Effect of Oxygen Consumption, T4 and T3 Assay Following Repeated Oral Administration of Evans Healthy Bitters in Wistar Albino Rats.

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Abstract: This study was carried out to evaluate the Effect of Evans Healthy Bitters on oxygen consumption, T3 and T4 levels in albino wistar rats. Twenty (20) female albino rats weighing 120 – 130g were randomly assigned to four groups A, B, C and D and were allowed to acclimatize for 7 days. Animals in group A served as control received food and water ad libitum while groups B, C and D received 0.25, 0.5 and 1ml of EHB orally daily for the period 28 days. Weight changes, food and water intake was monitored weekly. Oxygen (O2) consumption was measured at the end of period and the T3 and T4 assayed using standard methods. Body weight changes as well as food intake were significantly (P<0.01, P<0.001) higher in B, C and D when compared with A. Water intake was significantly higher in D compared with A. Result of oxygen consumption showed a significant (p<0.001) increase in groups B, C and D compared with A. In the T3 and T4 assay, T3 was significantly (p<0.001) higher in B, C and D compared with A while T4 was significantly (p<0.001) higher in B and C but changes not statistically significant in D. The results show that EHB might cause an increased secretion of T3 and T4 which might enhance increase oxygen consumption, food and promote growth in the albino rat when taken at moderate concentration but might possibly triggers obesity in humans since it increases metabolic rate.

Key words: Evans Healthy Bitters, oxygen consumptions, thyroxine, triiodothyronine.

INTRODUCTION

Herbal formulations have served as important therapeutic sources available to man in search for remedies for diseases management (Ogbonnia et al., 2008). Nature has provided the main source of natural drugs (Doughari et al., 2008). Plant-derived biologically active compounds used as drugs has led to compoundment of potent medicaments (Houghton and Raman, 1998) that have shown enormous benefit in relieving man’s health challenges in developed and developing countries (Mythilypriya et al., 2007). Most of these plant base medicines consist of two or more products known to have nutritive and phytoconstituents with multiple physiological activities that could alleviate disease condition (Pieme et al., 2006; Okakudjejo et al., 2008).

In Nigeria, National Agency for Food and Drug Administration Control (NAFDAC) regulates drug compoundment. Evans Healthy Bitters (EHB) is one of such drugs produced by Evans Medical Plc, Ogun State, Nigeria. It is formulated in a manner that the components exhibit a synergetic effect in the management of disease condition. It consists of Cassia augustifolia (16.6mg), Alhagi camelorum (22.20mg), Commiphora myrrha (5.33mg), Andrographis paniculata (22.20mg), Aloe barbadensis (40mg), Crocus sativus (1.06mg) and H2O QS. EHB is said to be used to manage a wide range of human ailments such as poor digestion, chronic constipation, poor appetite, jaundice anemia, immunological disorders, diarrhea, malaria infection, inflammation (Schultz, 2010; Grover and Vert, 2010). Some of the components have anti-hepatotoxic as well as ability to moderate the thyroid gland (Bando and Mann, 2006; Ayedum, 1998). We find it worthwhile to investigate the drug effect on oxygen consumption, T3 and T4 assay in the laboratory rat.

MATERIALS AND METHODS

The drug sample (Evans Healthy Bitters) was obtained from Evans Medical Plc, Ogun State, Nigeria while other samples, kits and reagents used were of analytical grade.

Animals Preparation:

Twenty (20) wistar albino rats weighing 120 – 130g were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were housed in metabolic cages in the Animal House and fed with rat chow (psiyzer Aba, Nigeria) and water ad libitum. The animals were randomly assigned.
into four (4) groups (A – D) of five rats and allowed to acclimatize for 7 days with 12 hours light and dark cycle and temperature 28\(^\circ\) + 2\(^\circ\)c and humidity of 40 – 60% before start of the experiment.

