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Nephrotoxicity associated with Orlistat in normal and obese female rats

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Abstract

Obesity is a global health concern associated with high morbidity and mortality. Therapeutic strategies include synthetic drugs and surgery, which may entail high costs and serious complications. Orlistat is a pancreatic lipase inhibitor licensed for the treatment of obesity. The current study was carried out to elucidate the modulating effect of Orlistat against obesity induced kidney toxicity in female rats. A total of 50 female rats were divided into five groups (G1, Control; G2, Orlistat; G3, Obesity; G4, Co- treated Orlistat with Obesity; G5, Post- treated Obesity with Orlistat rat group). The current study revealed that a significant increase in serum urea, creatinine, while a significant decrease in the levels of sodium, potassium, calcium and chloride ions levels in treated rats with Orlistat while a significant increase in serum urea, creatinine, sodium, potassium and chloride ions levels in obesity group when compared with control group. In contrast; a significant decrease in serum urea, Creatinine, sodium, potassium and chloride ions levels in treated obese rats with Orlistat when compared with obesity group. So; Orlistat induced renal toxicity when used for treatment of obesity and self-recovered obese rats is safe and better than the use of Orlistat in treatment of obesity.

Keywords: Obesity, Orlistat, kidney functions, Electrolytes, Toxicity, Rat.

1 Introduction

Obesity is a chronic metabolic disease that is characterized by excess body fat which is generally accompanied by increase in body weight and this happens when the body mass index (BMI) in an adult is greater than 30 units (Huang et al., 2015; Sánchez et al., 2018). Obesity has become a major health problem all over the world and is associated

with substantial increases in morbidity, premature mortality, impaired quality of life and with an increased risk of complications including insulin resistance, hypertension, dyslipidemia, diabetes mellitus, atherosclerosis, coronary heart disease, and certain cancers (Drew *et al.*, 2007; Wang et al., 2015; Campbell et al., 2016; Defo et al., 2017).

Anti-obesity medication or weight loss drugs are pharmacological agents that reduce or control weight

by altering either appetite, or absorption of calories. Some anti-obesity drugs can have severe, even lethal side effects and these side effects are often associated with the medication's mechanism of action (Huang et al., 2015; Defo et al., 2017). Orlistat is anti-obesity medication that is inhibitor of gastric and pancreatic lipases that acts in the gastrointestinal tract (Amin et al., 2014). Orlistat when administered with fat-containing foods, it partially inhibits the hydrolysis of triglycerides, thus reducing the subsequent absorption of monoglycerides and free fatty acids, leading to a reduced fat absorption by around 30% (Choussein et al, 2009). The current study was carried out to elucidate the modulation effect of orlistat against obesity induced kidney toxicity in female rats.

2. Materials and Methods

Orlistat Supplementation

Orlistat drugs were obtained from the pharmacy in Cairo, Egypt. Orlistat drug was marketed as a prescription under the trade name Xenical by Roche in most countries and also known as tetrahydrolipstatin.

Experimental animals

The experiment was performed on 50 male albino rats (*Rattus norvigicus*) weighing 120 g (± 10) and of 10-12 weeks age. They were acquired from laboratory farms, Zoology Department, Faculty of Science, Tanta University, Egypt.

The rats were held in suitable plastic cages for one week before the experimental work for acclimation with a new room conditions and maintained on a standard rodent diet and water available ad libitum. The temperature in the animal room was adjusted to $23 \pm 2^\circ\text{C}$ with a relative humidity of $55 \pm 5\%$. Light was on a 12 h light/dark cycle. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt.

Experimental groups

A total of 50 rats were equally divided into five groups (10 rats each).

Group 1, Control group included rats received no treatment;

Group 2, Orlistat group included rats received (12 mg/kg) of orlistat daily dissolved in saline (1ml/kg) by intraperitoneal injection for four weeks (Amin et al., 2015).

Group 3, obese group in which rats were fed on high fat diet for six weeks. Also, saline solution was

administered intraperitoneally to the rats of this group (1ml/kg).

Group 4, post-treated group included obese rats that treated with orlistat or four weeks.

Group 5, Self-treated group included obese rats that left for another four weeks without receiving any treatment.

At the end of the experimental period, animals were fasted overnight and for clinical chemistry blood samples from each rat were collected from the eyes by retro-orbital puncture using blood capillary tubes without heparin as per requirement under mild ether anaesthesia.

