

Original Article

ORLISTAT AND GARLIC CURTAILED ANTIOXIDANT ENZYMES' ACTIVITIES AND AMELIORATED HIGH FAT DIET INDUCED OBESE SPRAGUE-DAWLEY RATS

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ABSTRACT

Background: The global prevalence of obesity is on the increase, hence the development of a safer and more effective anti-obesity therapeutic approach is becoming more of an imperative. **Objective:** The study is to investigate whether Orlistat and Garlic can alter antioxidant property and lipid profile of high fat diet induced- obese Sprague-Dawley rats. **Methods:** Sixty-four albino Sprague-Dawley rats ($170.62 \pm 5.74\text{g}$), were divided into two equal groups. Group A rats were fed with commercial chow diet, while group B rats were fed with chow and fat emulsion to induce obesity in rats for 30days. The rats in group B were divided into 4 groups of 8 rats each) and treated with Orlistat drug (5mg/kg body weight), Garlic extract (5ml) and a combination of both for 5 weeks. **Results:** Treatment with Orlistat and Garlic extracts and a combination of both significantly ($p < 0.01$) reduced the lipid profile of the high fat induced obese rat. However, administration with Orlistat and Garlic in rats significantly ($p < 0.05$) ameliorated hepatic and renal functions as observed in high-fat diet treated groups. Serum ALT, AST activities, LDL, triglycerides and total cholesterol levels were significantly ($P < 0.05$) higher in the high fat group compared with control. **Conclusion:** Data of the study indicate that oral administration of Orlistat and / or Garlic extracts curtailed anti-oxidant enzymes' activities and ameliorated high-fat diet induced obese Sprague-Dawley rats.

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1. Introduction

Obesity is a chronic disorder that has reached a pandemic proportion worldwide (Flier, 2004; Haslam and James, 2005). Due to the swift increase in its prevalence and the severe health consequences, obesity is commonly considered one of the most serious health challenges of the early 21st century (WHO, 2005). In 2000, an estimated 15–20% of the populations in established market economies were clinically obese ($\text{BMI} > 30\text{kg}/\text{m}^2$). These rates have steadily risen over time, increasing the risk of comorbidities associated with obesity (Seidell, 2000). Notably, the metabolic syndrome, which is associated with obesity, has been found in nearly a quarter of the population of the United States (Ford et al., 2008). These trends appear to be related to the typical Western diet (Van Dam et al., 2002) and decreased physical activity (McTigue et al., 2002).

'Orlistat' is a drug that acts by preventing the absorption of fats from the human diet, thereby reducing caloric intake (Torgerson et al., 2004). It is known that orlistat is the saturated derivative of lipstatin, a potent natural inhibitor of pancreatic

lipases isolated from the bacterium, *Streptomyces toxytricini*. However, due to simplicity and stability, orlistat rather than lipstatin was developed into an anti-obesity drug (Haslam and James 2005).

Garlic (*Allium sativum* Lin.) contains a variety of active compounds, such as selenium and germanium that exhibit sulphur antioxidant property, as well as vitamins A, C and E which help scavenge harmful free radicals and also eliminate low density lipoprotein from blood, thereby increasing high density lipoprotein in the blood (Miller, 2010). Presently, it is considered to be useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type-2, hypertension, cataract and disturbances of gastrointestinal tract (Gardner, 2007).

Many authors have published articles on orlistat and several others have reported the use garlic in obesity treatment. However, there is still no published article that has checked the combined effect of orlistat and garlic in the treatment of Obese rats. The current study compare the singular and combinative effect of orlistat and garlic in the management of obesity.

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The objective of the study is to study the use of high-fat diets to induce obesity and also determine the subsequent combined effect of orlistat and garlic treatment on antioxidant enzymes' activities and lipid profile in adult obese Sprague-Dawley rats.

