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Comparative physiology study of side effect between Xenical and Lipo-6 Supplements which treated obese rabbets

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ABSTRACT

Obesity is rapidly becoming a health problem, and it is considered a metabolic disease of epidemic proportions. Obesity is also associated with many comorbidities such as hyperlipidemia, fatty liver, atherosclerosis, cancer. Use of over-the-counter weight-loss supplements has become more common in the world; Xenical and Lipo-6 are two examples of nutritional supplements that do not pass safety research before being sold to the consumer.

The ability of Xenical and lipo-6 supplements on DNA fragmentation in human lymphocytes was studied, where these cells represent important defense line of the body. On other hand, thirty white rabbits Iraqis in this experiment, weighing 2.5 to 3.0 kg were employed, which divided randomly to the three group (A: Control group, B: Xenical group, C: Lipo-6 group). In the end of experiment, all animals synthesized for collection blood to complete blood count examination and then all animal scarified for taking tissues samples to histopathological examination.

The results showed that, the effects of two different types of medications on DNA fragmentation in human lymphocytes were examined. Xenical, at high concentrations (500 µg/ml), caused a significant fragmentation of lymphocytes' DNA ($p \geq 0.05$); DNA fragmentation percentages were 68.5 percent after 24 hours and reached 76.4 percent after 48 hours. the lipo-6 at high concentration displayed apoptotic activity against healthy cells after 24 hours of exposure; DNA fragmentation was 80.13%. After 48 hours, the proportion of DNA fragmentation increased to 89.71 percent. As well as, the present study were conducting to determine toxicity of drug in rabbits in order to scientific information about its safety. The results of this study, reveals a histological sections for (liver, lung, heart, spleen, and intestine) in rabbits groups treated with Xenical and Lipo- 6 two capsule per day. pathological alterations in the treated rabbits' intestines, including macrophage and lymphocyte infiltration indicating chronic inflammation, furthermore, intestine section showed Macro vesicular fatty change. The spleen histological Samples founded with highly-blood foci (bleeding), highly infiltration with lymphocytes, also section exhibit highly proliferative cells with abnormal blood collections in both groups treated with Xenical and Lipo-6,in comparative to normal spleen. the lung tissue inflammation, the production of air vacuoles, damage to the alveolar sac and found a significant RBC cast in the heart muscle.

Introduction:

Obesity is rapidly becoming a health problem, and it is considered a metabolic disease of epidemic proportions. Whereas, the World Health Organization (WHO) has stated that obesity with type 2 diabetes is a major global health problem (1-2) and obesity a greater risk than malnutrition (3).

Obesity is also associated with many comorbidities such as hyperlipidemia, fatty liver, atherosclerosis, cancer (4-5). Which may soon become the most common cause of preventable cancer, bypassed cigarette smoking (6).

Obesity has been recognized by raise in the number and size of adipocytes in the adepose tissue (7). Use of over-the-counter weight-loss supplements has become more common in the world; Xenical and Lipo-6 are two examples of nutritional supplements that do not pass safety research before being sold to the consumer.

XENICAL (Orlistat) is a noval anti-obesity agent used in the treatment of obesity and its comorbidities, it has been prescribed to obese people and to those who do not meet their aims in lifestyle and nutritional interventions (8). It is widespread and commonly used due to its widespread availability, affordable price, and significant effect on weight loss compared to other drugs (9).

The lipase of (gastric and pancreas) is inhibited by Orlistat which doing that by inhibiting the absorption of dietary fats and prevent hydrolysis of triglyceride to the absorbed fatty acids and glycerol, at the end triglycerides are prevented from liver enters (10, 11). It also has a utilized role on body weight and glucose tolerance by amendment the composition of gut microbiota (12).

Several studies on obese rats showed the protective and therapeutic role of orlistat against metabolic dysfunction-associated fatty liver disease in rats through the activation of Nrf2 signaling pathway (13), Improvement in metabolic variables (13, 14, 15), oxidative stress (13, 16, 17). As well as a role in improving fertility in obese men (18).

