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Amelioratory Effect of Aqueous Leaf Extract of *Origanum majorana* on some Liver Function and Histological Alteration of the Liver in Experimentally Obese Rats

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Abstract

Obesity's dangers are becoming more widely recognized around the world. Obese people are more likely to develop a variety of serious diseases, including fatty liver. The purpose of this study was to investigate the effect of *Origanum majorana* on light microscopic changes in the liver, as well as hematology and some liver enzymes in experimentally obese albino rats. Four groups of twenty-four male albino rats were formed. For three months, the control group was fed a standard laboratory diet and received saline orally by gavage tube. Obese rats were given a high-fat diet. The marj group received 20 mg/kg of aqueous extract of marjoram via gavage tube. The obese/marj group received the same treatment as the obese group and received 20 mg/kg of aqueous extract of marjoram daily via gavage tube. The liver and visceral fat weights of animals were recorded, blood samples for hematology and liver functions were estimated. Liver samples were fixed in 10% formol saline then stained by Hematoxylin and Eosin for histological examination. Obese animals had significantly higher liver, visceral fat weights, and liver function than the control group. Histologically, there was an increase of fatty degeneration in the obese group. However, the obese group that received the aqueous leaf extract of *Origanum majorana* was lower in all the measured and examined liver parameters than that of the control group. In this study the aqueous leaf extract of *Origanum majorana* may ameliorate the obesity induced abnormal changes in the hepatocytes.

KEYWORDS
Liver, Obesity, Rats, *Origanum majorana*, Hematology

INTRODUCTION

Obesity is a global disorder that affects both developed and developing countries; it is the world's second leading cause of preventable death (World Health Organization, 2009). High-fat and high-cholesterol diets raise blood cholesterol (CHO) and triglycerides (TG), both of which cause oxidative stress, a major contributor to the development of nonalcoholic fatty liver disease (Awad *et al*., 2016). Obesity has an impact on nonalcoholic fatty liver disease, the most common liver disorder worldwide (Yu *et al*., 2019). Obesity is distinguished by triacylglycerol accumulation within liver cells (Ezquer *et al*., 2011). Obesity can lead to more serious liver conditions such as non-alcoholic steatohepatitis, liver fibrosis, and liver cirrhosis, as well as, in rare cases, liver carcinoma (Vernon *et al*., 2011).

Medicinal plants are also important for treating different diseases in humans because they have been proven to have various properties that protect the liver (Nasri *et al*., 2014). Marjoram (*Origanum majorana*) is a valuable plant with proven health benefits. It possesses diverse qualities that aid in combatting detrimental elements within the body, preventing infections, safeguarding the liver and heart, inhibiting stomach ulcers, diminishing inflammation, inhibiting cell growth, and combating fungus (Bina and Rahimi, 2017).

This work was performed to study amelioratory effect of aqueous leaf extract of *Origanum majorana* against high fat diet as a model of experimental obesity focusing on the biochemical changes in the blood related to the liver function as well as light microscopic examination of the liver.

MATERIALS AND METHODS

Origanum majorana

Origanum majorana leaves were used in this study, this product was purchased from a local market. The aqueous extract was prepared following the method of Vuong *et al*. (2013).

Experimental animals

A total of twenty-four adult male albino rats weighing between 120 to 130 g were maintained in plastic-cages with stainless steel wire lids. They were housed for seven days, for adaptation to laboratory conditions, before the initiation of the experiments under constant temperature and humidity. Animals were provided with rat diet and clean tap water without restriction throughout the experiment.

Experimental groups and design of work

Four groups of twenty-four male Albino rats were formed. For three months, the control group was fed a standard laboratory diet and received saline orally by gavage tube. Obese group rats were given a high-fat diet according to Woods *et al*. (2003). The marj group received 20 mg/kg of aqueous extract of marjoram

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via gavage tube according to Rababa'h *et al*. (2020). The obese/ marj group received the same dietary treatment as the obese group and received 20 mg/kg of aqueous extract of marjoram daily via gavage tube.

