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Orlistat Induce Renal Toxicity, DNA Damage, and Apoptosis in Normal and Obese Female Rats

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Abstract: Obesity has been linked to several chronic diseases, including fatty liver disease, fatty heart disease, diabetes mellitus, hypertension, obstructive sleep apnea, and cancer. The purpose of the current study was to determine how Orlistat affected renal toxicity, DNA damage, and apoptosis that were brought on by obesity in normal and obese female rats. There were four groups made up of a total of 20 female rats (G1, Control; G2, Orlistat; G3, Obesity; G4, treated obesity with Orlistat rat group). The results of the present investigation showed that orlistat treatment resulted in kidney injury, DNA damage, and P53 mutations as well as a large increase in serum urea and creatinine levels as well as a significant drop in sodium, potassium, calcium, and chloride ions levels. Therefore, when administered to treat normal or obese rats, orlistat caused kidney damage; as a result, doctors should carefully monitor it.

Keywords: Obesity, Orlistat, Kidney, Toxicity, DNA Damage, Rat

Introduction

A person is deemed obese if their Body Mass Index (BMI) is greater than 30 because they have too much body fat, which is often accompanied by an increase in weight (Huang *et al*., 2015; Heindel *et al*., 2017). With significant increases in morbidity, premature mortality decreased quality of life, and an increased risk of complications like insulin resistance, hypertension, dyslipidemia, diabetes mellitus, coronary heart disease, atherosclerosis, and some cancers, obesity has become a significant global health issue (Wang *et al*., 2013; Campbell *et al*., 2016).

Anti-obesity drugs, sometimes known as weight loss meds, are pharmacological compounds that reduce or control weight by influencing either appetite or calorie absorption. Some anti-obesity drugs may cause negative, even deadly, adverse effects, and these side effects are frequently related to the drug's mechanism of action (Huang *et al*., 2015; Defo *et al*., 2017). In 1998, the Food and Drug Administration (FDA) gave orlistat its approval for weight loss.

Furthermore, the first gastrointestinal lipase inhibitor used to treat obesity is orlistat (Heal *et al*., 2012).

Orlistat is a gastrointestinal lipase inhibitor. Within three to five days of oral administration, orlistat is nearly completely removed from the feces, absorbs very little systemically, and exhibits little accumulation (Henness and Perry, 2006; Wang *et al*., 2013; Lee *et al*., 2021). Orlistat inhibited intestinal lipase, reducing the absorption of dietary fat (Joyce *et al*., 2020). In an endeavor to have a graceful form and obvious muscles, many people use medications to enhance their muscles, but doing so has several unfavorable side effects (Tousson *et al*., 2012; 2016; Alm-Eldeen and Tousson, 2012; Elmasry *et al*., 2018). In the current investigation, normal and obese female rats were used to better understand how Orlistat affected renal toxicity, DNA damage, and apoptosis caused by obesity.

Furthermore, the use of various medications, such as orlistat, in the quest for fitness and a thinner body resulted in a variety of adverse effects and health issues.

Materials and Methods

Animals

The study employed 20 mature female albino rats, all of whom were albinos. Each was 120-130 g in weight. Rats were housed in individual, well-ventilated cages with unrestricted access to regular food and water whenever they need it. Rats were kept in a controlled setting with a constant temperature of 25°C and a cycle of 12 h of light and 12 h of darkness. Rats were given two weeks to adjust before the experiments started.

Rats used in this study were bought from the central animal house of Tanta university's faculty of science. The institutional ethics committee approved all animal procedures and all procedures were carried out per the principles and recommendations for the care and use of experimental animals in the NIH protocol.

Drugs and Chemicals

Orlistat was purchased as a capsule manufactured by Global Napi Pharmaceuticals (GNP), Penta Pharma, Egypt, under the brand name regimax.

Experimental Design

The animals were divided into equal groups of four (5 rats each). Over the course of 10-12 weeks, rats were employed in the following experiments.