On the other hand, LIPO-6 is a potent and wildly popular fat- burner that uses liquid capsules of maximum strength for superior absorption and quick results. Lipo6 is sympathomimetic substances it's depend on the process of lipolysis is started by activating beta adrenergic receptors in adipose tissue by raising the serum concentration of catecholamines (19). Where Synephrine and Yohimbine activate ß-3

adrenaline receptors, thus exhibiting lipolytic and thermogenetic capacity as it enhances norepinephrine levels.

A few studies on the toxicity and negative effects have been published. Therefore, in order to gather scientific data concerning the safety of dietary supplements, the present research were carried out to detection the toxicity of dietary supplements in rabbits.

Material and methods:

Xenical and lipo-6 preparation

preparation of samples

Xenical samples were obtained from Baghdad market, were prepared by dissolving one capsule (120mg) in 2m of D. W, the final concentration (12µg/ml). while (lipo-6 120) liquid capsule each one contain 275mg gradients were prepared by cut one side of it and squeeze its component in clean container. Serial concentrations of Xenical and lipo-6 (250, 500µg/ml) were prepared and used in this study.

complete blood count

EDTA blood samples were collected from each animal (from jugular vein), and transfer directly to hematological lab to studying the complete blood counts depending on procedures of (Harmening,2009) (20) all blood cells counts (RBCs, WBCs and platelets), also studying any morphological change of cells, as well as studying differential WBCs.

Note: on each EDTA sample tube we fixed all information's about animal, drug types and time of collections.

Cytogenetic study Solutions, buffers and dyes for the cultivation of blood lymphocytes:

Phosphate buffer saline (PBS) was created using the following (21).

The sodium bicarbonate solution was made in accordance with (22).

The fetal calf serum (FCS) was made in accordance with (23).

Antibiotic preparations: it was made in accordance with (24).

Colcimied suspension, employed in the cytogenetic investigation to halt cell division in the tropical phase (Metaphase), was made by dissolving (10) mg of colcimied in 10 ml of phosphate buffer saline while keeping the preparation immediately usable when used and away from light**.**

Hypotonic Solution**:** A 0.75 g of potassium chloride (KCl) was dissolved in 100 ml of distilled water to create the hypotonic solution, which was then autoclaved and kept at

4 °C until needed. It was prepared as RPMI-1640 (Roswall Park Memorial Institute) medium (25).

It was made as Maintenance Medium (Serum Free Medium SFM) (26).

For the purpose of detecting DNA fragmentation, solutions and buffers were made as (27).

TE buffer, PH 7.4: To make this solution, 10 mM of Tris HCL and 1 mM of EDTA were added to DW. After bringing the pH to 7.4, adding DW to make the solution a liter, sterilizing it in an autoclave, and storing it at 4°C until use.

TTE solution: TE buffer and Triton-X100 were combined in this solution at a 0.2 percent concentration ratio.

Solution of trichloroacetic acid (TCA) at 25%: It was made by combining 100 ml of sterile DW with 25 gram of TCA, and it was then kept at 4 °C until it was needed.

Solution of trichloroacetic acid (TCA) (5%): It was made by combining 5g of TCA with 100 ml of sterile DW, then cooling it to 4C until usage.

Diphenylamine reagent (DPA): Using a magnetic stirrer, 1.5 g of DPA was dissolved in 100 ml of glacial acetic acid to create this reagent. Following the addition of 1.5 ml of sulfuric acid, 20 ml of this mixture received 0.1 ml of acetaldehyde.

Collection of Blood samples

Each individual had 5 ml of peripheral blood aspirated, and the blood was then promptly transferred to sterile, heparinized vacutainer tubes for lymphocyte isolation. lymphocyte removal from blood samples as (28).

Identification of the impact of Xenical and lipo-6 on the viability of lymphocytes removed from healthy people as (29).

Method of Boyum (1968) (27) was required for the quantitative method of DPA reagents to detect Xenical and lipo-6-induced apoptosis on cells.

Experimental animal:

Thirty white rabbits Iraqis in this experiment, weighing 2.5 to 3.0 kg were employed. They were given access to unlimited amounts of water and stock diets of cabbage and carrot. Animals were randomly assigned to three groups, with group A and group B each consisting of ten animals. Groups A and B received daily oral doses of Xenical and lipo-6, whereas group C, the "negative control group," only received water and food.