Blood and liver sampling

At the end of the experiment, blood samples were taken from retro orbital plexus of all rats under light anesthesia before decapitation and after overnight fasting. Blood samples were then collected in EDTA tubes and clean dry plain tubes without anticoagulant. The blood in EDTA was used immediately for hematological testing according to Mehmood *et al*. (2018). Serum of non-hemolyzed samples was thoughtfully transferred into clean dry Eppendorf tubes that were kept frozen at -20°C till used for biochemical analysis.

Liver/body weight and visceral fat /body weight ratio

The livers of rats of all experimental groups and visceral fats were dissected and weighed. The organs/body weight ratio was calculated as follow: liver or visceral fat weights/ body weight x100.

Biochemical parameters

Cholesterol was determined using a commercial kit (Egyptian company for Biotechnology (S.A.E), Egypt, REF: 230 001), TG were determined using a commercial kit (Egyptian company for biotechnology (S.A.E), Egypt, REF: 314 001). High density lipoprotein (HDL) was determined using a commercial kit (Vitro Scient, Egypt, REF: 1581), low-density lipoprotein (LDL) (Vitro Scient, Egypt, REF: 1591), very-low-density lipoprotein (VLDL) (Biocompare, U.S.A REF: IT3133), alanine aminotransferase (ALT) (SPINREACT company, Spain, REF: SP41274), aspartate aminotransferase (AST) (SPINREACT, Spain, REF: MD41264) and gamma-glutamyl transferase (GGT) (BioMed - γ- Cat. No30175). Total protein (TP) was estimated using a commercial kit (Vitrescent, Egypt, REF: 13501). All procedures were carried out according to the enclosed manufacturer's pamphlet.

Histological examination

For the light microscopic study, the liver was removed, excised, washed, fixed, and dehydrated. The tissues were then placed in molten soft paraffin, then hard paraffin, and cooled. The blocks were cut into thin sections and placed on glass slides. The slides were warmed, dried, and stained with Hematoxylin and Eosin (H&E) stain to study the liver's structure.

Statistical analysis

One-way analysis of variance (ANOVA) was used, with Tukey post-hoc test for multiple comparisons. Data was represented as means and standard errors. P-value of the measured parameters less than 0.05 was considered significant.

RESULTS

Effect of marjoram on liver/ body and visceral fat/body weight ratio of obese rats

The liver body weight ratio, and body/visceral fat ratio of control and different treated groups are demonstrated in Table 1. The liver body weight ratio and Body/ visceral fat ratio showed significantly increased values in obese group in comparison to control group. Obese/marj group showed significantly decreased liver/body ratio and body/visceral fat ratio in comparison to obese group.

Table 1. Effect of marjoram on liver/ body and visceral fat/body weight ratio of obese rats.

Parameters	Groups			
	Control	Marj	Obese	obese/marj
Liver/body ratio	2.81±0.05^{c}	2.66±0.60^{d}	3.20±0.03^{a}	3.00±0.02^{b}
visceral fat /body ratio	3.12±0.06^{c}	3.08±0.10^{c}	3.36±0.07^{a}	3.21±0.09^{b}

Data are expressed as means ± SEM, n=6. Data is statistically analyzed using One-way ANOVA followed by Tukey multiple comparisons test. Data followed by different superscript letters in the same row is statistically significant P≤0.05.

Effect of marjoram treatment on hematological parameters

Hemoglobin levels (HB%), red blood cell count (RBCs), platelets count (PLTs), and white blood cells (WBCs) count are demonstrated in Table 2, for control and different treated groups. The obese group resulted in a significant decrease in RBCs, PLTs, and significant increase in WBCs count compared to control group. On the other hand, data showed treatment of obese rats with marjoram increased RBCs, PLTs, and decreased WBCs comparison to obese group.

Table 2. Effect of treating obese rats with marjoram on hematology parameters.