Rats were included in Group 1's Control group, which received no treatment; Group 2: For four weeks, rats in the orlistat group received daily intraperitoneal injections of the medication (12 mg/kg) diluted in saline (1 mL/kg) (Amin *et al*., 2015). Group 3: The rats in this group of obese rats were fed a high-fat diet for six weeks before receiving an intraperitoneal injection of saline solution (1 mL/kg). For four weeks, Group 4 provided orlistat to the obese rats.

After one night of starvation at the end of the experiment, rats were individually given blood samples for clinical chemistry from their eyes using retro-orbital punctures and blood capillary tubes without heparin while being lightly sedated with ether. Blood samples were collected, and left to clot for 10 min at room temperature and then the serum was extracted by centrifuging at 3000 rpm for 10 min. Following collection, the serum was kept at-80°C for analysis.

Kidney Functions and Electrolytes in Serum

Utilizing the methods described in (El-Moghazy *et al*., 2014; Mutar *et al*., 2020), urea and creatinine were determined by SERA (2020); El-Masry *et al*. (2020) used commercial kits to calculate the blood electrolyte concentrations (potassium, sodium, calcium and chloride ions) (Sensa core electrolyte, India).

Comet Assay

DNA damage in the renal tissues from the various research groups was analyzed and quantified using the comet test (single-cell gel electrophoresis) technique. This strategy was adapted from previously released materials (Elgharabawy *et al*., 2021; Abd Eldaim *et al*., 2019a). The comet's tail lengths were measured from the middle of the nucleus to the end with a 40x increase to count and estimate the size of the object. GelRed-stained DNA is seen under a fluorescence microscope with a 40x objective to demonstrate DNA damage. To determine the quantitative and qualitative extent of DNA damage in the cells, (El-Masry *et al*., 2019) report that Kinetic Imaging, Ltd.'s (Liverpool, UK) Komet 5 image analysis software was used in conjunction with a CCD camera to measure the length of DNA migration and the percentage of migrated DNA. The program calculates the tail moment last. Each sample's 50-100 randomly selected cells are typically analyzed.

Histopathological Examination

According to Tousson (2016), kidney tissue samples were stained with Hematoxylin And Eosin (H&E) for histological inspection after being fixed in a 10% formalin solution for 48 h.

Immunohistochemical Detection of P53 Immunoreactivities

According to (Tousson *et al*., 2014; 2020), the expression of apoptotic P53 immunoreactivities in the kidney sections was discovered utilizing the Avidin-Biotin Complex (ABC) technique.

Statistical Analysis

Means and SEM were used to represent the data. One-way ANOVA was used to compare the data between the orlistat group and other groups. The p-value was considered significant at 0.05. Analysis was done utilizing (Graphpad prism, Graphpad Software, Inc, La Jolla, CA, USA).

Results

Changes in Kidney Functions and Electrolytes

When compared to controls, Table 1 showed that; levels of urea, creatinine, sodium, potassium, and chloride in sera were considerably higher in obese (G3) individuals (G1). Additionally, when compared to control (G1) or obese rats, treatment of normal with orlistat (G2) demonstrated a substantial increase in levels of urea and creatinine and a significant decrease in sodium, potassium, calcium, and chloride ions (G3). Contrarily, when compared to control (G1) or obese rats, treatment with orlistat (G4) led to significantly lower levels of sodium, potassium, calcium, and chloride ions.

DNA Damage in Kidney

When compared to the normal control group, the obese group (G3, kidney)'s tissues showed a substantial increase in DNA damage ($P<0.05$), as shown by an increase in tail length, tail DNA%, and tail moment (G1). However, whether normal or obese rats were treated with orlistat (G2 and G4), DNA damage was shown to be increased relative to normal control (G1) or obese rat (G3) groups (Table 2, Fig. 1). On the other hand, the normal control (G1) group showed no discernible variation in DNA damage (tail-length).

