animals and humans.

This study was conduct to confirm the weight reducing effect of O2LS .and its possible relation with 5-H metabolism, involved in the regulation of appetite and satiety.

## MATERIALS AND METHODS

### *Preparation of suspension*

One capsule dissolved in 10ml of water.

### *Experimental protocol*

Pakistan bred Albino Wistar male rats whose weight was between 280-320gm. All animals were placed in single cages under 12 hours light-dark cycle and control room temperature (23±2$^{o}$C) with free access to specially prepared diet and normal water for one week, prior to starting the experiment so that rats could adopt themselves to new conditions. The test group received 2ml of O2-Lean suspension. 2ml water suspension contains 0.096gm of capsule. The control group received normal water equivalent to that of aqueous O2LS.

Weighed amount of food was placed in the hopper of all cages. Body weight and food intake was monitored weekly when 15-19% body weight reduction was observed in herbs treated rats, rats were decapitated using guillotine. Blood and brain samples were obtained and preserved at -70$^{o}$C for neurochemical estimations. Untreated rats were also decapitated at the same time.

### *Neurochemical estimation*

### *Estimation of monoamines and their metabolites by high performance liquid chromatography with electrochemical detection (HPLC-EC)*

Estimation of monoamines and their metabolites in the whole brain samples of rats was made by HPLC-EC method as reported by[23].

It is highly sensitive and sophisticated technique developed in 1970. HPLC is associated with development of stationary phase with small type of silica particles. In HPLC, the small particles of 5nm in diameter can resist the high pressure of mobile phase which does not occur in the thin layer chromatography. Performance is increased using high pressure pump and small particle, the separation is possible quickly in short time.

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 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 0.8 to 1.0V.

After detection, message from the detector may be recorded by an integrator .Each sample was identified by comparing with the retention time of standard[23-24].

***Mobile phase***

The composition of mobile phase was Methanol 10% (HPLC grade 18%), Sodium dihydrogen phasphat 1.56%, octyl sulfate salt 0.18%, EDTA 0.05%, and Orthophasphoric acid to adjust pH at 2.9.

***Extraction medium***

The extraction medium contains Perchloric acid 0.1M, Sodium *meta*-bisulphate 0.1%, EDTA 0.01% and cystein 0.01%.

***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. The homogenate were allowed to stand for 10-15 minutes to help precipitation .Supernatant transfer in Eppendorf tubes was centrifuged at 12,000rmp for 20-25 minutes at 4$^{o}$C. Supernatants were used for the estimation.

***Extraction of tryptophan from plasma***

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

***Calibration of HPLC-EC system***

Before analyzing the samples by HPLC the system was calibrated using following standards separately to identify their respective retention time. Finally a solution of stranded in the following concentration was made:

1. 5-Hydroxyindol acetic acid (5-HIAA):100ng/ml
2. 5-Hydroxytryptamine cretinine sulphate (5-HT-Cs):200ng/ml i-e 86.5 ng/ml 5-HT
3. L-TRP:1000ng/ml

***Statistical analysis***

The significance differences between the mean of the treated and untreated groups were analyzed by student's *t*-test. Values of $p<0.05$ were considered as significant. Data expressed in figures as mean±standard deviation (SD).

## RESULTS

***Behavioral effect of repeated administration of O2LS in rats***

***Effect of repeated administration of O2LS on weekly food intake and body weight in rats***

Figure 1 shows the effect of repeated administration of O2LS on weekly food intake and body weight. Statistical analysis by student's *t*-test revealed a significant decrease ($p<0.01$) in food intake and body weight as compared to control.

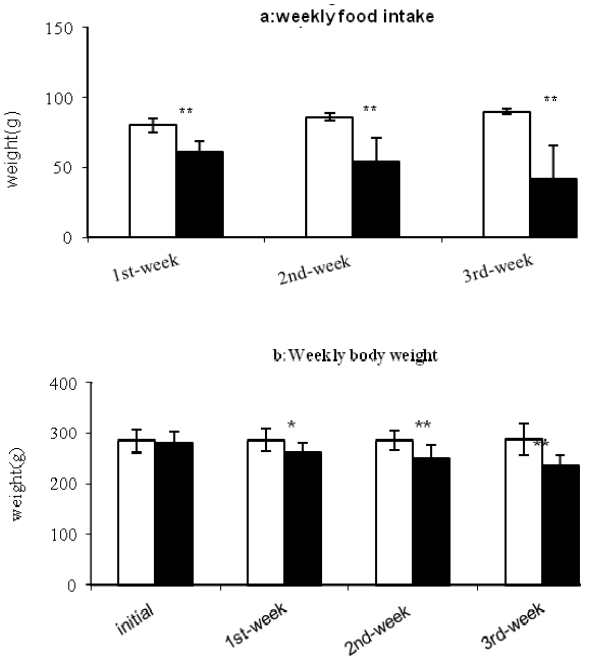


**Figure 1:** Effect of repeated administration of O2LS on food intake and body weight of rats. Values are mean±SD (n=12) significant difference by Student *t*-test **p<0.01 and *p<0.05 from respective controls.