Histopathological and histochemical studies

After intramuscular injections of xylazine (3 mg/kg of body weight) and ketamine hydrochloride (35 mg/kg), all animals were rendered unconscious and killed as a group. Each glass jar containing 10% formaline that contains the entire collection of animal organs is labeled with a number and special symbols for each sample. After that, the histopathology lab received the samples for analysis. structural changes in the tissues following drug treatment, with all tissue slices stained with hematoxylin and eosin.

The results and discussion

Cytotoxic research. In the current study, the effects of two different types of medications on DNA fragmentation in human lymphocytes were examined. Xenical, at high concentrations (500 µg/ml), caused a significant fragmentation of lymphocytes' DNA ($p \geq 0.05$); DNA fragmentation percentages were 68.5 percent after 24 hours and reached 76.4 percent after 48 hours (table 1).

Current results showed that, the lipo-6 at high concentration displayed apoptotic activity against healthy cells after 24 hours of exposure; DNA fragmentation was 80.13%. After 48 hours, the proportion of DNA fragmentation increased to 89.71 percent (table 2). When compared to untreated cells, there were significant differences ($p \geq 0.05$), and the current results demonstrated low percentages of DNA fragmentation (21.14 percent and 21.78 percent, respectively) after various exposure times (24 and 48). (table 1;2).

Table 1: DNA fragmentation percentage of Xenical

Value of LSD	Concentration			Time (hrs)
	250	500	0	
7.883 *	57.75± 2.27	68.5± 1.81	21.14 ± 1.45	24
7.025 *	67.54 ± 3.51	76.4± 2.42	21.78 ± 1.62	48
----	5.217*	6.983*	NS	LSD

Table 2: DNA fragmentation percentage of lipo-6

Value	concentration	Time(hrs)

of LSD	250	500	0	
7.883 *	61.82±2.89	80.13 ± 2.58	21.14 ± 1.45	24
7.025 *	71.44±3.456	89.71± 2.52	21.78 ± 1.62	48
----	5.217*	6.983*	NS	LSD

It is clear from the results above that Xenical and Lipo-6 2 have a harmful effect that varies with time. Additionally, the data demonstrated a substantial difference between treated and untreated cells' percentages of DNA fragmentation at various incubation times.

The results of this study, reveals a histological sections for (liver, lung, heart, spleen, and intestine) in rabbits groups treated with Xenical and Lipo- 6 two capsule per day employing (H&E, X200). Figure [1,2] displayed pathological alterations in the treated rabbits' intestines, including macrophage and lymphocyte infiltration indicating chronic inflammation, furthermore, intestine section showed Macro vesicular fatty change., this results agreed with the results of complete blood picture, where neutrophils number reach to36, while lymphocytes number reach to 62, for rabbits treated with Xenical, while cells reach to (51), (44) for rabbits treated with Lipo-6.

According to a study by Greaves (2011) (30), micro vesicular fatty change is more likely a sign of toxicity while macro vesicular fatty change is related with metabolic abnormalities and is typically easily reversible. These findings are consistent with (31) which demonstrated that orlistat increased cell proliferation. Hyper-proliferation and aberrant crypt foci are premalignant lesions that increase the risk of colon cancer progression.

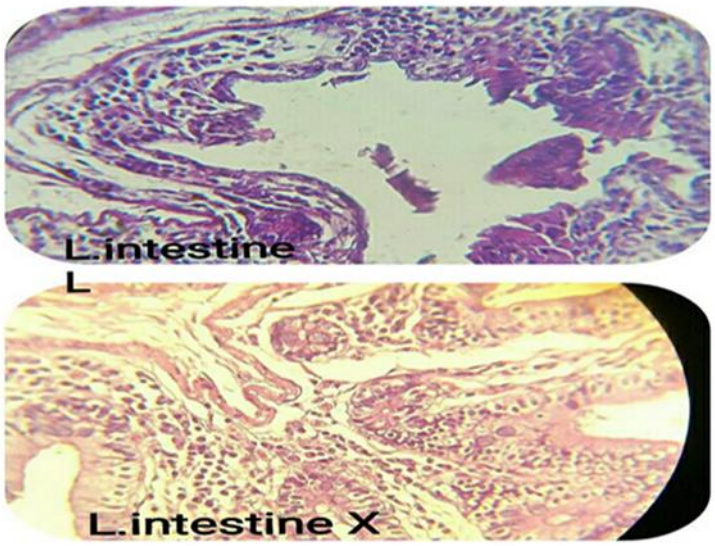

Figure [1] section in large intestine (L intestine) of rabbits treated with Xenical (X) and Lipo-6(L)