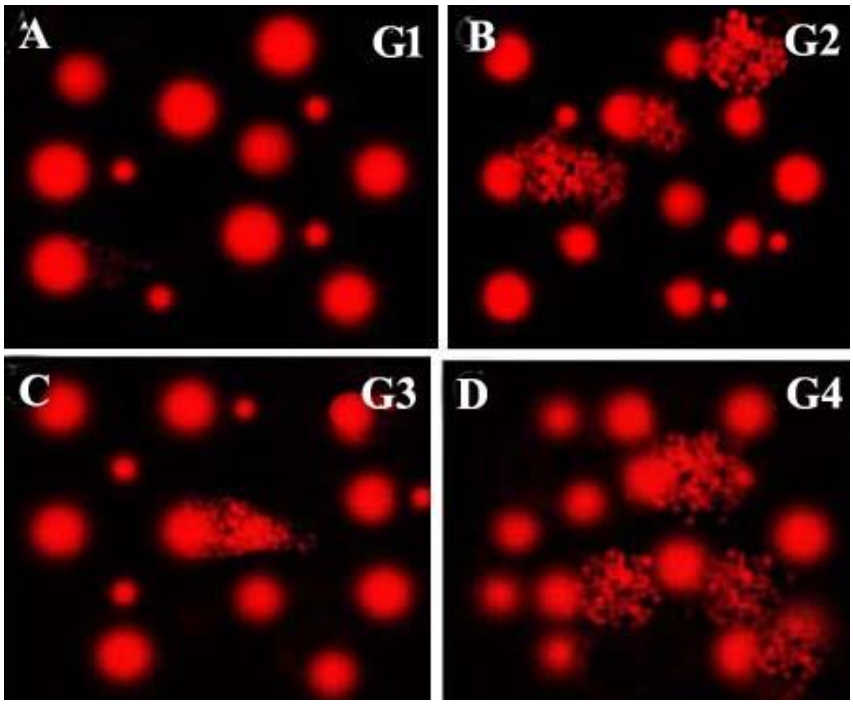

Fig. 1: DNA damage in kidney tissues in the control group by comet assay. Where G1, normal control; G2, Orlistat; G3, obesity; G4, treated obese rats with Orlistat