MATERIALS AND METHODS

Reagents, Diagnostic Kits, Orlistat and Garlic

Commercially available reagents and enzyme kits for alanine amino transferase (ALT), aspartate amino transferase (AST), triacyl glycerol, cholesterol, total protein and HDL were purchased from Randox ® (Randox Laboratories, N.Ireland). 'Orlistat' (Xenical) was purchased from a reputable Community Pharmacy at Yaba, Lagos, Nigeria. The Orlistat rat dose was 5mg/kg according to Hala et al. (2011) was used for the study. Garlic was purchased from Mushin market, Lagos, Nigeria.

Animal Grouping

Sixty-four albino Sprague-Dawley rats (170.62±5.74g) were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The rats were housed metallic cages under hygienic condition. They were fed on basal diet and acclimatize in a 12:12-h light-dark cycle, temperature and humidity controlled environment for 2 weeks. The guidelines for ethical conduct in the care and use of animal research were strictly adhered to in accordance with the APA (2010).

The rats were divided into two equal groups A and B. Group A rats were fed with commercial chow diet while group B rats were fed with chow and Fat emulsion for 30 days to induce obesity, with the 65% of the energy of this diet derived from lipids. All the food items were weighed daily fed with water ad libitum. The body weight of obese rats reached 250.16 ± 7.45g after a period of first 4 weeks. After 30 days, 60 rats were divided into five equal groups as follows:

Group A: Positive Control (Control) fed with rat chow for 5 weeks.

Group B: Obese rats fed with rat chow and fat emulsion for 5 weeks (HFD).

Group C: Obese rats fed with rat chow and treated with 30mg/Kg body weight orlistat (HFD OR) for 5 weeks.

Group D: Obese rats fed with rat chow and treated with garlic for 5 weeks. (HFD GA).

Group E: Obese rats fed with rat chow and treated with 30mg/Kg body weight orlistat and garlic for 5 weeks. (HFD OR&GA).

Plant Extract Preparation

Aqueous garlic extract was prepared from locally available garlic bulbs purchased at Mushin market. The garlic bulbs were peeled on crushed ice. Then 50g of the peeled garlic was cut into small pieces and homogenized in 70 ml of cold and 0.9% NaCl, in the presence of some crushed ice. The homogenisation was carried out in a Waring blender at high speed using 30-sec bursts for a total of 10 min.

The homogenised mixture was filtered 3 times through cheesecloth and the filtrate was centrifuged at 2000 RCF for 10 min and the clear supernatant was diluted to 100ml with normal saline. The concentration of the garlic preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50g/100 ml). The aqueous extract of garlic was stored in small aliquots at 4 °C for further use.

Preparation of High Fat Emulsion

This was done according to the method of Wang et al., (2005) with a few modifications. A constant volume of 100 ml emulsion shall contain 20g lard (source of saturated fat), 5g cholesterol, 1g sodium glutamate, 5g sucrose, 20 ml Tween 80 and 30 ml propylene glycol was prepared by adding distilled water and storing at 40°C.

Biochemical Analysis

After treatment period, the rats were deprived of diets for 8 h. The rats were anaesthetized by over-exposure of CO₂ gas and exsanguinated through cardiac puncture. Blood samples were rapidly centrifuged at 3,000 g for 15 min at 4°C. The extracted serum was stored at -20°C before analysis. Hepatic and renal tissues were quickly removed, weighed, placed in ice-cold saline and stored at -20°C for further studies.

Preparation of Liver Homogenate

The liver was separated by sacrificing the rats of all groups (Group 1 to Group 5) after 30 min observation period. Liver tissues were homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer pH 7.4 followed by centrifugation (5000g 10 min at 4°C). The supernatant was used to prepare aliquots of homogenates, which were used to carry out superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) assays.

Serum alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel (1957). Serum superoxide dismutase and catalase activities were assayed according to Flohe and Gunzler (1984). Lipid peroxidation was determined with spectrophotometric measurement of the amount of malondialdehyde equivalents with thiobarbituric acid and was expressed as thiobarbituric acid reactive substances (TBARS: Malondialdehyde nmol / mg protein) (Ohkawa et al., 1979).

