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Abstract

This research aimed to examine the beneficial effects of bitter orange extract on rat suffering from fatty liver. Thirty-six male albino rat with a weight of 160 ± 5 g were randomly allocated into six groups (6 each) . The 1st group has been fed on the basal food (-ve control). while 30 rats were fed on high-fat diet and fructose to trigger fatty livers and divided into a positive control group fed on basal diet and four treated groups with (4mg/kg, 8mg/kg, 10mg/kg, and 12mg/kg) bitter orange extract for six weeks. The outcomes illustrated that every fatty liver groups managed with bitter orange extract caused by a various diminution in FI, feed effectiveness ratio and BWG. The outcomes demonstrated a significant reduction in ALP, aspartate aminotransferase, alanine aminotransferase enzyme activity, blood urea, creatinine, and uric acid, total cholesterol, triglycerides, low-density lipoprotein and VLDL in the serum nevertheless demonstrated significant rises in values of serum high-density lipoprotein cholesterol and malondialdehyde whereas enhancing in serum glutathione for the managed groups as than the (+ve) control group. It can be recommended that the incorporating bitter orange extract into the diet due to their hypolipidemia and antioxidant properties, which offer a safer therapeutic approach against fatty liver and help mitigate its complications especially for those exposed to consume saturated fats and fructose for enhancing functions of kidney and liver.

Key words: High fat diet - Malondialdehyde – Liver function-Lipid profile – Citrus fruits - Rats.

تاثير مستخلص اللارنج على الفئران التى تعانى من الكبد الدهنى

الملخص العربى

هدفت هذه الدراسة إلى دراسة التأثيرات المفيدة لمستخلص اللارنج المر على الفئران بالكبد الدهني. تم استخدام ستة وثلاثين فأرًا أبيض ذكرًا يزن ١٦٠ ± ٥ جم . تم تغذية المجموعة الأولى (ن = ٦) على النظام الغذائي الأساسي (مجموعة ضابطه سالبه). وتم تغذية باقي الفئران علي غذاء عالى الدهون والفراكتوز للاصابة بالكبد الدهني ثم اعادة تقسمهم الي المجموعة الثانية كمجموعه ضابطه موجبه. تم تغذية المجموعات (٣-٦) على نظام غذائي عالي الدهون والفركتوز مضافًا إليه (٤ مجم / كجم و ٨ مجم / كجم و ١٠ مجم / كجم و ١٢ مجم / كجم) مستخلص اللارنج المر لمدة ٦ أسابيع. اوضحت النتائج أن جميع مجموعات الكبد الدهني المعالجة بمستخلص اللارنج المر ادت الى حدوث انخفاض ملحوظ في معدل زيادة وزن الجسم الغذاء المتناول ونسبة كفاءة التغذية. أظهرت النتائج انخفاضًا كبيرًا في نشاط إنزيمات AST و ALT و ALP والكرياتينين واليوريا في الدم وحمض البوليك للمجموعات المعالجة مقارنة بالمجموعه الضابطه السالبه. أشارت النتائج إلى أن مجموعات الفئران التي اتبعت نظامًا غذائيًا عالي الدهون وتناولت بمستويات مختلفة من مستخلص اللارنج المر أدت إلى انخفاضات ملحوظة في مستويات الدهون (TC,TG,LDL and VLDL) ولكنها أظهرت زيادات ملحوظة في مستوى HDL مقارنةً بالمجموعة الضابطة السالبه. وقد خفضت مجموعات مستخلص اللارنج المر مستوى المالونديالدهيد، بينما رفعت مستوى الجلوتاثيون في سيرم الدم. وتوصي الدراسة بضرورة دمج مستخلص البرتقال المر في النظام الغذائي لخصائصه المضادة للأكسدة وخفضة لنسبة الدهون في الدم، مما يوفر نهجًا علاجيًا أكثر أمانًا ضد امراض الكبد الدهني وتساعد في التخفيف من مضاعفاته خاصة لأولئك المعرضين لاستهلاك الدهون المشبعة والفركتوز

الكلمات المفتاحيه : الغذاء العالى الدهن – المالوندهيد– وظائف الكبد – مستويات الدهون– الفاكهة الحامضية – الفئران.