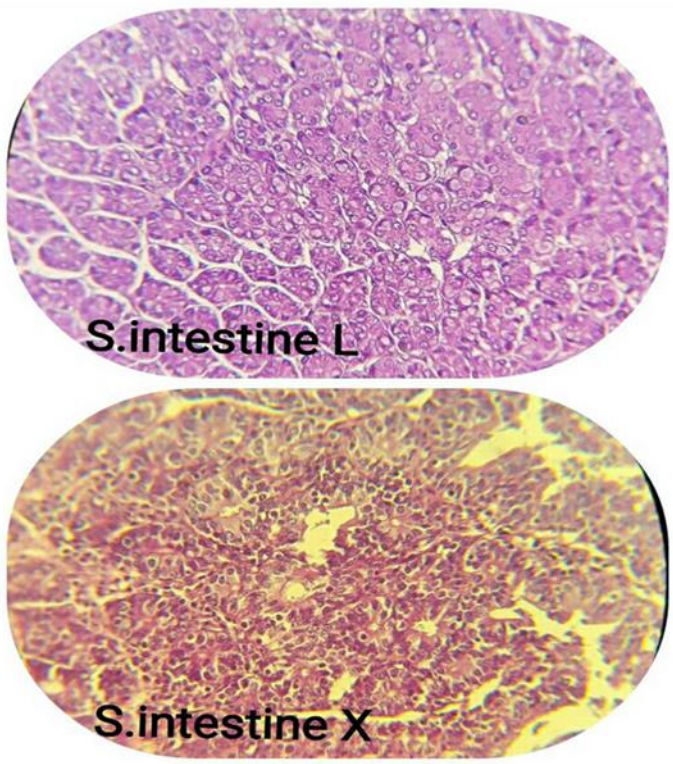

Figure [2] section in small intestine (S intestine) of rabbits treated with Xenical (X) and Lipo-6(L)

Hematoxylin and eosin histopathological examination of the paraffin jelly-rolled spleen was done. Samples founded with highly-blood foci (bleeding), highly

infiltration with lymphocytes, also section exhibit highly proliferative cells with abnormal blood collections in both groups treated with Xenical and Lipo-6,in comparative to normal spleen (figure 3), It was plausible to assume that the increase in the concentration of these stimulants open the possibility of negative effects on blood pressure (32) which may result in bleeding because of how the Central Nervous System (CNS) reacts to caffeine and other stimulant substances. According to Seifert et al. (33), taking the dietary supplement Acceleron® for 24 hours at dosages of 52 mg of p-synephrine and 704 mg of caffeine had no negative effects on blood pressure (BP) or heart rate (HR).

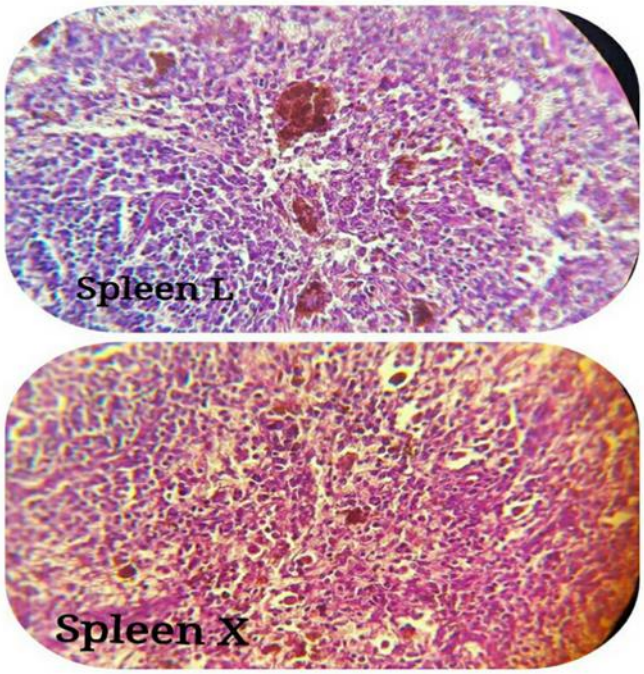

Figure [3] section of spleen in rabbits treated with Xenical (X) and Lipo-6(L)