Parameters	Groups			
	Control	Marj	Obese	obese/marj
HB% (g/100 mL)	13.31±0.37^{a}	13.29±0.06^{a}	12.80±0.90^{c}	12.96±0.06^{b}
RBCs (X10^{12}/L)	6.86±0.20^{a}	6.79±0.20^{a}	6.49±0.31^{c}	6.63±0.91^{b}
PLTs (X10^{9}/L)	761.00±1.12^{a}	768.01±0.07^{a}	735.04±2.00^{c}	749.03±1.82^{b}
WBCs (X10^{9}/L)	13.57±0.10^{c}	13.50±0.05^{c}	14.91±0.27^{a}	14.52±0.10^{b}

Data are expressed as means ± SEM, n=6. Data is statistically analyzed using One-way ANOVA followed by Tukey multiple comparisons test. Data followed by different superscript letters in the same row is statistically significant P≤0.05.

Effect of marjoram treatment on biochemical analyses

All the measured biochemical parameters except the HDL and TP showed significant increase in obese group compared with control group (Table 3). On the other hand, HDL showed significant decrease in obese compared with control group. The treated obese group with marjoram modulated the biochemical change induced in obese group.

Light microscopic examination of the liver

Examination of sections obtained from the liver of control and marjoram groups revealed classic hepatic lobules. Rows of liver cells are arranged in the form of radiating cords surrounding the portal areas. Hepatocytes are separated from one another by narrow blood sinusoids. Blood sinusoids were lined by endothelial cells and Kuepfer cells (Fig. 1 a & b). On the other hand, the obese group showed many histological variations such as loss of cellular details, maculation and fatty degeneration around the portal area (Fig. 1c). Histological sections of treated obese rats with marjoram group showed normal arranged hepatic architecture with few cells showed hydropic degeneration (Fig. 1d).

Table 3. Effect of marjoram treatment on biochemical analyses.

Parameters	Groups			
	Control	Marj	Obese	obese/marj
AST (U/L)	19.92±0.09^{c}	19.53±0.06^{d}	65.01±0.31^{a}	35.71±0.24^{b}
ALT (U/L)	22.23±0.05^{c}	22.12±0.13^{c}	45.12±0.24^{a}	33.43±0.18^{b}
GGT (U/L)	21.26±0.17^{c}	20.53±0.21^{c}	105.01±2.43^{a}	41.81±0.79^{b}
TP (g/dL)	5.27±0.33^{b}	7.61±0.01^{a}	3.91±0.01^{d}	3.70±0.09^{c}
CHO (mg/dL)	104.03±0.19^{c}	102.12±1.12^{d}	222.03±0.92^{a}	124.02±0.55^{b}
TG (mg/dL)	50.31±0.95^{c}	47.05±0.37^{c}	127.10±0.93^{a}	75.01±0.37^{b}
HDL (mg/dL)	26.22±0.23^{b}	30.14±0.61^{a}	24.61±0.15^{d}	25.51±0.21^{c}
LDL (mg/dL)	68.01±0.67^{c}	67.74±1.08^{c}	116.21±0.98^{a}	72.45±1.10^{b}
VLDL (mg/dL)	10.06±0.11^{c}	9.40±0.72^{d}	26.02±0.18^{a}	15.27±0.70^{b}

Data are expressed as means ± SEM, n=6. Data is statistically analyzed using One-way ANOVA followed by Tukey multiple comparisons test. Data followed by different superscript letters in the same row is statistically significant P≤0.05.