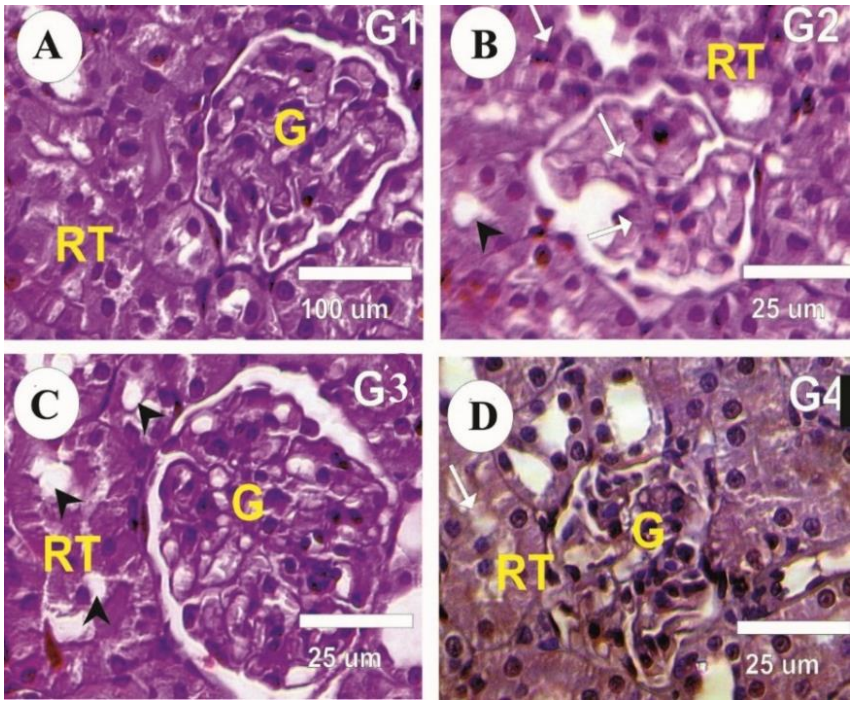

Fig. 2: Photomicrographs of rat kidney sections in the different groups under study stained with Haematoxylin and Eosin. A: Rat kidney sections in the control (G1) group revealed a normal structure of the renal cortex which comprised renal corpuscles (G), and proximal and distal convoluted tubules. B: Kidney sections in treated rats with orlistat (G2) revealed severe changes in malpighian corpuscles lost their characteristic configuration, moderate cell infiltration (arrows), marked atrophied and vacuolated (arrowheads), moderate disrupted convoluted tubules with mildly dilated blood vessels. C: Kidney sections in the obese rat group (G3) revealed mild atrophy in renal tubules (arrowheads) with a few inflammatory cells. D: Kidney sections in obese rats treated with orlistat revealed moderate atrophied glomeruli, moderate cell infiltration (arrows), and moderate disrupted convoluted tubules

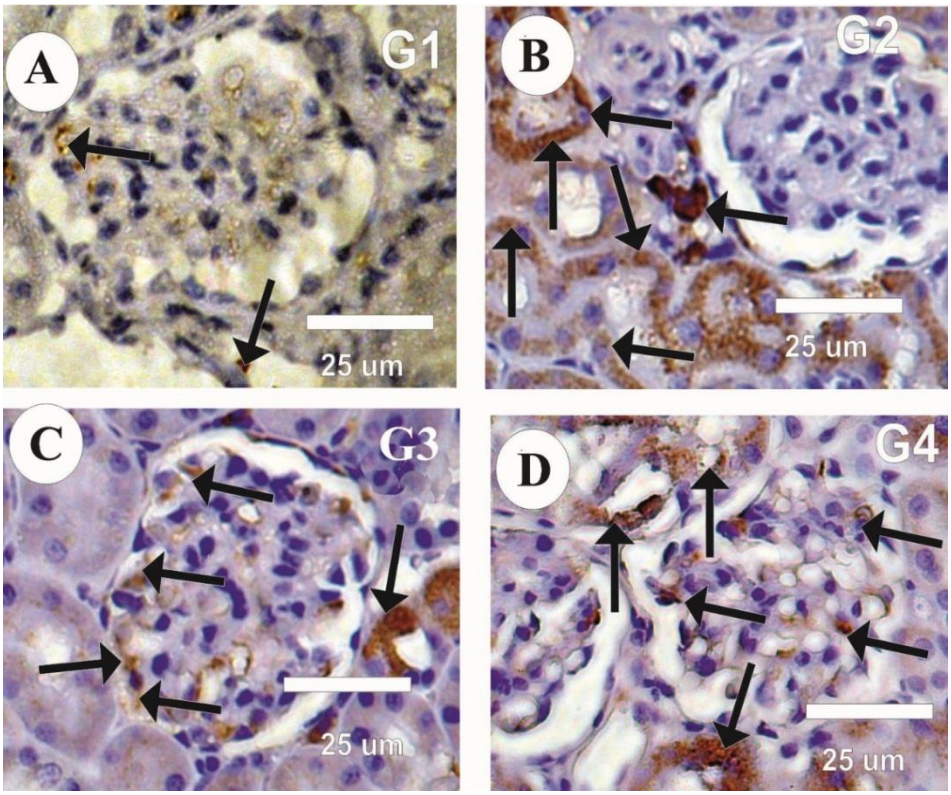

Fig. 3: Photomicrographs of rat kidney sections in the different experimental groups stained with P53-ir. A: Faint positive reaction for P53-ir (arrows) in renal tubules in control. B: Moderate positive reactions for P53-ir (arrows) in kidney sections in treated rats with orlistat. C: Mild to moderate positive reactions for P53-ir (arrows) in kidney sections in obese rats. D: Mild to moderate positive reactions for P53-ir (arrows) in kidney sections in treated obese rats with orlistat

Table1: Changes in kidney functions and electrolytes levels in different groups under study