Figure [4] displays the locations of Xenical and Lipo-6 in the heart and lung tissues. Following oral medication delivery, the rabbit group displayed lung tissue inflammation, the production of air vacuoles, and damage to the alveolar sac. Recent heart surgery found a significant RBC cast in the heart muscle (figure 5). These

findings were consistent with Thomas et al. (34).'s case report of a myocardial infarction in a guy using Nutrex Lipo-6 Black® for three weeks continuously without any known risk factors. While there was proofed that a one dose of sympathomimetic drugs can increase BP, HR, and sympathetic nerve activity in the body (35, 34, 36).

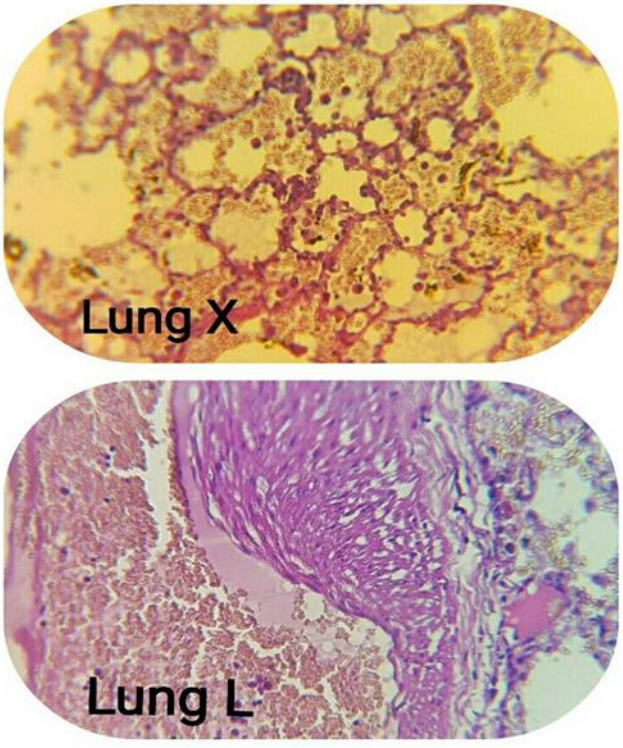

Figure [4] section of lung in rabbits treated with Xenical (X) and Lipo-6(L)

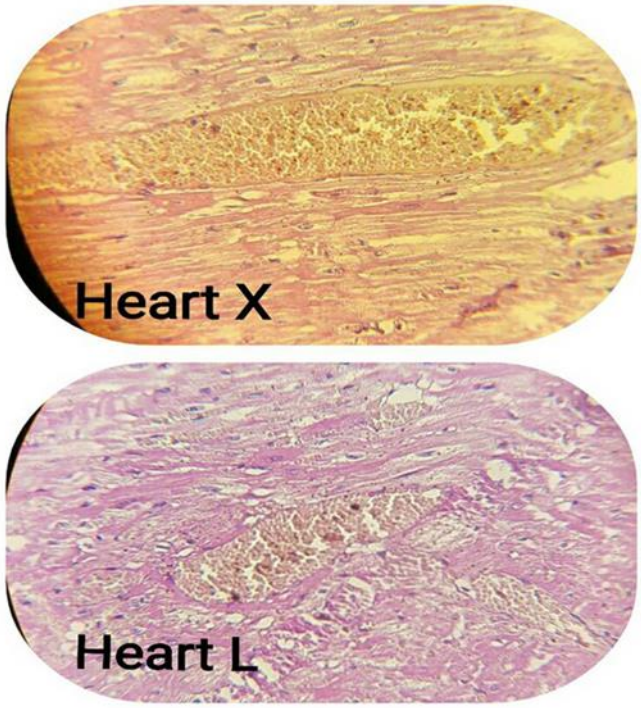

Figure [5] section of heart in rabbits treated with Xenical (X) and Lipo-6(L)

Xenical and Lipo-6 showed formation of giant tumor cells where cells contains two to three nucleus as well as, infiltration of macrophages and lymphocytes infiltration referring to chronic inflammation, , this results agreed with the results of complete blood picture, where neutrophils number reach to36 , while lymphocytes number reach to 62, for rabbits treated with Xenical, while cells reach to 51,44 for rabbits treated with Lipo-6, current results came in harmony with Roche Laboratories Inc (2009) (37) they reported severe liver injury with hepatocellular necrosis or acute hepatic failure in patients treated with weight loss drugs .