***Neurochemical effect of repeated administration of O2LS in rats brain***

***Effect of repeated administration of O2LS on whole brain 5-HT and 5- HIAA levels in rats***

Figure 2 shows the effect of repeated administration of O2LS on brain 5-HT and 5-HIAA levels in rats. Statistical analysis by student's *t*-test revealed a significant increase (P<0.01) in 5-HT while significant decrease in 5-HIAA levels as compared to control.

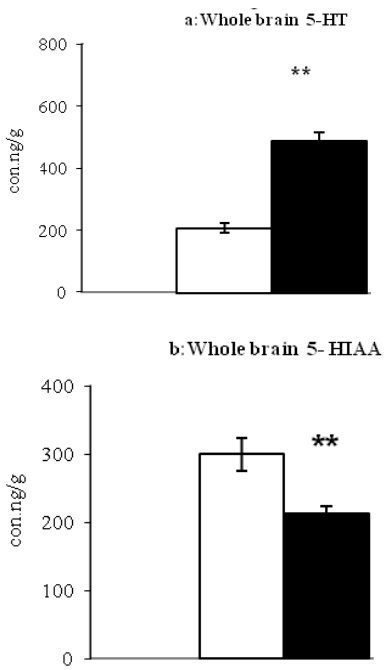


**Figure 2:** Effect of repeated administration of O2LS on 5-HT and 5-HIAA level of rats brain. Values are mean±SD (n=12) significant difference by Student *t*-test **p<0.01 from respective controls.

***Effect of repeated administration of O2LS on whole brain tryptophan levels in rats***

Figure 3 shows the effect of repeated administration of O2LS (three weeks) on brain tryptophan levels in rats. Analysis of data by student's *t*-test revealed a significant increase (p<0.01) in brain tryptophan levels as compared to control.

***Effect of repeated administration of O2LS on plasma tryptophan levels in rats***

Figure 3 shows the effect of repeated administration of O2LS on plasma tryptophan levels in rats. Analysis of data by student's *t*-test revealed a significant increase (p<0.01) in plasma tryptophan levels as compared to control.

## DISCUSSION

The use of natural products for reducing body weight has increased, based on reliability, safety and cost compared with synthetic medecine[25], or surgical procedures[26], which may have limitation[27].

Today obesity and its deleterious effect on health have been challenge for scientist .The attenuation of food intake as induced by an increase in serotonergic efficacy has been a target of obesity pharmacotherapies. Researchers identified an inverse relationship between the biogenic amines neurotransmitters serotonin and food intake.

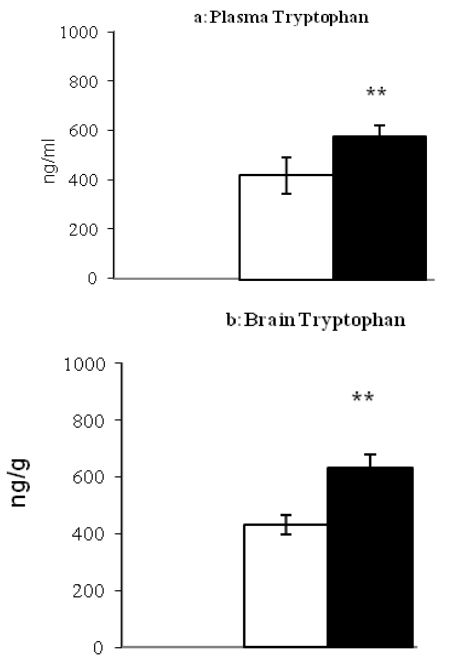


**Figure 3:** Effect of repeated administration of O2LS on brain and Plasma TRP of rats. Values are mean±SD (n=12) significant difference by Student *t*-test **p<0.01 from respective controls.

More specifically, a selective reduction in serotonin bioavailability was associated with hperphagia and subsequent weight gain, while diminished in food intake was induced by an increase in serotonin efficacy[21].in the present study also monitored decrease in Body weight, food intake (Figure 1) and increased in 5-HT metabolism (Figure 2) following the repeated administration of O2LS .decrease body weight could be compensatory response to high 5-HT turnover in whole brain. This is consistent with previous finding that decrease 5-HT levels in brain develop hyperphagia and obesity[28-29] while increase level of this neurotransmitter produce hypophagic effect and reduce body weight[19-22]. O2LS also increased both plasma and brain TRP level figure 3. It has been reported by several researchers that tryptophan is essential amino acid and precursor of 5-HT. its high concentration in plasma increased availability of TRP to brain that lead to increase syntheses of 5-HT[30-31]. This study, therefore indicate

relationship between increased level of TRP and 5-HT concentration. O2LS increased 5-HT metabolism in brain and this increase the satiety and decrease in body weight.

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