Introduction

(NAFLD) refers to Non-alcoholic fatty liver disease is marked by accumulation of triglycerides in hepatocytes, which results in cellular damage. This damage may give rise to non-alcoholic steatohepatitis (NASH), cirrhosis, and fibrosis as a repairing response, it has the potential to advance to hepatocellular carcinoma (HCC). **(Mohamed *et al*.,2022).** Non-alcoholic fatty liver disease, currently is deemed the most frequent liver illness universal, affects up to a quarter of the people. NAFLD, characterized by hepatic steatosis, is correlated with numerous adverse outcomes and high mortality. Furthermore, fatty aciduptake and de novo lipogenesis production outstrips fatty acid oxidation and export, leading to hepatic steatosis. Hepatic fatty acid uptake beside de novo lipogenesis is enhanced in NAFLD, whereas compensatory fatty acid oxidation is inadequate to normalize lipid levels and, by inducing oxidative stress, may contribute to cellular damage and disease progression, especially when the function of mitochondrial is impaired and increased peroxisomal and cytochromal oxidation **(Radwan and Mansour, 2023).**

Because to lifestyle factors and risk factors like metabolic syndrome (MS), type 2 diabetes, hypertriglyceridemia, and obesity, NAFLD has a significant incidence among the general population **(Perumpail *et al*.,2017).** Almost 25% of people worldwide have NAFLD **(Younossi *et al*.,2016).** Type 2 diabetes, dyslipidemia, insulin resistance (IR) and central obesity are the key risk factors for NAFLD, just as they are for the other elements of the metabolic syndrome. NAFLD is regarded as the metabolic syndrome hepatic component **(Younossi *et al*.,2011).** Hepatic inflammation histologically is one of the most significant risk factors. Other variables that have been linked to disease progression or extensive fibrosis include: older age, diabetes mellitus, raised serum aminotransferases, body mass index (BMI) ≥28 kg/m215, ballooning degeneration and Mallory hyaline on biopsy **(Argo *et al*.,2009).**

About half of all cases of acute inpatient liver failure are caused by drug-induced liver damage. Specifically, cancer patients frequently experience hepatotoxicity from some cancer chemotherapy medications. Since the majority of chemotherapy medicines are lipophilic, up to 85% of cancer patients may experience hepatic steatosis. This steatosis makes hepatocytes more susceptible, which could ultimately result in irreparable hepatocellular damage (**Joshi *et al*., 2014).** According to **Maor and Malnick (2013),** the formation of reactive oxygen species, which is thought to cause tumor cells to undergo apoptosis, may be the primary mechanism for liver damage brought on by chemotherapy.

Citrus fruits constitute one of the most highly regarded categories of fruits due to their significant nutritional and pharmacological value. They also rank among the most extensively cultivated crops worldwide **(Vargas-Canales *et al*., 2020)**. The botanical name for citrus is *Citrus L*., a genus within the family rutaceae, which comprises 28 recognized species. This genus encompasses a diverse range of cultivated natural hybrids, including oranges, lemons, grapefruits, limes, mandarins, and citrons. They are widely cultivated in areas classified as tropical or subtropical, as well as in many other non-tropical areas, which together produce more than 100 million tons annually worldwide **(Liang *et al*., 2024; Liu *et al*., 2024).**

The leaves, fruit, flowers as well as other parts of *C. aurantium* act as rich sources of bioactive substances, involving as essential oils, alkaloids, tannins, coumarins, terpenoids, flavonoids, in addition to vital minerals and vitamins **(Mansour,2019;Abdallah *et al*., 2024).** These metabolites demonstrate a significant range of health-enhancing characteristics, like antioxidant, antibacterial, anti-inflammatory, anxiolytic, antidiabetic, in addition anticancer impacts. **(Sevindik *et al*., 2021; Nagarajan *et al*., 2023 & Ellouze *et al*., 2024).**

Citrus fruits involve a various of secondry metabolites, like coumarins, phenolic acid, alkaloids, flavonoids, limonoids, carotenoids, in addition to crucial oils, among others. These

substances illustrate several biological properties advantageous to human health, involving anti-inflammatory, antioxidant, cardiovascular, anticancer, in addition to neuroprotective impacts **(Fernández-Cabal *et al*.,2025).** Accordingly, this study aimed to assess the possible effects of bitter orange extract in mitigating fatty liver disease induced by high-fat diet and fructose male rats.