Lipid Profile

The Serum cholesterol, triacyl glycerol, HDL and LDL levels were determined using commercially available kits from Randox (RandoxInc, N/Ireland). The composition of reagents, principles, procedures and formulae used have been taken from the instruction manuals of the kits provided by the manufacturer. LDL-Cholesterol (LDL-C) was calculated from triacyl glycerol, total cholesterol, and HDL-Cholesterol (HDL-C) concentrations using the following Friedwald formula (Friedewald et al., 1972):

LDL-C = Total-C - HDL-C - (TG/2.2). Values shall be expressed as mg/dl.

Adiposity Indices

Body weights were measured and the Lee index was calculated by the cube root of body weight (g/naso-anal length/cm), for which a value equal to or lower than 0.300 was classified as normal at month 3 of life. For values higher than 0.300, the rats were classified as obese (Bernardis, 1970).

Statistical Analysis

The data were expressed as means ± S.E.M. All statistical analyses were performed using statistical package for social sciences (SPSS) 22.0 statistical software. Significant differences among the treatment means were determined using analysis of variance (ANOVA). Results were considered to be statistically significant at P values less than 0.01 (P<0.01) according to the posthoc ANOVA statistical analysis.

RESULTS

The results of the effect of Orlistat and Aqueous Garlic extracts on Serum Lipid profile of rats are presented in Fig.1. The serum cholesterol, triacyl glycerol and low density lipoprotein levels of rats fed high fat diet, high fat diet and orlistat or garlic were significantly higher than differences (p<0.01) than in control. However, the serum high density lipoprotein levels of rats fed these respective diets were significantly higher than in control (Fig. 1).

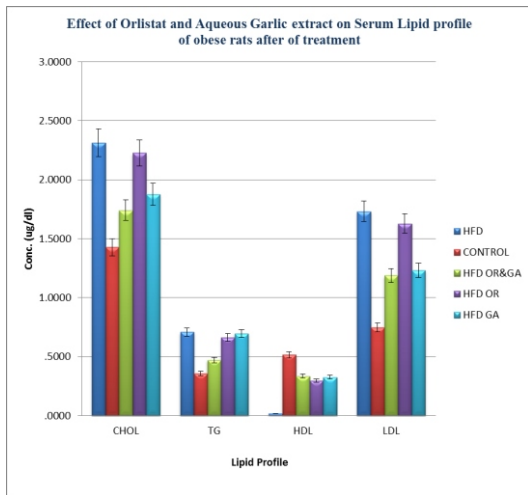


Fig. 1: Effect of orlistat and garlic extracts on serum lipid profile of rats

The results of the effect of orlistat and garlic extracts on serum AST and ALT activities of rats are presented in Fig.2. The serum cholesterol, triacyl glycerol and low density lipoprotein levels of rats fed high fat diet, high fat diet and orlistat or garlic were significantly higher than differences (p<0.05) than in control. However, the serum high density lipoprotein levels of rats fed these respective diets were significantly higher than in control (Fig. 1).

Low density lipoprotein levels of rats fed high fat diet, high fat diet and orlistat or garlic were significantly higher than differences (p<0.01) than in control. However, the serum high density lipoprotein levels of rats fed these respective diets were significantly higher than in control (Fig. 1).