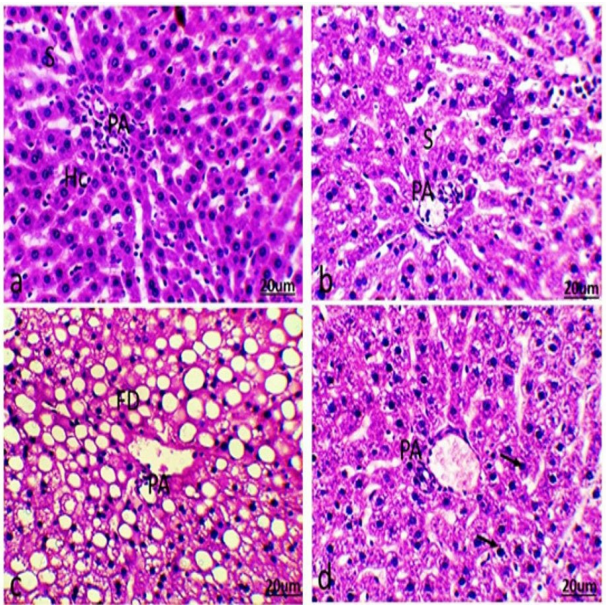

Fig. 1. Photomicrographs of liver sections obtained from various experimental groups and stained by Hematoxylin and Eosin stains. (a): control group. (b) marjoram group demonstrated normal portal area (PA) structure of classic hepatocytes (HC) between them blood sinusoids (S). (c): liver section of obese group showed highly disturbed portal area (PA) surrounded by fatty degenerated cells (FD). (d): liver section treated obese rats with marjoram showed cell hepatocytes have vacuolated cytoplasm (arrow) surrounded the portal area. (H&E, 400X).

DISCUSSION

Obesity has been linked to a variety of liver diseases, including nonalcoholic fatty liver disease, which is defined by an increase in triacylglycerol contents in hepatocytes with or without inflammation or fibrosis (Yamaguchi *et al*., 2007). In the present study, liver /body weight ratio and visceral fat/ body ratio showed a significant increase in obese group compared to control group. These results coincided with de Castro *et al*. (2013) who reported that there is an increase in the liver weight with the high fat diet. The observed increase in liver/ body weight ratio of obese group may be attributed to increase accumulation of triacylglycerol within liver cells. These results confirmed by Ezquer *et al*. (2011) who reported that high fat diet may be cause nonalcoholic fatty liver disease which distinguished by triacylglycerol accumulation within liver cells. Tanaka *et al*. (2016) reported that the increase of the liver/ body weight ratio may be due to lipid deposition in hepatocytes.

The observed increase of visceral fat/ body ratio in obese group may be attributed to greater energy impact from high fat diet. These results confirmed by Bray *et al*. (2004) who reported that high-fat diets have caused obesity where energy from fat has a greater impact on body weight gain than energy from non-fat sources. Moreover, high-fat diet is associated with higher basal plasma insulin levels and resistance to the metabolic effects of insulin which cause inhibition of lipolysis (Ouwens *et al*., 2007). On the other hand, treatment of obese rats with marjoram group showed significant decrease in the liver /body weight ratio, visceral fat/ body ratio in comparison to obese rats. This result was approved by Ahmed *et al*. (2009) who reported that the intake of water extract of marjoram markedly lowered liver weight gain and recommended the usefulness of marjoram for treating obesity accompanied by hyperlipidemia. The observed decrease liver /body weight ratio and visceral fat/ body ratio in obese group may be attributed to improve digestion and the ability of marjoram to reduce plasma lipids. Moreover, marjoram declines the levels of CHO, TG, and LDL and increases the HDL level (Desouky *et al*., 2015).

Obese rats resulted in a significant decrease in HB%, RBCs, PLTs and significant increase in count of WBCs. These results coincided with Tussing-Humphreys *et al*. (2012) who reported that obesity may disrupt iron homeostasis, resulting in iron deficiency anemia and RBCs count decline. They added that the association between obesity and iron deficiency may be due to increased hepcidin levels mediated by chronic inflammation. Hepcidin is a small peptide hormone that functions as a negative regulator of intestinal iron absorption. Treatment of obese rats with marjoram compared to obese group modulated that change. Ramadan *et al*. (2013) observed improvement of HB% obese group and attributed their results to the richness of marjoram in iron beside its ability in reducing inflammatory effect induced by high fat diet intake. Moreover, marjoram herb has one of the highest antioxidant levels among herbs which has anti-inflammation potential as well as hemogram promoting effect (Sharangi and Guha, 2013).