Materials and Methods

Materials:

1-Bitter orange:

Bitter orange (***Citrus aurantium)*** was purchased from the Agricultural Research Center, Giza, Egypt.

2-Rats:

Adults male albino rat (36) of Sprague- Dawely strain with a weight of (160 ± 5g) have been purchased the Laboratory Food Technology Research Institute, Agricultural Research Center Ministry of Agriculture, Egypt.

3-Chemicals and kits for biochemical analysis:

Vitamins, minerals, cellulose, high fat diet, fructose, in addition casein have been attained from Al-Gomhoria Company, Egypt. Kits were bought from Gama Trade Company, Dokki, Egypt.

Methods:

plant extract:

Fresh better orange *(Citrus aurantium*) has been rinsed, with water and cut into slices, then extracted seeds & dried utilizing a hybrid convective drying system from the Solar Energy Department at the National Research Center in Dokki, Egypt, at temperatures of 30 to 40 degrees Celsius. After soaking dried citrus aurantium in 10 ml of 70% ethanol, it was sonicated for 1 hour at 37°C. Following 0.5 hour centrifugation at 3000 rpm, the suspension was filtered. A suitable amount of ethanol was added to a volumetric measuring flask to get the filtrate's volume down to 25 ml according to **(Verep *et al*., 2023).**

Biological Experiment:

A high-fat emulsion (HF), that involved of milk powder 80 g, saccharose 150 g, sodium deoxycholate 10 g, corn oil 400 g,

cholesterol 100 g, Tween-80 36.4 g, propylene glycol 31.1 g, cooking salt ten g, mineral combination 1.5 g, vitamin combination 2.5 g, in addition to distilled water 300 ml, has been organized as defined by (**Zou *et al*., 2006 ; Rasoul *et al*., 2020).** The high-fat group has been given high-fat emulsion (10 ml/kg) by gavage every day. In preparation for the development of the HF model, 36 rat were prepared for adaption by being kept in hygienic conditions and fed a basal meal for a period of one week **(Reeves *et al*., 1993)**. Subsequently this week, rat has been randomly separated into 6 groups. The first group (n=6) has been nourished on the basal diet as negative control group (-ve). The second group (+ve) has been fed on a high-fat food and fructose to stimulate fatty liver. Groups 3-6 have been fed on a high-fat food and fructose supplemented with 4mg/kg, 8mg/kg, 10mg/kg, & 12mg/kg bitter orange extract, correspondingly.

Blood sample gathering:

At finish of the nourishing duration (6 weeks), the animals have been subjected to overnight fasting and lightly anesthetized with ether. Blood has been drawn into dry, clean, plastic tubes for centrifugation and samples were taken from eyes. Blood samples have been centrifuged to gain serum, which were subsequently stored at 20 degrees Celsius in clean, well-stopped vials till analysis.

Nutrition and growth variables:

In accordance with **Chapman *et al*., (1959) ,**percentage of body weight gain (BWG%), food intake (FI), feed efficient ratio (FER) have been detected.

Biochemical analysis:

serum aspartate aminotranseferase (AST) and Alanine aminotransferase (ALT), alkalinephosphatase (ALP), creatinine (Cr) , urea and uric acid were measured depending on the methods of (**Zaia *et al*., 2000 , Bergmeyer *et al*., 1985 , Henry, 1974,Fossati *et al*., 1980 and Schultz, 1984),** respectively. Serun high-density lipoprotein (HDLc), Triglyceride (TG) and Total cholesterol (TC), has been conducted in accordance with **(Lopes-Virella *et al*., (1977); Trinder and Ann (1969) & Allain *et al*., (1974)**

correspondingly. serum low density lipoprotein LDLc & very low density lipoprotein VLDLc were calculated as defined by **Friadwald *et al*., (1972)** equations .

LDL-c (mg/ dl) = TC-(HDL-c+ VLDL-c).