Groups	Creatinine (mg/dl)	Urea (mg/dl)	K++ (mmol/l)	Na (mmol/l)	Ca++ (mg/dl)	Cl- (mmol/l)
G1	0.5800#±0.07	26.50#±0.36	4.3400#±0.05	135.800#±0.42	1.00300#±0.02	99.200#±0.410
G2	0.8300$±0.04	61.20$±0.74	3.3400$±0.13	124.700$±0.23	0.89300$±0.09	95.700$ ±0.323
G3	0.7660*±0.02	51.00*±0.71	5.2400*±0.23	152.300*±0.72	0.98400*±0.03	108.600*±0.350
G4	0.730*$±0.04	50.5*$±0.59	2.79#*$±0.11	88.4#*$±0.76	0.773#*$±0.03	80.2#*$±0.350

Data are expressed as mean ± S.E.M of 5 observations. Where G1, Control; G2, Orlistat; G3, Obesity; G4, treated obese rats with Orlistat. Significantly different ($P < 0.05$)

Table 2: Comet assay parameters obtained by image analysis in cells of all groups after the treatment experiment

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
G1	2.0	98.0	$1.47±0.12^{d}$	1.52	2.230
G2	15.0	85.0	$4.91±0.31^{c}$	4.35	21.360
G3	4.5	95.5	$1.99±0.28^{d}$	2.05	3.190
G4	25.3	74.7	$7.54±0.39^{b}$	6.93	52.080

Different superscript letters in the same column of tail length showed a significant difference at $P<0.05$. Where G1, normal control; G2, Orlistat; G3, obesity; G4, treated obese rats with Orlistat

Histological Examination of the Kidney in Different Groups

The glomerulus and renal tubules are part of the normal histological structure of the renal corpuscle, according to a histological investigation of the kidney tissues from the control (G1) group (Fig. 2A). However, kidney sections from the orlistat-treated group (G2) of rats showed severe alterations, including malpighian corpuscles that had lost their characteristic configuration, moderate cell infiltration, marked atrophied and mild vacuolated tissue, moderately disrupted convoluted tubules and mildly dilated blood vessels (Fig. 2B).

The obese rat group (G3 kidney)'s sections showed mild to moderate nephron degeneration, mononuclear cell infiltration, and congested renal blood vessels. The renal tubules, on the other hand, appeared to be more or less normal-looking, with only mild atrophy and a small number of inflammatory cells (Fig. 2C). Conversely, orlistat-treated obese rats' kidney sections showed mildly atrophied and lobulated glomeruli, moderately disturbed convoluted tubules and mildly atrophied glomeruli (Fig. 2D).

Kidney P53 Immunoreactivity

The figure shows the detection and distribution of P53 immunoreactivity in kidney slices from the various research groups (3A-3D). P53 faintly positive responses in renal tubules are visible in the kidney portion of the control group (Fig. 3A). On the other hand, orlistat-treated rats' kidney sections showed somewhat positive P53 responses (Fig. 3B). On the other hand, kidney slices from obese rats showed modest to moderately positive P53 responses (Fig. 3C). When orlistat was administered to obese rats, mild to moderately positive responses to P53 was seen in the kidney sections (Fig. 3D).

Discussion

Obesity, a global health issue, is associated with high rates of morbidity and mortality. Obesity also increases the risk of diabetes, heart disease, stroke, and colon cancer (Grundy, 2004). Obesity, in particular its role in the emergence of renal diseases, poses risks to the kidneys that have only lately come to light (Abrass, 2004; Tang *et al*., 2012). Obesity prevalence has considerably increased over the past 20 years in both industrialized and non-industrialized countries and is expected to continue to rise. More than 250 million persons globally are estimated to be obese, while 500 million adults are estimated to be overweight (Gasbarrini and Piscaglia, 2005).

The bulk of currently available anti-obesity drugs either prevent the absorption of dietary fat or reduce food intake by acting on noradrenergic or serotonergic receptors (e.g., Orlistat) Another strategy for managing obesity is the use of orlistat, a gastric lipase inhibitor. The present study was designed to elucidate the effects of orlistat on kidney injury, damage, and changes in P53 immunohistochemistry induced by obesity in female rats.