Blood samples were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 r.p.m for 10 min and the serum were collected, serum was separated and kept in clean stopper plastic vial at -80°C until the analysis.

Kidney functions and electrolytes in serum

Sera were used for the determination of urea and creatinine according to the methods described by Patton and Crouch (1977) and Henry et al. (1974), respectively. Sodium and potassium ions concentration were determined using the method of Henry et al. (1974).

Statistical Analysis

Data were expressed as mean values \pm SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $p < 0.05$ for the biochemical data.

3. Results

Toxicity

Many of side effects were appears on rats after Orlistat administration as loss of body weight, loss and changes in colour of hairs, and bloody eyes, and many of tumours appearance in different location in animals.

Changes in relative body and kidney weights

Rat relative body and kidney weight levels in obesity group were significantly increased when compared with control. In contrast; a significant decrease in relative body weight level in treated rats with Orlistat when compared with control and obesity groups (Figures 1&2). On the other hand; a significant decrease in relative body weight level in treated obese rats with Orlistat when compared with obesity groups. In contrast; in significant decrease

were detected in relative kidney weight level in treated obese rats with Orlistat or self-treated obese rats when compared with obesity groups (Figures 1&2).

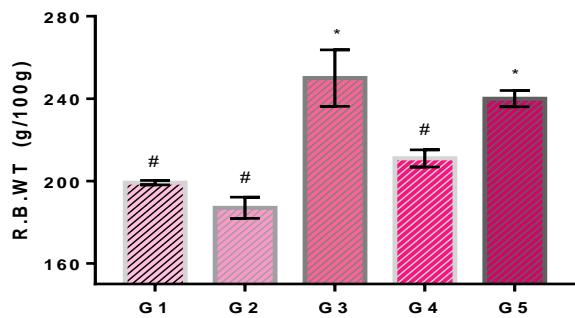


Figure 1: Changes in relative body weight levels in different groups. Values are expressed as means \pm SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

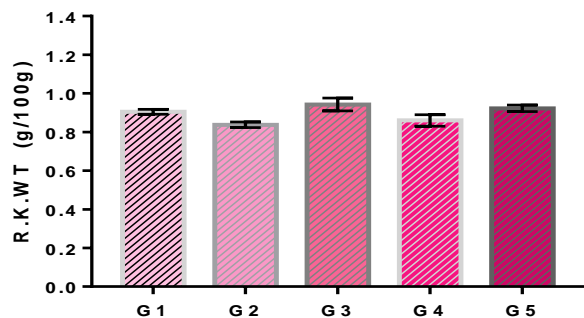


Figure 2: Changes in relative kidney weight levels in different groups. Values are expressed as means \pm SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

Orlistat induce renal toxicity:

Figures (3&4) showed a significant increase in serum creatinin and urea levels in treated rats with Orlistat when compared with control, also a significant increase in serum creatinin and urea levels in obese rats when compared with control. On the other hand; treatment of obese rats with Orlistat revealed in significant changes in creatinin and urea levels when compared with obese rats group. Self-treated obese rats revealed insignificant decrease in creatinin and urea levels when compared with obese

or treated obese rats with Orlistat groups (Figures 3&4).

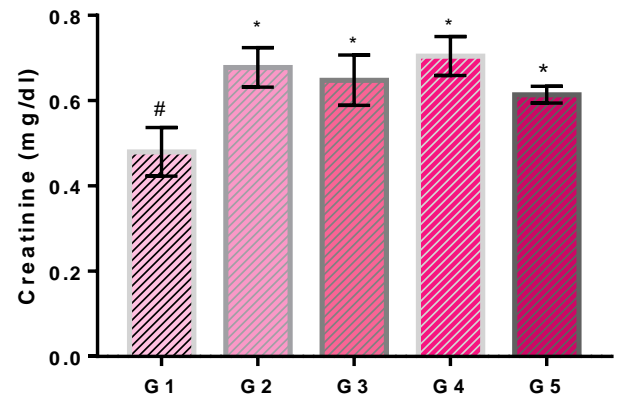


Figure 3: Changes in serum creatinin levels in different groups. Values are expressed as means \pm SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