VLDL-c (mg / dl) = (Triglycerides /5)

Serum malondialdehyde (MDA) and glutathione have been assessed in accordance with **(Draper and Hadly, 1990; Hamad *et al*., 2020).**

Statistical analysis:

The SPSS application was used to examine the data. To determine whether there were statistically noteworthy variations among the groups, an ANOVA was performed (**SPSS, 1986).**

Results and Discussion

Table (1): Impact of bitter orange extract on body weight gain, feed intake and feed efficient ratio of rats with fatty liver and control

Variables / Groups	BWG (%)	FI (g /day/rat)	Feed efficient ratio
Control(-Ve) group	١٣٨.٤٥ ± 1.٧٤ b	٩.٤٥ ± 0.٧٦ a	0.523 ± 0.٥٧ c
Control (+Ve) group	14٣.٢٥ ± 1.٧٤ a	٩.٩١ ± 0.٥٢ a	0.515 ± 0.٦٣ a
4 mg/kg bitter orange extract	١٣٩.٨٥ ± 1.٥٦ b	٩.٦٨ ± 0.٥٧ a	٠.٥١١ ± 0.٢٨ a
8mg/kg bitter orange extract	١٣٧.٧٥ ± 1.٦٥ b	٨.٧٤ ± 0.٨٤ b	0.5٠9 ± 0.٨٢a
10 mg/kg bitter orange extract	13٣.٦٢ ± 1.٢٤ c	٧.٦٢ ± 0.٦٥ c	0.626 ± 0.٩١ b
12 mg/kg bitter orange extract	١٢٨.٧٤ ± 2.٦٥c	٧.٦٣ ± 0.٣٨ c	0.602 ± 0.٨٧ b

*Values at the same column with different letters are significantly different at P<0.05.

*Values are expressed as means ±SE.

Table (1) illustrated that there was a significant (P<0.05)rise in body weight percent for the control group (+ve) when comparison with the negative control group (143.25 ± 1.74 VS 138.45 ± 1.74%), respectively. A significant reduce in the percentage of BWG has been additionally discovered in the groups rich diet in fats and fructose that were fed different levels of bitter orange extract when than the (+ve) control group. Significantly increased in FI has been observed in the (+ve) control group in compression with groups of rats nourished on bitter orange extract. It was also noted that there were not significant changes between the healthy and fatty liver groups in the level of FI. Table (1) showed also, increase in FER in (+ve) control group when in comparison with the negative control group.

Therefore, it was found that the palatability and taste of food had an impact on feed intake. The outcomes indicated that decreased feed intake and body weight were accompanied by a rise in the percentage of *C. aurantium* in rats with fatty liver. This result demonstrates the general enhancement of body functions caused by bitter orange extract (BOE) and suggests the capability of BOE to detoxify the harmful impacts of a high-fat diet. This is because BOE has a high concentration of polyphenolic components, which in turn is responsible for its powerful antioxidant activity, which in turn is responsible for its detoxifying properties. Moreover, **Suryawanshi ,(2011)** stated that bitter orange in relieves flatulence and in addition, its general aids in digestion that could aid in enhancing the absorption & therefore improves FER.

Our study was agreement with **Ullah *et al.*, (2014)** who showed that mixed administration of *C. aurantium at* a dose of 200 mg / kg per day for a duration of 21 days significantly reduced in body weight. Consequently, it can be clarified by (**Ramadan, 2017)** who stated that ant obesity impact of *C. aurantium* contains synephrine that is a trigger with comparable characteristics as ephedrine and caffeine. It states to have comparable impacts through suppressing appetite, enhancing metabolism, in addition raising energy expenditure**.** The rise catabolism associated by anorexia and the

reduce of feed intake can be etiologies of loss in the body weight observed in injection of glycerol. Accordance to **(Karagozlu *et al.*, 2016).** Citrus peel management diminished BWG and reduced mesenteric fat, epididymal fat, plasma and hepatic (TG) concentrations.

Table (2): Impact of bitter orange extract on serum liver enzymes of rats with fatty liver and control

Variables / Groups	ALT	AST	ALP
	(U/L)		
Control(-Ve) group	٣٢.٩١ ±1.٣٥ e	٤١.٦٤ ±0.٢٥ d,e	٨٣٧.٥٨ ±1.٢٣ c,d
Control (+Ve) group	٦٦.٤٥±1.٥٦ a	١٢٣.٦٥ ±2.٢٦ a	٨٧٢.٥٤ ±1.٤٧ a
4 mg/kg bitter orange extract	٥٥.٣٦ ±1.٢٥ b	٥٥.٣٥ ±1.٥٧ b	٨٥١.٩٤ ±1.٨٤ b
8mg/kg bitter orange extract	٥٠.٧٤ ±1.٧٤ b	٤٩.٥٤ ±0.٩١ c	٨٤٥.٢٧ ±1.٢٤ b,c
10 mg/kg bitter orange extract	٤٣.٥٨ ±2.٨8 c	٤٨.٥٧ ±1.٥٤ c	٨٤٢.٧١ ±1.٥٢ c
12 mg/kg bitter orange extract	٣٨.٣٥±1.٥٦ d	٤٥.٧٤ ±0.٤٥ d	٨٤١.٣٦ ±1.٦٣ c,d

*Values at the same column with different letters are significantly different at P<0.05.