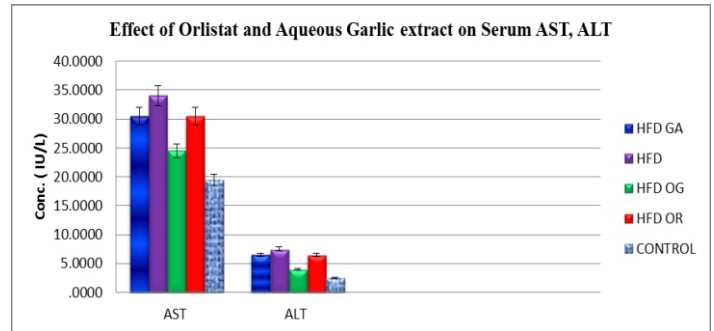


Fig. 2: Effect of orlistat and garlic extracts on serum alanine amino transferase and aspartate amino transferase in the rats . The results of the effect of orlistat and garlic extracts on the activities of the serum alanine amino transferase and aspartate amino transferase in the rats are presented in Fig.2. The activities of serum alanine amino transferase and aspartate amino transferase in the control rats were significantly lower than in rats administered the respective drugs and fed the respective diets. Administration of high fat diet produced significant (p<0.05) decrease in the activities of serum alanine amino transferase (ALT) and alkaline phosphatase (ALP) with respect to control. Treatment with orlistat and garlic extracts produced significant (p<0.05) reduction in the activities of serum ALT and ALP compared to the high fat diet group. The combination of orlistat and the garlic extracts led to a greater decline in the activities of serum enzymes than in other treated groups.

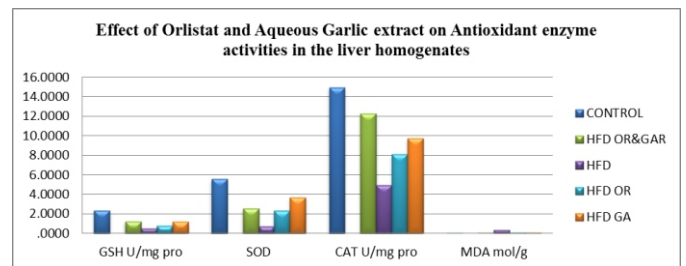


Fig. 3: Effect of orlistat and garlic extracts on activities of superoxide dismutase, catalase and levels of reduced glutathione and malondialdehyde in the rat liver homogenates

The results of the effects of orlistat and garlic extracts on activities of superoxide dismutase (SOD), catalase and levels of reduced glutathione and malondialdehyde (MDA) in the rat liver homogenates are shown in Fig.3. Treatment with orlistat and garlic extract led to the significant decline in MDA levels, while the combination of orlistat and garlic treated group showed the highest decline in levels. Superoxide dismutase (SOD) activity in

HFD group has shown significant decline with respect to the control. Treatment with orlistat and garlic extract led to the significant increase in SOD activity (Fig.3).

Results of the study showed that rat treated with orlistat showed no significant ($p>0.05$) difference in the serum MDA, GSH levels, SOD and catalase activities compared with control. But these treated rats showed a significant ($p<0.05$) increase in the serum MDA, GSH levels, SOD and catalase activities compared with control. There was no significant ($p>0.05$) increase in serum SOD and catalase activities in the rats treated with garlic, orlistat and combination of both.

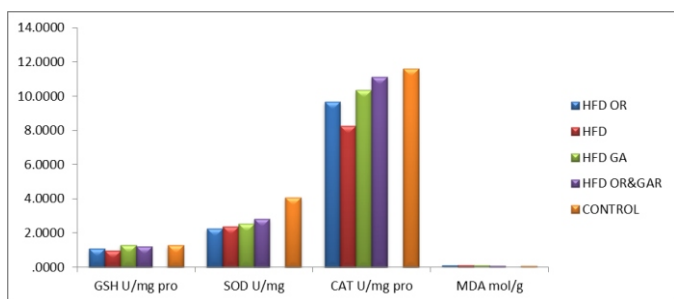


Fig. 4: Effect of orlistat and garlic extracts on antioxidant activities of the rat kidney homogenates

The results of effects of orlistat and garlic extracts on antioxidant activities in the rat kidney homogenates are shown in Fig. 4. There were no significant differences in the antioxidant activities of the rat kidney homogenates in the control and rats administered or fed the respective drugs and diets. However, The activities of SOD and CAT of kidney homogenates of the control were significantly higher than in rats administered or fed the respective drugs and diets (Fig.4).