In the current investigation, obese rats' serum levels of urea and creatinine were considerably greater than those of control rats. Additionally, orlistat-treated rats exhibited considerably greater serum levels of urea and creatinine when compared to control rats, demonstrating that the drug damages the kidneys. However, orlistat therapy significantly changed the levels of urea and creatinine when compared to the group of obese rats. Orlistat may result in acute renal injury, according to (Courtney *et al*., 2007; Singh *et al*., 2007; Weir *et al*., 2011).

The concept of orlistat-induced AKI was developed by Singh and colleagues (Singh *et al*., 2007). In a patient with underlying stage III Chronic Kidney Disease (CKD), among other comorbidities, the onset of orlistat therapy was accompanied by an increase in serum creatinine concentration, an increase in urine oxalate concentration, and the presence of calcium oxalate crystals in the renal tubule lumen. These results vanished a month after the use of orlistat was stopped. Weir *et al*. (2011) found that orlistat inflicted acute kidney impairment in humans. Obesity was associated with other risk factors such as hypertension, diabetes, and dyslipidemia to raise the renal function test and cause proteinuria, as seen by the increased blood creatinine found in these people (Matsushita *et al*., 2009).

The regulation of the two univalent cations of fundamental physiological relevance, potassium, and sodium, throughout the body is one of the kidney's key roles (Alm-Eldeen and Tousson, 2012; Hasan *et al*., 2022). The metabolism of calcium is a subject that the kidney takes very seriously (Salama *et al*., 2013). In the current investigation, orlistat-treated rats had significantly lower blood levels of sodium, potassium, calcium, and chloride ions when compared to control rats; in contrast, obese rats had significantly higher serum levels of sodium, potassium, and chloride ions when compared to control rats. As opposed to the obese rat group, obese rats treated with orlistat or self-treated with orlistat had a substantial decrease in blood sodium, potassium, calcium, and chloride ions. Although the pathogenic mechanism is not fully understood. López-Martínez *et al*. (2020) postulated that obesity has a high prevalence and is linked to kidney illness. In a white lady with underlying chronic renal illness, Orlistat caused Acute Kidney damage (OIAKI) related to acute oxalate nephropathy, according to (Humayun *et al*., 2016; Karamadoukis *et al*., 2009).

López-Martínez *et al*. (2020) assumed that; the prevalence of obesity is high and it is associated with kidney disease, although the pathogenic mechanism is incompletely understood. Humayun *et al*. (2016; Karamadoukis *et al*., 2009) who find that; Orlistat induced acute kidney injury secondary to acute oxalate nephropathy in a white woman with underlying chronic kidney diseases.

Apoptosis, or" programmed cell death " plays a critical role in how mammalian cells behave in a variety of pathological circumstances (Tousson *et al*., 2020; El-Aarag *et al*., 2021; Abd Eldaim *et al*., 2019b; 2021). Many external signals are involved in controlling apoptosis; p53 is one of the mechanisms that has been the subject of the most research (Tousson *et al*., 2018; Aldubayan *et al*., 2019). The incidence of apoptotic cells and the expression of

cytoplasmic p53 were both very low in the control kidney sections. In contrast to the control and obese groups, rats treated with orlistat showed a considerable increase in the expression of cytoplasmic p53 in the hepatocytes of the renal tubules in the kidney. According to recent findings, orlistat treatments for normal or obese rats significantly boost P53 expression.

Conclusion

While reversible gastric and pancreatic lipase inhibitor orlistat may help people lose weight, it also causes DNA damage, renal toxicity, tissue damage, and electrolyte disturbances. People who desire to use Orlistat to lose weight should therefore use caution.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability

Data supporting our study results are accessible from the relevant author whenever needed.

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Author's Contributions

Shahenda Anter Fatoh and Ahmed Flayyih Hasan: Wrote the first draft of the manuscript.

Haneen Mushtaq Hameed and Zainab Haytham Razooki: Performed the statistical analysis and approved the final manuscript.

Ehab Tousson: Designed the study, and wrote the protocol, read and approved the final manuscript.

All authors managed the analyses of the study and managed the literature searches.

Ethics

The institutional ethics committee approved all animal procedures and all procedures were carried out per the principles and recommendations for the care and use of experimental animals in the NIH protocol.

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