*Values are expressed as means ±SE.

The results in Table 2 show that rats nourished on high fat diet and fructose (positive control group) had increased in serum alanine aminotransferase (ALT) activity with an average value of 66.45 ± 1.56 U/L when in comparison with the negative control group (32.91 ± 1.35 U/L). The outcomes demonstrated a diminution in serum ALT activity in four level in rat fed a high-fat, high-fructose food supplemented with bitter orange extract than the positive control group.

Data indicated that feeding the rats a high-fat and fructose food (positive control group) led to a significant rise in AST activity with an average value of 123.65 ± 2.26 U/L than the negative control

group (41.64 ± 0.25 U/L). Nevertheless, a significant reduction in serum AST level activities than the positive control group was observed when rat was fed bitter orange extract in the diet at any intake level.

Table 2 illustrated, that serum ALP activity increased significantly in positive group in comparison with healthy group. Also note a significant decrease in serum concentrations of ALP in rat nourished on HFD and fructose supplemented with bitter orange extract compared to the (+ve) group.

Liver function parameters of the serum concentration of enzymes ALP, AST, ALT, in addition to bilirubin are utilized to determine the existence of liver illness or possible damage to the liver and any type of liver damage that may etiology a enhance in ALT **(Ezeigwe *et al.*, 2022).** The release of aspartate aminotransferase and alanine aminotransferase from the cytosol happens when there is injury to hepatocytes, particularly in membrane harm **(Chikwendu *et al.*, 2015).** The outcomes illustrated that there was a significant diminution in the concentrations of alanine aminotransferase, aspartate aminotransferase, in addition to alkaline phosphatase when than the values of the normal control group and non-significant variance have been seen in total protein albumin, & total bilirubin within all the groups. This could however suggest the non-toxic impact of *C. aurantium* on the liver. No adverse effect on liver function was also observed in the examination performed by **(Jung *et al.*, 2017)** on pre-workout nutritional supplement with and without psynephrine.

The administration of *Citrus aurantium* L. peel essential oil at a dosage of 250 mg / kg of body weight diminished the raised serum concentration of enzymes (alanine aminotransferase, aspartate aminotransferase, LDH, in addition ALP,) triggered by Carbon tetrachloride (CCl4), leading to a subsequent recovery towards normalization in comparison with to the CCl4 group (**Ogaly *et al.*, 2015**). CCl4 treatment was shown to significantly elevate serum concentrations of liver transaminases (AST & ALT) compared to the normal group. Furthermore, these authors, Ocimum basilicum

curial oil significantly reduced liver transaminase levels (**Hsouna *et al.*, 2019)**.

In this respect **(Alam *et al.*, 2014)** demonstrated that naringin avoided the elevation of hepatic marker enzyme activity (ALP, AST and ALT) diminished lipid accumulation and fibrosis in the livers of obese mice subjected to a High-fat diet, high carbohydrate. Naringin supplementation enhanced mitochondrial respiration in these rats, indicating a reduction of dysfunction of mitochondrial compartment and lipid power expenditure by the liver.