**Treatment procedures:**
- **Group A:** received food and water only throughout the period of experiment.
- **Group B:** received food, water and a daily oral administration of 0.25ml of Evans Healthy Bitter.
- **Group C:** received food, water and a daily oral administration of 0.5ml of Evans Healthy Bitters.
- **Group D:** received food, water and in addition a daily oral administration of 1ml of Evans Healthy Bitters.

Throughout the period of 28 days the cages, food trough and drinking bottles were kept clean weight changes were recorded as well as food and water intake.

**Measurement Of Weight Changes:**
Weight changes were taken weekly using animal weight balance for the period of 28 days.

**Measurement Of Water Intake:**
A known volume of water was placed in a feeding bottle for each animal in the four groups. The amount of water that was left each day was measured using a measuring cylinder and subtracted from the known volume, and was recorded.

**Measurement Of Food Intake:**
Each animal has a feeding trough that holds a known quantity of food in the metabolic cage. The left-over everyday was measured with metler 163 (Switzerland) and deducted from the weight of food that was given.

**Measurement Of Oxygen Consumption:**
The method described by the staff of the Department of Pharmacology, Edinburg University (1970) and modified by Okwari and Ettarh (1999) was used. The system consists of an air tight chamber for the animal connected to an air filled bottle. The bottle has three entering to it. One from an O2 – cylinder, the second from a burette and third from a 20ml syringe use to adjust the pressure or determining O2 use by the animal. The lower compartment of the animal chamber contains soda lime to absorb the carbon dioxide expired. The upper carries a tube connected to a water manometer.

The air space of the apparatus was flushed with pure oxygen. The connections to the atmosphere were closed and a few minutes are allowed for thermal equilibrium to be reached. As the animal takes up oxygen and its expired CO2 was absorbed by the soda lime, the amount of gas in the apparatus reduced and the pressure recorded as the manometer tends to fall. The pressure can be restored to the atmospheric pressure by running in water from the burette. If the pressure is adjusted at the beginning and end of timed period, the volume of water needed represent the volume of O2 taken up by the animal during the period. Alternatively by using syringe to adjust the pressure the volume of O2 use is known.

**Collection of Blood:**
Blood samples from control, and test animals were obtained by cardiac puncture. Animals were placed in chloroform and diethyl vapour (1:1). They were removed and their thorax was carefully exposed and blood was collected with sterile syringe and needles into test tube and left to stand for one hour.

**T3 and T4 Assay:**
T3 (Triiodothyronine) and T4 (Thyroxine) assay T3 and T4 activities were assayed in serum using standard diagnostic kit:DRG T3 Triiodothyronine ELISA EIA–1780 and DRG T4Thyroxine EIA 1781 manufactured by DRG International, USA. Briefly after colour development was stopped, the absorbance was measured spectrophotometrically at 450nm.

**Statistical Analysis:**
Data collected were expressed as mean ± standard error of mean. Students test was used for the analysis P-values of 0.01 and 0.001 were graded as significant.

**Result:**
Table 1 presents the results of weight changes food intake and oxygen consumption in wistar albino rats. The results of body weight changes, food intake were significantly (P<0.01) higher in groups B,C and D compared with group A. Water intake was shown to be significantly (P<0.001 ) higher in group D compare with group A . The result of Oxygen consumption were significantly (P < 0.001) higher in test groups (B,C and D)
than in A. Table 2 presents the result of T3 and T4 assay. The results showed that T3 and T4 levels were significantly (P<0.001) higher in groups B and D only.