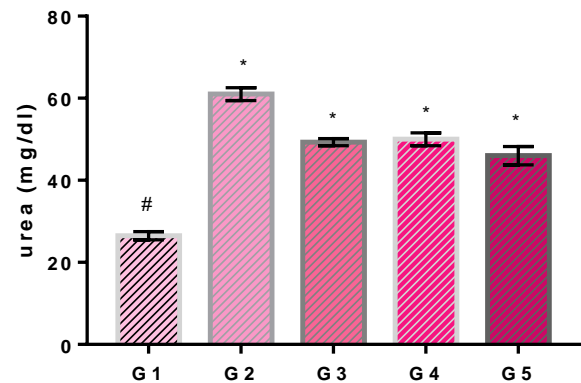


Figure 4: Changes in serum urea levels in different groups. Values are expressed as means \pm SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

Changes in electrolytes:

Figures (5-8) showed the changes in electrolytes in different groups. a significant decrease in serum sodium, potassium, calcium and chloride ions levels in treated rats with Orlistat when compared with control, in contrast a significant increase in serum sodium, potassium and chloride ions levels in obese rats when compared with control. On the other hand; calcium ions levels revealed in significant changes

between obese and control rats (Figures 5-8). A significant decrease in serum sodium, potassium, calcium and chloride ions in treated of obese rats with Orlistat or in self-treated obese rats when compared with obese rats group (Figures 5-8).

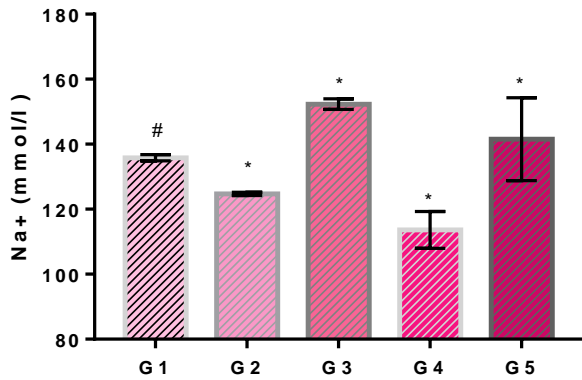


Figure 5: Changes in serum sodium ions (Na⁺) levels in different groups. Values are expressed as means ± SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. #Significantly different from obesity, *Significantly different from control.

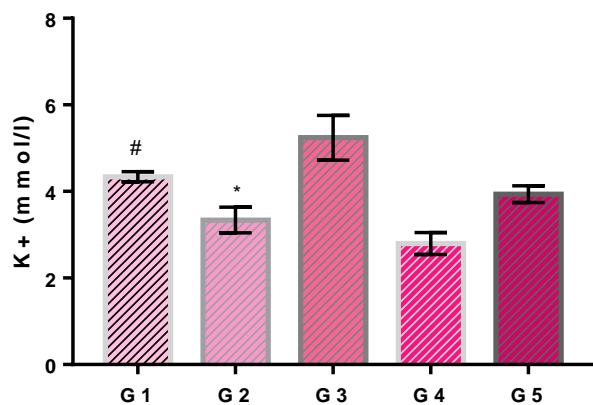


Figure 6: Changes in serum potassium ions (K⁺) levels in different groups. Values are expressed as means ± SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

4. DISCUSSION

Obesity is a global health concern associated with high morbidity and mortality. Obesity also increases the risk of diabetes, cardiovascular disorder, stroke and colon cancer. The combination of these chronic diseases is called metabolic syndrome (Grundy

2004). However, renal risks induced by obesity, especially its role in initiation and progression of renal diseases, have only been recently recognized (Abrass 2004, Tang *et al.* 2012). Orlistat is a gastrointestinal lipase inhibitor used as an adjunct treatment of obesity. The current study was carried out to elucidate the modulation effect of orlistat against obesity induced kidney toxicity, injury and P53 immunohistochemical alterations in female rats.