Table (3): Impact of bitter orange extract on serum kidney functions of rats with fatty liver and control

Variables / Groups	Urea	Creatinine	Uric acid
	mg/dl		
Control(-Ve) group	٤٠.٣٢ ± 1.٢٧ c	0.٦٢ ± 0.٨٤ d	4.٢٤ ±0.٦١ b
Control (+Ve) group	٤٧.٤١ ±0.٣١ a	1.٧٢ ±0.٥١ a	5.٧٤ ±0.٥٤ a
4 mg/kg bitter orange extract	٤٥.٦١ ± 1.٦٤ a	1.٥٧ ±0.٢٧ a,b	4.٨٤ ±0.٨٢ a
8mg/kg bitter orange extract	٤٥.٣٤ ±0.٨٤ a	1.٣٥ ±0.٢8 b,c	4.٣١ ±0.٦١ b
10 mg/kg bitter orange extract	٤٢.٦٢ ±.1.٧٢ b	0.٨٤ ±0.٦٢ d	3.٨٢ ±0.٢٧ c
12 mg/kg bitter orange extract	٤٢.٥١ ± 0.٧٤ b	0.٣٥ ±0.٣٥ e	3.٥١ ±0.٧٣ c

*Values at the same column with different letters are significantly different at P<0.05.

*Values are expressed as means ±SE.

Table (3) shows the outcomes of the impact of bitter orange extract on renal function (concentration of uric acid, creatinine, in addition to urea in the serum) in rats with fatty liver. When the rats have been fed on a diet rich in fructose and fat, the level of urea in the serum raised significantly with an average value of 47.41±0.31 mg/dl than the (-ve) control group (40.32±.1.27 mg/dl). Whereas the group of rats that were nourished a fructose and high-fat diet supplemented

with bitter orange extract at any level consumption illustrated a significant diminution concentration of urea in serum to normal concentrations than the positive control group with average values of 45.61 ± 1.64, 45.34 ± 0.84, 42.62 ± 1.72 & 42.51 ± 0.74, respectively.

Table 3 data shows that the (+ve) control group that has been feed on a HFD and fructose had an increase in creatinine concentration with an average value of 1.72 ±0.51 mg/dl in comparison with the (-ve) control group (0.62±0.84 mg/dl). The group of rats that have been nourished a high-fat fructose supplemented with bitter orange extract showed a significant decreased in the concentration of creatinine in comparison with the (+ve) control group.

Impact of bitter orange extract on the concentration of serum uric acid of rats fed on HFD demonstrated in Table (3). The information revealed that uric acid established a significant increase in the (+ve control group) with an average value of 5.74±0.54 mg/dl than the (-ve) control group (4.24±0.61 mg/dl). Nevertheless, when rats have been treated with bitter orange extract at four levels, they illustrated a significant diminution in uric acid in comparison with the (+ve) control group.

High concentrations of blood urea nitrogen and creatinine might be a mark of an underlying condition influencing the kidneys, **Raj ,(2014)** but non-significant rise in blood urea nitrogen or creatinine has been noticed which can be an indication that *C. aurantium* fruit juice have no toxic or adverse impact on renal function. This outcome in line with the research work conducted by **(Jung *et al.*, 2017).** In their research, the safety of a pre workout nutritional supplement with and without p- synephrine which showed no adverse impacts on the function of kidney was investigated.

Kidney lipotoxicity and lipid accumulation might drive fibrosis, oxidative stress, and, inflammation resulting in renal dysfunction **(Mitrofanova *et al.*, 2023).** Multiple investigations have demonstrated that high-fat diet-induced lipid accumulation may lead to alterations in renal function & structure, involving an elevation in

urine microalbuminuria and a decrease in glomerular filtration rate, partially because of a prooxidant/antioxidant imbalance **(Serra-Majem *et al*.,2019).** It has been demonstrated by **Martínez-García *et al*., (2015)** that the accumulation of lipids in the kidneys and insulin resistance (IR) is correlated with oxidative stress, inflammation, & stress in endoplasmic reticulum in podocyte cell lines that have been managed with palmitic acid.

Long-term high-fat diet consumption was demonstrated to etiology ectopic lipids in the kidney accumulation and activate pro-fibrotic pathways inside that organ **(Almatroodi *et al*., 2021).** Also, numerous researches have additionally illustrated that HFD might trigger kidney proximal tubular injury (**Yamamoto *et al*., 2017; Xu *et al*., 2019).** High-fat high-fructose (HFHF) food nourished rats demonstrated anomalous deposition of lipids in kidney. Accumulation of the lipid in the kidney that was observed in the research represented enhanced oxidative stress and accompanying inflammation that result in renal injury **(Wulandari *et al*., 2025).**

Table (4): Impact of bitter orange extract on lipid profile in the serum of rats with fatty liver and control