Obese rats resulted in a significant increase in count of WBCs this results coincided with Veronelli *et al*. (2004) who reported that WBCs count is elevated in obesity. The observed WBCs increase in obese group may be attributed to inflammatory stress of obesity. Veronelli *et al*. (2004) reported that elevated WBCs count due to impaired glucose tolerance, and WBCs count is associated with macro- and microangiopathic complications in type 2 diabetes and obesity. Treatment of obese rats with marjoram compared to obese group modulated that increase. This result was approved by Ramadan *et al*. (2013). The observed decrease in WBCs count may be attributed to anti-inflammatory effect of marjoram whereas marjoram herb has high contents of antioxidant and anti-inflammatory potential (Arranz *et al*., 2015).

The obese group showed a significant increase in ALT, AST and GGT when compared to the control group. These results coincided with Hajifathalian *et al*. (2020) who reported that overweight and obesity are significantly associated with elevated biochemical markers ALT, AST and GGT of the liver. The observed increase in AST, ALT, GGT in obese group may be explained by Yang *et al*. (2014) who reported that high-fat diet cause lipid accumulation and increased infiltration of inflammatory cells in the liver evidenced by the increased plasma activities of the AST, ALT, GGT and ALP enzymes, these enzymes are considered markers of hepatic dysfunction. The observed increase may be explained by Yang *et al*. (2014) who reported that the raised levels of AST and ALT might be due to the leakage of these enzymes from the hepatocytes into the blood stream after cellular damage (El Hak *et al*., 2022). Treatment of obese rats with marjoram showed significant decrease in ALT, AST and GGT. This result was in agreement with the works of El-Ashmawy *et al*. (2005) who reported that *O. majorana* played an important role in ameliorating liver functions and induced a significant decrease in serum activities of transaminases of the obese rats. This showed a hepatoprotective ability of marjoram against obesity -induced hepatic damage. The reduction in the ALT, AST and GGT by *O. majorana* might be attributed to the stabilizing ability of the cell membrane thus preventing enzyme leakage and restoring the integrity of the cells via its antioxidant potential (Anusha *et al*., 2011).

Obese group compared to control group showed a signifi-

cant increase of CHO, TG, LDL, VLDL, and significant decrease in HDL. This results coincided with Ugwor *et al.* (2022) who reported the obese rats that fed with high fructose and high lipid diet had significantly higher body weight and total body Lipids (TG, CHO, LDL, VLDL and free fatty acids) with significantly reduced HDL. The previous results may be attributed to effects of a high-fat diet in CHO, TG, LDL and VLDL absorption, synthesis and lipoprotein processing. These findings help in the accumulation of hepatic steatosis (Chung *et al*., 2013).

Treatment of obese rats with marjoram showed significant decrease in CHO, TG, LDL, and VLDL while increased HDL level. These results were approved by Ahmed *et al*. (2009) who found that marjoram reduced blood CHO, TG, LDL and VLDL while increased HDL levels of obese rats fed with high fructose, high fat diet. Moreover, Desouky *et al*. (2015) found that marjoram declines the levels of CHO, TG, and LDL while increased the HDL level. The previous results may be attributed to effects of marjoram antioxidant perorates Kim and Jeong (2019). Histological examination of liver of obese group showed steatosis with vacuolated hepatocytes which may be caused by high fatty acids influx and low fatty acids utilization in the hepatocytes (Hijmans *et al*., 2014).

CONCLUSION

This study confirmed that obesity is responsible for remarkable structural and functional abnormalities in the liver. This study showed the possible amelioratory effects to these changes with the aqueous leaf extract of *Origanum majorana* supplementation via improving hematology and biochemical parameters.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related.

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