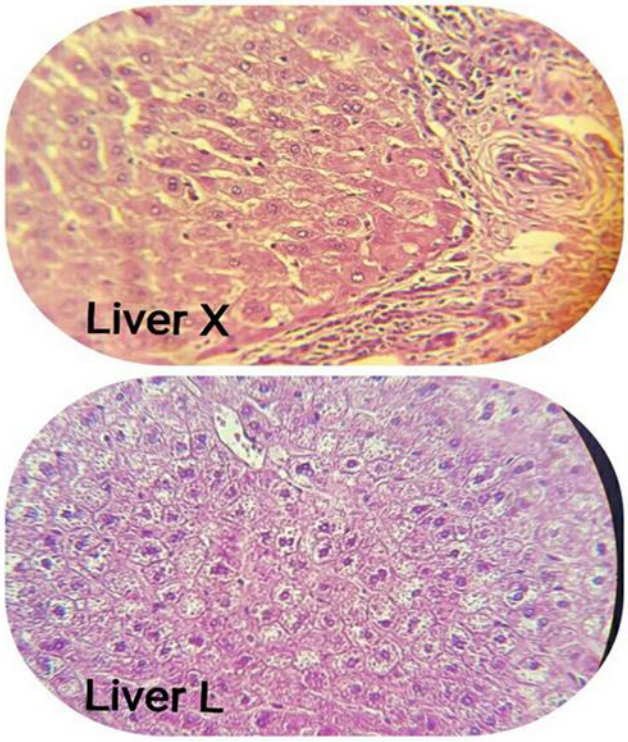

Figure (3-6) section of liver in rabbits treated with Xenical(X) and Lipo-6

According to certain research, orlistat may raise the risk of colon cancer. Orlistat produced indirect genotoxic effects at different concentrations, as demonstrated by Garcia et al. (38). In rats given orlistat, the number of aberrant crypt foci (ACF) dramatically increased, and orlistat also promoted cell proliferation. Hyper-proliferation and aberrant crypt foci are premalignant lesions that increase the risk of colon cancer progression. Orlistat increased the amount of undigested fat in the colon and created free radicals that induced colonic epithelial cell growth.

Lipid per-oxidation results from the production of reactive oxygen species (ROS) in feces. These oxidizing substances deteriorate lumen membranes and speed up cell death. Leila and others, 2015 (31).

Additionally, the peroxidation of lipids results in the formation of cytotoxins. There are some worries that using orlistat may lead to a higher risk of colon cancer. There is a link between Orlistat use and an increase in colonic preneoplastic indicators, according to recent preliminary studies on rats (38).

However, according to another study on colorectal cancer, orlistat dramatically reduced cell growth and elevated levels of caspase-3 and death in vitro when compared to the control group. Orlistat's antitumor activity was dosage dependent. Additionally, orlistat reduced cell growth by 50% at 25 mM and by 100% at 50 mM. A study conducted in vivo also revealed that orlistat prevented cell

growth. However, unless at extremely high doses, such as 200 mM of orlistat, activation of FASN remained unchanged (39).

Conversely, Orlistat might have negative effects that affect the digestive system directly or indirectly. Because of its poor systemic absorption and rapid first-pass metabolism, orlistat has a lower bioavailability than other anti-obesity drugs (less than 1%; McNeely and Benfield 1998) (40).

As a result, its direct systemic side effects are less frequent. The side effects of orlistat that are most frequently mentioned are steatorrhea, bloating, oily spotting, fecal urgency, and fecal incontinence. Between 16 and 40 percent of people appear to be having at least one of these side effects (41). Fecal incontinence affects about 7% of people and is arguably the most upsetting psychosocial side effect. The fat-soluble vitamins A, D, E, and K may not be as well absorbed when using orlistat, according to some research (42). According to an RCT looking at the effects of orlistat on vitamin D absorption, bone turnover, and bone density, orlistat causes a relative increase in bone turnover as well as a net resorption of bone, probably as a result of vitamin D and calcium malabsorption (43).