Variables / Groups	TC	TG	HDL-c	LDL-c	VLDL-c
	(mg/dl)				
Control(-Ve) group	13٠.٣٤ ±٤.٢٥^{d}	٩٥.٤١ ±1.٥٢^{e}	٣٤.٣٥ ±.٨٤ b	٧٦.٢٨ ±4.٤١ ^{c}	2٣.٥١ ±0.٤٢ b
Control (+Ve) group	24٣.٣١ ±٤.٤١^{a}	18١.٢٧ ±1.٥١^{a}	٢٩.٣٤ ±1.٩١^{d}	1٥٩.٧٥±٢.٥١^{a}	3١.٦٢ ±.٧٢ a
4 mg/kg bitter orange extract	1٤٨.٤0 ±1.٣٢^{b}	1٣٩.٣٢ ±2.٩٤^{b}	٣٢.٦٢ ±.1.٦٢ ^{c}	8٢.٣٢ ±٤.4١ ^{b}	2٠.٣٦ ±0.٣٦ b
8mg/kg bitter orange extract	1٤٦.٧١ ±٣.٩٢^{b}	1٢٧.٦٢ ±2.٣٢^{c}	٣٦.٧١ ±.1.٢٥^{b}	٧٨.٧٤ ±2.٢٣ ^{c}	١٦.٥٤ ±0.٦٣ c
10 mg/kg bitter orange extract	14٣.٦٥ ±1.٥١^{b,c}	1٠٦.٤١ ±3.٦٧^{d}	4٠.٥١ ±2.٧٢^{a}	٧٤.٦٢ ±٤.٥١ d	١٦.٤١ ±0.٨٢ c
12 mg/kg bitter orange extract	141.٢١ ±٢.٣١^{c}	10٠.٥١ ±2.٧٤^{d}	41.٦٤ ±1.٤٥^{a}	٧٤.٣٥ ±2.٦٣ d	١٤.٥١ ±0.٩١^{c,d}

*Values at the same column with different letters are significantly different at P<0.05.
*Values are expressed as means ±SE.

Triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low lipoprotein cholesterol l(VLDL-C), increased significantly in the (+ve) control group, as in comparison with the (-ve) control group, as observed in table (4). Nevertheless, concentration of serum high density lipoprotein cholesterol (HDL-C) was significantly (P<0.05) reduced. Foods supplemented with bitter orange extract demonstrated significant diminution (P<0.05) in the mean values of serum lipid profile. Nevertheless, serum HDL-C has been enhanced significantly (P<0.05), in comparison with the (+Ve) control group. Groups of rats had HFD, fructose and nourished on bitter orange extract showed enhancing lipid profile in comparison with the (+ve) control group. These modifications recommend importance of bitter orange extract based food in the avoidance or treatment of cardiovascular associated disorder. In this respect **Wu *et al.*, (2013)** stated that (HFD) stimulated enhanced serum Total Cholesterol and Triglycerides concentrations in comparison with those nourished on the (LFD). In this setting **kelleya *et al.*, (2015)** stated that citrus flavonoids have (+ve) impacts versus obesity like reduce in Triglyceride & LDLC.

The significant reductions in the concentration of LDL triglycerides, and cholesterol in extract managed groups than the untreated positive control rat group indicates that extract of *C. aurantium* can possess hypolipidemic activity(**Sharma *et al.*, 2008)**. Findings correspond with the earlier reports by (**Sule *et al.*, 2016),** who stated reduction in serum lipids, LDL, VLDL fraction and triglycerides in normal in alloxan-induced diabetic rats managed with alcoholic extract of *C. aurantium*. Excessive accumulations of glucose, cholesterol, triglyceride and LDL have been implicated in obesity.

According to the outcomes of our investigation, the impact of CS on the improvement of NAFLD may be largely attributed to the increases in the up regulation of fibroblast growth factor 21

(FGF21) and the metabolic pathways that are related with it. In addition to being the first source of circulating of FGF21, the liver is also responsible for the majority of the beneficial impacts that FGF21 has on the metabolism as a whole **(Ejaz *et al.*, 2016).** Numerous lines of prove have illustrated that administration of FGF21 or transgenic overexpression reduction hepatic and serum concentrations of lipids, diminish body weight, & alleviate the progress of NAFLD **(Liu *et al.*, 2015).**

Table (5): impact of bitter orange extract on serum malondialdehyde and glutathione of rats with fatty liver and control