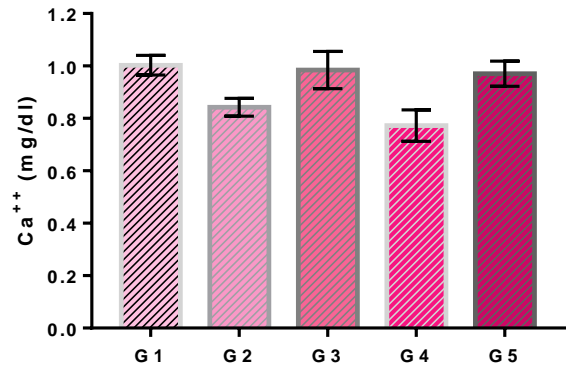


Figure 7: Changes in serum calcium ions (Ca⁺⁺) levels in different groups. Values are expressed as means ± SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

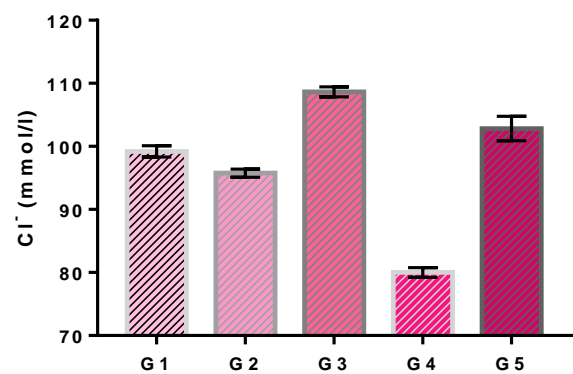


Figure 8: Changes in serum chloride ions (Cl⁻) levels in different groups. Values are expressed as means ± SE; n = 10 for each treatment group. G1, Control; G2, Orlistat; G3, obesity; G4, post-treated obesity with Orlistat; G5, Self-treated obesity. # Significantly different from obesity, *Significantly different from control.

In the current study; a significant increase in serum creatinine and urea levels in obese rats when compared with control. Also significant increases in serum creatinine and urea levels in treated rats with Orlistat when compared with control rats indicating Orlistat induce renal toxicity.

On the other hand; treatment of obese rats with Orlistat revealed in significant changes in creatinine and urea levels when compared with obese rats group. Self-treated obese rats revealed insignificant decrease in creatinin and urea levels when compared with obese or treated obese rats with Orlistat groups. Courtney *et al.* (2007); Singh *et al.* (2007) and Weir *et al.* (2011) reported an association between orlistat and acute kidney injury. In 2007, Singh and colleagues provided the initial suggestion of orlistat-induced AKI [Singh *et al.* 2007]. In a patient with underlying stage III chronic kidney disease (CKD; among other comorbidities), initiation of orlistat treatment coincided with an increase in serum creatinine concentration, an increase in urine oxalate concentration and the presence of calcium oxalate crystals in the lumen of the renal tubules. These findings resolved 1 month after discontinuation of orlistat.

Courtney and colleagues reported similar findings in a 55-year old woman with diabetic nephropathy (Courtney *et al.* 2007). After 5 months of orlistat therapy, the patient's estimated glomerular filtration rate (eGFR) declined from a baseline of 66 ml/min/1.73 m² to 12 ml/min/1.73 m² with a documented accumulation of calcium oxalate crystals in the renal parenchyma. In 2007, McLaughlin and Macdougall reported a case series of 33 obese patients with CKD (stages III or IV) taking orlistat for more than 6 months (MacLaughlin and Macdougall, 2007).

Weir *et al.* (2011) reported that; orlistat induced acute kidney injury in humans. In obese people, increased serum creatinine was observed, suggesting that obesity caused elevation in renal function test and produced proteinuria, concomitantly with other risk factors such as hypertension, diabetes and dyslipidemia (Matsushita *et al.*, 2009). Renal triglyceride accumulated in obese rats accompanied by hypoalbuminemia and elevated blood urea nitrogen.

In the current study; a significant decrease in serum sodium, potassium, calcium and chloride ions levels in treated rats with Orlistat when compared with control, in contrast a significant increase in serum sodium, potassium and chloride ions levels in obese rats when compared with control. On the other hand; a significant decrease in serum sodium, potassium, calcium and chloride ions in treated of obese rats

with Orlistat or in self-treated obese rats when compared with obese rats group. Weisinger *et al.* (1974) reported that massive obese patients developed nephrotic range proteinuria. Obesity that is related to glomerulopathy is considered an increasing cause of end-stage renal disease. Potential mechanisms by which obesity affects renal physiology include: altered renal hemodynamics, insulin resistance, hyperlipidemia, activation of reninangiotensin aldosterone system, inflammation and oxidative stress. Also, increases in both glomerular filtration rate and renal plasma flow are observed in both obese subjects and animals (Chagnac *et al.*, 2000).

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