The authors contend that weight loss alone can account for these effects. Except for vitamin D, all fat-soluble vitamins were reduced according to the XENDOS study. Numerous people who use orlistat for an extended period of time may require multivitamin supplements that contain fat-soluble vitamins (44). It should be recommended to patients to take them at least two hours before or after taking orlistat (45)

Additionally, it is believed that taking a lipase inhibitor with a fatty diet may enhance the presentation of free fatty acids to the lower gastrointestinal tract, which will increase oxalate absorption and raise the risk of kidney stones and renal impairment (46).

It was logical to assume that a high concentration of these stimulants open the possibility of negative effects on blood pressure because Nutrex Lipo-6 Black® is wealthy in caffeine and other stimulants of the Central Nervous System (CNS). In fact, researchers discovered that caffeine, whether consumed orally or administered intravenously, caused a hypertensive response, at least during the initial hour after exercise recovery (47, 48).

However, when the commercial supplement Ripped Fuel, which contains 304 mg of caffeine and 21 mg of synephrine, was administered, the subjects' diastolic BP

increased significantly but not their systolic BP (32).

These findings align with the current research. Acute ingestion of the commercial supplement Metabolife356®, which contains 12 mg of efredra alkaloids, 40 mg of caffeine, and 16 other substances, elevated systolic blood pressure, according to McBride et al. (49) Prior research mainly looked at HR, BP, and electrocardiographic behavior when assessing the cardiovascular effects of thermogenic substances. The differential presented by study (32) also looked into the cardiac autonomic nervous activity. Given that alterations in autonomic nerve activity occurred before an increase in blood pressure, this variable is significant in clinical practice (49). It represents one of the most often embroiled processes in hypertension.

Previous research does in fact demonstrate harmful cardiovascular effects from ephedrine consumption over time (35) Ephedrine-containing products were outlawed by the FDA in 2004 (50), which led to a rise in the commercial use of chemicals like psynephrine and octopamine. However, as Stephensen et al. (51) and Bui et al. (36) showed alterations in blood pressure and heart rate with usage of these drugs, the negative effects of these ephedrine substitutes do not seem to be discounted. In contrast, Seifert et al. (33) discovered that using the supplement Acceleron® at dosages of (52 mg and 704 mg) of (p-synephrine and caffeine) respectively, over the course of day had no negative effects on BP and HR.

The sympathomimetic ingredients used in the other dark side of thermogenic supplements are taken from Asian native plants of the genus Ephedra, which are natural suppliers of alkaloids, primarily ephedrine (52). However, supplements containing ephedra and ephedrine alkaloids were linked to serious neurological side effects such cardiovascular and cerebrovascular accident, seizures, myocardial infarction, and sudden death (35, 53). Ephedrine present in dietary supplements was linked to 16,000 adverse events, according to Shekelleet al. (54).

For this reason, new formulations with sympathomimetic-like effects rather than ephedrine have entered the market. Nutrex Lipo-6 Black is a commercial product that has been heavily marketed and is free of ephedrine. It entails switching ephedrine for synephrine while maintaining the synergistic effects of caffeine, yohimbine, and diiodine in its formula.

As Nutrex Lipo-6 Black® incorporates sympathomimetic compounds into his recipe, ephedrine-related side effects may also manifest in this supplement.

Nutrex Lipo-6 Black® effect have already been clarified by Thomas et al. (34) in a case of myocardial infarction in a guy who was using Nutrex Lipo-6 Black® continuously for three weeks despite having no known risk factors. Nevertheless, very few studies have attempted to clarify these effects to date. While studies show that only caffeine is able to completely eliminate post-exercise hypotension (a drop in BP following exercise in a very short time), there is proof that a one dosage of sympathomimetic substances can enhance cardiovascular effects in sympathetic nerve activity, H R, and BP (35,36). With research showing that the only substance that can reverse post-exercise hypotension, which is a drop in BP following exercise in a very short time, is caffeine (55). Regardless of the results of infarctions or strokes, consumers of Nutrex Lipo-6 Black® may already be experiencing negative effects.

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