Table 1: Weight, Food intake, Water intake and O2 intake of EHB fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight changes(g)</th>
<th>Food intake (g)</th>
<th>Water intake (ml)</th>
<th>O2 Consumption g/h.w (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>150.00±0.00</td>
<td>14.72±0.06</td>
<td>22.40±0.13</td>
<td>0.36±0.003</td>
</tr>
<tr>
<td>B</td>
<td>158.20±2.26</td>
<td>16.55±0.20</td>
<td>22.56±0.09</td>
<td>0.55±0.02</td>
</tr>
<tr>
<td>C</td>
<td>157.60±1.03</td>
<td>17.75±0.09</td>
<td>23.04±0.31</td>
<td>0.53±0.007</td>
</tr>
<tr>
<td>D</td>
<td>162.60±1.25</td>
<td>18.25±0.08</td>
<td>23.52±0.17</td>
<td>0.59±0.002</td>
</tr>
</tbody>
</table>

Data expressed * = SEM ** = P<0.01 *** = P<0.001 n = 5

Table 2: Weight, T3 and T4 levels of EHB fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>0.68±0.03</td>
<td>1.23±0.03***</td>
<td>1.15±0.07***</td>
<td>1.06±0.05***</td>
</tr>
<tr>
<td>T4</td>
<td>2.20±0.20</td>
<td>5.12±0.11***</td>
<td>2.88±0.04***</td>
<td>3.70±0.25***</td>
</tr>
<tr>
<td>T3:T4</td>
<td>0.31±0.004</td>
<td>0.25±0.005**</td>
<td>0.40±0.004</td>
<td>0.29±0.005**</td>
</tr>
</tbody>
</table>

Data expressed * = SEM ** = P<0.001 n = 5

Discussion:
This study investigated Evans Healthy Bitters (EHB) on weight, water, food intake, oxygen consumption, T4 and T3 assay in rat. The results on body weight changes as well as food intake showed significant increase in the test groups when compared with control (A). These effects might be caused by one of the agents in the EHB. *Commiphora myrrha* has been reported to moderate the thyroid function (Ayedun, 1998; Bado and Mann, 2006). Thyroid hormones (T3 and T4) are known to exhibit general and specific effect on growth especially in growing children. Increased food intake provide substrate for cellular metabolism. Metabolic rate increase due to the action of T3 and T4 and increased nitrogen excretion, if food intake were not increased endogenous proteins and fats stores are catabolized which might result in weight lost (Barrett et al., 2010; Hall, 2011). Body weight therefore depends on food intake and utilization. Mechanisms that drives eating (hunger) and those that restrain (satiety) operate to control short term eating behavior and long term control of body weight and composition (Smith, 2000).

Water intake was significantly higher in group D than in control or groups B and C. Water intake results when thirst is sensed. Controlling drinking is largely regulated by the interaction of positive and negative feedbacks that maintain body fluid homeostasis. Food intake and drinking often but not always occur together in time. Physiologically, intake of fluid during eating increases due to increase in plasma osmolality that occurs as food is absorbed and possibly the action of an effect of one or more of the gastrointestinal hormones on the hypothalamus (Barrett et al., 2010; Hall, 2011).

The rate of oxygen consumption per gram per body weight was significantly increased in the test groups than in control. It is possible that this increase might be due to the effect of EHB or *Commiphora myrrha* which is a constituent of EHB which has been thought to moderate thyroid activity (Ayedun, 1998; Bando and Mann, 2006). Parallely, T3 and T4 were increased significantly in test groups compare with control. T3 and T4 stimulates and caused increased in oxygen consumption and glucose uptake (Kvetny and Matzen 1989; Hall, 2011). Other researchers reported that T3 and T4 regulate tissue oxygen consumption and thermogenesis (Boket et al., 1977, Mcnabb, 2000; Hulbert, 2000). Tyroxine and T3 circulate in plasma almost entirely bound to thyroid binding globulin. The unbound hormone diffuse into tissues and exert their metabolic effects (Hall, 2011). Increase in serum T3 and T4 levels in test group suggest hyperactivity of thyroid gland that may lead to thyrotoxicosis which is also supported by the report that T3 : T4 might be responsible for hormonal disorders like thyrotoxicosis and thyroiditis among others (Mortoglou and Candiloros, 2004). It might be logical to conclude that EHB has a tremendous therapeutic and pharmacological potentials, which might and very be relevant to children when taken at moderate concentration but might possibly triggers obesity in adult since it increases metabolic rate.

REFERENCES