Variables Groups	alondialdehyde	Glutathione
Control(-Ve) group	٥٨.٢٥ ±3.٦٤ f	4.٥٢ ±0.٥٧ a
Control (+Ve) group	١٤٥.٤٧ ±3.٤١ a	2.٤٧ ±0.٦٥ c
4 mg/kg bitter orange extract	10٠.٨٤ ±2.٥١ b	2.٣٥ ±0.٤٧ c
8mg/kg bitter orange extract	٩٥.٦٥ ±1.٣١ b,c	3.٦٤ ±0.٥٢ b
10 mg/kg bitter orange extract	٨٧.٧١ ±2.٧٤ d	4.٣٢ ±0.٧٤ a
12 mg/kg bitter orange extract	٦٨.٤٥ ±2.٦٥ e	4.٥٤ ±0.٧٤ a

*Values at the same column with different letters are significantly different at P<0.05.

*Values are expressed as means ±SE.

Positive control group, significant rise in average value of malondialdehyde in positive control group when in compression with the normal group. It decreased significantly (P<0.05) as a result of feeding bitter orange extract to high-fat fed rats, in compression with the control group (+ve). The examined sample of bitter orange extract had beneficial impacts on malondialdehyde levels .

Table 5 showed significant decrease (P<0.05) in the mean value of glutathione activity in positive group comparison with the healthy group in rats fed on high-fat diet. Moreover, it was found that feeding rats with bitter orange extract levels increased the average values of glutathione activity significantly when in comparison with the corresponding values of the +ve control group.

Diets, mainly great in carbohydrates or fat, are correlated with oxidative stress by enriching amounts of lipid peroxidation and

protein carbonylation products whereas diminishing the antioxidant defense state **(Babel and Dandekar, 2021).** Nucleic acid, Proteins, in addition to lipids have functional and structural damage because of oxidative stress. MDA, 4-hydroxynonenal, & isoprostane may be generated by oxidation of lipids, essentially unsaturated fatty acid **(Grootveld *et al.*, 2020).** Antioxidant enzymes, involving Glutathione Peroxidase (GPx), are crucial in sustaining reactive oxygen species (ROS) homeostasis and protecting cells from oxidative damage **(Zhang *et al.*, 2020).** In research by **Wulandari *et al.*, (2025),** illustrated that HFHF led to oxidative stress, proved by alterations in concentrations of GPx & malondialdehyde in the renal tissues. 6-G management attenuated high-fat high-fructose -diet-induced oxidative stress by improving the renal GPx activity and diminishing levels of renal malondialdehyde.

Orange bitter peel has been discovered to be a new potent natural antioxidant **(Tundis *et al.*, 2012;karoui and Marzouk 2013).** The anti-inflammatory and the antioxidant impact of the BOP is because of its great content of the polyphenols, particularly, quercetin, hesperidin, the naringenin, in addition rutin, which have been stated earlier to have great antioxidant activity. **Kim *et al.*, (2012)** stated earlier that the anti-inflammatory impact of the Korean *Citrus aurantium* has been observed to be because of proinflammatory mediators' inhibition by obstruction mitogen activated protein kinase (MAPK) & nuclear factor-kappa B (NF-ƘB) pathways .

30 , 300 mg / kg of body of p-synephrine and 4000 mg / kg of body of the bitter orange extract that included 300 mg p-synephrine led to significant rises in hepatic diminished glutathione (GSH), which is an antioxidant and tissue protectant, whereas the great dose of the bitter orange extract reduced liver malondialdehyde content (a marker of lipid damage & lipid peroxidation). As well as, p-synephrine enhanced catalase which eliminates thepro-oxidant hydrogen peroxide. 30 & 300 mg / kg of p-synephrine in addition to 400 mg / kg per day and 2000 mg / kg of the bitter orange extract (30 and 150 mg of p-synephrine, correspondingly) additionally significantly suppressed glutathione peroxidase activity,

consequently protecting GSH **(Arbo *et al*., 2009 ;Deshmukha *et al* ., 2017).**

Conclusions

Finally, It could be concluded that from the obtained results of consumption Egyptian bitter orange extract that enhanced of fatty liver health, inhibitor of hypercholesterolemia and improved antioxidant enzyme in rats fed on high fat and fructose diet.

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