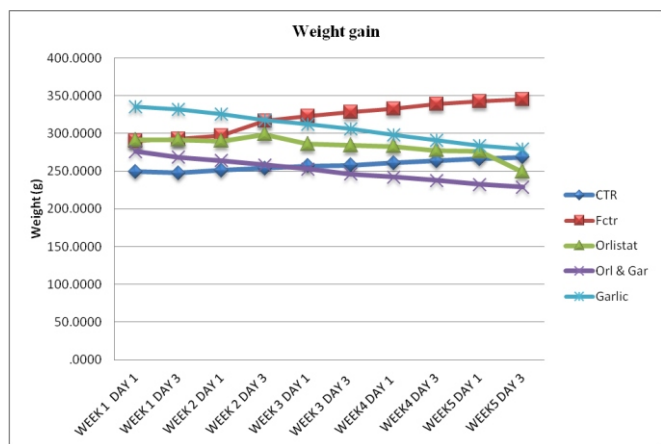


Fig. 5: Body weight gain of rats administered orlistat and garlic for 30days

The body weight gain of rats administered orlistat and garlic for 30days is presented in Fig. 5. There was significant reduction in the body weight of rats administered or fed the respective drugs and diets for 30 days as compared to control rats

DISCUSSION

The results from this study showed that regular administration of garlic extract (5ml) when combined with orlistat has hypolipidaemic effect on obese Sprague-Dawley rats. The rats administered or fed the respective drugs for 30 days showed a significant ($P<0.05$) decrease in the serum cholesterol, LDL and triacyl glycerol compared with high fat diet group. Orlistat works by inhibiting gastric and pancreatic lipases, the enzymes that break down triglycerides in the intestine. When lipase activity is blocked, triglycerides from the diet are not hydrolyzed into absorbable free fatty acids, and are excreted undigested instead. Only trace amounts of orlistat are absorbed systemically; the primary effect is local lipase inhibition within the gastrointestinal tract after an oral dose. The primary route of elimination is through the faeces (Zhi et al., 1995). A high level of cholesterol is one of the most common problems among overweight or obese people, and this can, over a period of time, cause several other complications, including coronary heart disease and heart attacks (Abd El-Ghany et al., 2004).

Studies show that the consumption of garlic regulates plasma lipid and antioxidant property (Lau, 2006). Some unique properties of garlic, such as antilipidaemic and antioxidant potentials have been studied (Lewin and Popov, 1994). Although large studies were done to elucidate their mechanism of action (Lau, 2001), no further studies have been conducted to elucidate possible effects of garlic extracts consumption on the relationship between some haematological and blood lipid parameters. Cells have different antioxidant systems, such as glutathione and various antioxidant enzymes to protect various tissues from free radicals attacks. Apart from reduced glutathione, the antioxidant enzymes including SOD, CAT and GSH dependent enzymes may minimize or remove the oxygen radical cascade in cells (Lau, 2006, Erukainure et al., 2014).

Malondialdehyde level in high fat diet group has shown significant rise with respect to control group MDA level in high fat diet group has shown significant rise with respect to the control group. Catalase activity has also shown a significant decrease in the high fat diet group. Treatment with the orlistat drug and the garlic extract led to a significant increase in catalase activity.

The weight loss in rats observed in the garlic treated group is in agreement with previous research studies (Mahesar et al., 2010), which suggested that garlic causes a significant ($p<0.05$) reduction in body weight. This reduction in body weight is probably due to the action of allicin, a potent compound in crushed garlic that increases the body's metabolic rate by stimulating the adrenal gland to release adrenaline which increases the rate of fat metabolism in the body and in turn helps burn more calories and decrease body weight (Djankpa et al., 2012; Noaki et al., 2007).

CONCLUSION

Data of this study showed that regular administration of garlic extract when combined with orlistat drug has hypolipidaemic effect on obese Sprague-Dawley rats. The garlic extract also showed significant in-vivo anti-oxidant activity. Therefore, the anti-obesity activity reported in the present study indicates that the antioxidant property of garlic may be responsible for this action.

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