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Original research

Effect of combination therapy of melatonin and orlistat on high fat diet induced changes in lipid profiles and liver function parameters in serum of rats

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ABSTRACT

Background: Obesity is a global health problem and also a strong risk factor for type 2 diabetes mellitus, cardiovascular (CV) diseases. High-fat diet (HFD) induced rats was proved to be a useful model for obesity in humans. Orlistat is a synthetic anti-obesity drug approved by European Medical Association (EMA). Some studies shown, orlistat supplementation caused liver and kidney damage. Melatonin, an indolamine, produced by the pineal gland in a circadian rhythm, and it is one of the most powerful antioxidants known. Studies shown, it also exerts control over metabolic functions that determine fat accumulation and obesity. To compensate orlistat induced side effects, the aim of this study was to determine the impact of melatonin and orlistat alone and the combination therapy of both on lipid profiles and liver function parameters in serum of HFD induced rats.

Materials and methods: Male wistar rats (150–180 g) were divided into five groups with 6 animals each. **Group 1** was maintained as control with normal diet. The other animals were fed with HFD (8 weeks). After 8 weeks, the animals were subdivided into following groups such as **Group 2** (HFD control), **Group 3** (HFD + Orlistat, 6 weeks), **Group 4** (HFD + Melatonin, 6 weeks), **Group 5** (HFD + Orlistat + Melatonin, 6 weeks). Blood samples were taken before and after administration of drugs for the measurement of lipid profiles and biochemical parameters.

Results: The increased body weight along with increased glucose, triglycerides, cholesterol, LDL, VLDL while decreased HDL was observed in HFD induced rats. The liver function parameters such as SGOT, SGPT, ALP and LDH were also altered. The melatonin and its combination with orlistat supplementation significantly restored all the parameters especially liver function parameters, when compared to orlistat alone. Melatonin had great effect in HFD induced changes than orlistat without affecting food intake.

Conclusions: In overall findings, melatonin alone and its combination therapy with orlistat prevents obesity and its harmful effects than Orlistat alone.

1. Introduction

Over the past 25 years, obesity has become a worldwide concern of alarming proportion. Association of obesity with an increased risk of type 2 diabetes mellitus, heart disease, metabolic syndrome, hypertension, stroke, and certain forms of cancer is a major cause of morbidity and mortality. It is measured by body mass index (BMI), which is calculated as weight divided by height squared. Individuals with a BMI $\geq$25 kg/m^2 are classified as overweight and those with a BMI $\geq$30 kg/m^2 are considered as obese (Frayling et al., 2007). According to world health organization in 2015, worldwide obesity has more than doubled since 1980. Study shows, India is just behind US and China in this global hazard list of top 10 countries with highest number of obese people. The US topped the list with 13% of the obese people worldwide in 2013, while China and India together accounted for 15% of the world's obese population, with 46 million and 30 million obese people, respectively. According to the study, number of overweight and obese people globally increased from 857 million in 1980 to 2.1 billion in 2013 and this is one-third of the world's population (Ng et al., 2014).

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Study shows 42 million children under the age of 5 were overweight or obese in 2013 (WHO, 2015). In 2014, more than 1.9 billion adults, 18 years and older, were overweight and of these over 600 million were obese. Most of the world's population lives in countries were overweight and obesity kills more people than underweight.

Management strategies for weight reduction in obese individuals include physical interventions such as exercise, diet and surgery, behavioral therapies and pharmacological treatments. These strategies may be used alone or in combination for greater efficacy. Drugs used to induce weight loss may reduce appetite or increase satiety, reduce the absorption of nutrients, or increase energy expenditure. Orlistat (Xenical, gastrointestinal lipase inhibitor) was approved by the Food and Drug Administration (FDA) for long-term therapy of obesity and is a gastric and pancreatic lipase inhibitor that reduces dietary fat absorption by approximately 30%, has been in use for around ten years and proved to be useful in facilitating both weight loss and weight maintenance (Heck et al., 2000). Although orlistat has approved by FDA, studies showed it causes undesirable adverse effects, and serious health risks. The most commonly experienced side effects are gastrointestinal and include diarrhea, flatulence, bloating, abdominal pain and dyspepsia (Ionnides-Demos et al., 2010). Recently, severe liver injury also has been reported. The FDA received 32 reports of serious liver injury in patients using orlistat between 1999 and 2008, including 6 cases of liver failure (FDA, 2010). Recently study also stated that the modest efficacy, undesirable adverse effects, and serious health risks combine to highlight the deficiencies of orlistat and underscore the pressing need for other anti-obesity drug options (Kim et al., 2014).

Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone produced by the pineal gland and plays major role in circadian rhythm and its levels generally increase in the dark at night. Studies show, it is one of the most powerful antioxidants as well as its metabolites exerts direct antioxidative effects *via* scavenging of radicals and acts as indirect antioxidants by stimulation of antioxidative enzymes (Tan et al., 1993; Rodriguez et al., 2004). Recently studies also shown, it is involved in energy expenditure and body fat mass regulation and effectively controls weight gain, obesity and associated heart disease (Cipolla-Neto et al., 2014). Based on literature and to compensate the Orlistat induced side effects, we aimed to compare the efficacy and safety of melatonin and orlistat alone and the effect of combination therapy of both on lipid profiles and liver function parameters in serum of HFD induced rats.

2. Materials and methods

2.1. Experimental design

Totally thirty male Wistar rats (60 days of age) with body weight ranging from 150 to 180 g were purchased from Indian Institute of Science IISC, Bangalore. The experimental rats were quarantined in animal house for 10 days and were maintained 12:12 h's light/dark cycle at 20 °C ± 2 for 14 weeks with *ad libitum* of food and sterile reverse osmosis water. Rats were dewormed with an oral treatment of albendazole (10 mg/kg/bw) before initiation of the experiments. This study was approved by the Institute Ethical Committee, SRM Medical college hospital, SRM University, India (No. 088/835/IAEC-2014). Rats were divided into five groups with 6 animals each. Group 1 was maintained as control with normal diet. The other animals were fed with HFD (8 weeks). After 8 weeks, the animals were subdivided into following groups such as Group 2 (HFD control), Group 3 (HFD + Orlistat therapy, 6 weeks), Group 4 (HFD + Melatonin therapy, 6 weeks), Group 5 (HFD + Melatonin with orlistat therapy, 6 weeks). After end of the HFD treatment, the drugs such as orlistat and melatonin were given to the animals for 45 days. After the post treatment of drugs, all the parameters were estimated to assess the impact of drugs treatment.

Melatonin and orlistat were purchased from Sisco Research Laboratory (SRL), India and Meyer Organics Pvt. Ltd. India. The dose and duration of both the orlistat and melatonin therapy was selected according to the following literatures. Orlistat (200 mg/kg/day) was administered in food according to (Nishioka et al., 2003) and melatonin (10 mg/kg/day) was administered in drinking water according (Agil et al., 2013). The animals were weighed once in a 2 days. High fat diet [HFD] (g/kg) was procured from National Institute of Nutrition (NIN), Hyderabad, India used to induce obesity in experimental animals. It contained the following contents; Casein (20%) - 200 g; Starch - 425 g; Sucrose - 100 g; Cellulose - 50 g; Ground Nut oil - 175 g; Mineral mix - 35 g; Vitamin Mix - 10 g; L-Cystine - 3 g; Choline - 2 g]. The commercial kits to determine both the lipid profiles and liver function parameters in serum were purchased from ERBA diagnostics Mannheim Gmbh, Germany/Transasia Bio medicals Ltd. India.

2.2. Sample collection

Blood samples were collected from all the animals and the serum from blood samples were obtained by centrifugation at 1500 rpm for 10 min and stored at −20 °C and used for the estimation of glucose, lipid profiles and liver function parameters.

2.3. Estimation of lipid profiles

The lipid profiles such as total cholesterol, LDL, VLDL, HDL and triglycerides in serum were estimated using commercial kits by UV spectrophotometer (absorbance at 505/670 nm). All the results were expressed as mg/dl.

2.4. Estimation of glucose

Serum glucose was estimated using commercial kits by UV spectrophotometer (absorbance at 505/670 nm). The results were expressed as mg/dl.

2.5. Estimation of liver function parameters

The liver function parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and Lactate dehydrogenase (LDH) were estimated using commercial kits by UV spectrophotometer (absorbance at 505/670 nm). All the results were expressed as IU/L. The serum alkaline phosphatase (ALP) level was estimated with the absorbance of 405 nm.

2.6. Statistical analysis

Data were expressed as mean ± SD. The statistical significance was evaluated by one way analysis of Variance (ANOVA) using SPSS 19. Significant was considered at $p < 0.05$.

3. Results

3.1. Body weight

The effect of Melatonin, orlistat alone and the combination therapy of both on HFD induced body weight were represented in Fig. 1. After end of the total experimental period, the body weight was significantly restored after the post treatment of melatonin,