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Effect of Marjoram and Choline Mixture Consumption Against Liver Injury in Experimental Rats

Nabila M. Rashwan

Department of Home Economics Faculty of Education, Suez University, Ismaelia, Egypt

Abstract: Sixty albino male rats Sprague Dawley strain were administered paracetamol drug to induce liver injury and randomly classified into six groups (10 rats each). One served as control (+ve) and five treated rat groups which were marjoram extract, marjoram powder, choline, marjoram extract with choline and marjoram powder with choline rat groups. All experimental rat groups showed a significant increase in final body weight, body weight gain & food efficiency ratio; serum total protein, globulin, high density lipoprotein cholesterol (HDLc) and liver triglyceride, glycogen, superoxide dismutase (SOD), glutathione (GSH) & glutathione peroxidase (GPX) in comparing with control (+ve) rat group. On the other side, there were a significant decreased in serum alanine and aspartate aminotransferase (ALT, AST), alkaline phosphates (ALP), gamma glutamyle peptidase (γGT), albumin to globulin (A/G) ratio, cholesterol, triglyceride, low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and liver cholesterol, total lipids, malondialdehyde (MDA) and cholesterol /HDLc ratio in comparing with control (+ve) rat group. The value of albumin was significantly increased in marjoram powder, choline and marjoram extract with choline in comparing with control (+ve) rat group.

Key words: Paracetamol drug · Marjoram leaves powder · Extract · Liver · Rats

INTRODUCTION

Herbs and herb extracts have a range of beneficial properties, with those most studied to date being their *in vitro* antioxidant, antifungal and anti-microbial activities. Marjoram *(Majorana hortensis)* is the gray green leaf of the mint family (Lamiaceae or Labiatae). It is an aromatic perennial herb, specific to the Mediterranean climate, the herb originated in Egypt and Arabia. Marjoram is a traditional culinary and folk remedy herb used by many different cultures. It has been used for arthritis, rheumatism, muscle and nerve pain, headaches, circulatory disorders and respiratory infections [1, 2]. Choline is a water-soluble essential nutrient and chemically similar to the B-vitamins. It is important to get dietary choline although the human body can make some choline. It is generally recognized that choline and compounds derived from choline enter in structure of cell membranes, protecting livers from accumulating fat, as the precursor molecule for the neurotransmitter acetylcholine and also a major source for methyl groups via its metabolite, trimethylglycine (betaine) that participates in the S-adenosylmethionine synthesis pathways [3]. Livers play important roles in metabolizing (altering in some way) proteins, carbohydrates and fats from our food, producing cholesterol (which is then used in bile acids and fat digestion) and producing proteins necessary for blood clotting and fluid balance in the body in addition to that it is responsible for detoxification. Hepatic injuries such as necrosis and hepatic failure are often produced on exposure of the tissue to virus or many chemical agents. Liver necrosis is a complex process, characterized by the simultaneous activation of multiple deregulated pathways that culminate in the loss of cell membrane integrity causing leakage of cellular constituents. Hepatic failure is characterized by severe hepatic dysfunction results in jaundice, hepatic encephalopathy and coagulopathy [4]. The aim of the present study was to evaluate the effect of marjoram and choline on paracetamol induced liver injury in experimental rats.

MATERIALS AND METHODS

Materials

Chemicals: BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt.

Corresponding Author: Nabila M. Rashwan, Department of Home Economics, Faculty of Education, Suez Canal University, Ismaelia, Egypt.

Paracetamol tablets were obtained from Kahira Pharm &Chem. Ind. Co., Cairo, Egypt. Paracetamol, known as acetaminophen,used to relieve mild to moderate pain, including instances of tension headache, migraine headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, general pain, toothache, teething pain and period pain. Large dose of paracetamol can damage the liver. Liver damage in rats was induced by a single dose of 2 g/kg paracetamol p.o [5]. Choline was obtained from El Naser for Chemical Industry. The human recommended daily allowance was 550 mg. The rat dose was 49.5mg /kg body weight of rats in diet [6].

Marjoram Plant and Standard Diet: Marjoram plant was obtained from local market. The standard diet was performed according to Nelson [7].

Animals: Forty two Sprague Dawley strain male rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 176±0 g.

Methods

Preparation of Marjoram Powder and Extract: Marjoram leaves were dried in dry freezer and crushed into powder then added to standards diet in 5% in substitution of fiber. Marjoram ethanolic extract was prepared from marjoram powder according to Ryszard *et al.* [8]. 25 g marjoram powder mixed with 150 ml of 95% ethanol with stirring and repeated twice. The extract was evaporated to dryness using a Rotavapor and water bath under vacuum and stored at 4°C until further analyzed. Rats administered marjoram extract at dose 15 mg/kg dissolved in distilled water by stomach tube.

Animals and Treatment: After adaptation period (one week), rats were administered paracetamol by stomach gavage to fasting rats as a single dose to induce liver injury then classified into six groups (10 rats each). One served as control (+ve) and other five rat groups treated with marjoram extract, marjoram powder, choline, marjoram extract with choline and marjoram powder with choline. During the study period (8 weeks), the daily food intake and weekly body weight gain were recorded. Rats were sacrificed to obtain blood and liver.

Laboratory Analysis:

- Serum aminotransferase (ALT, AST), alkaline phosphates (ALP) enzymes activity and gamma glutamyle peptidase (γGT), were estimated according to Bergmeyer and Harder [9], Kind and King [10] and Henry [11], respectively.
- Serum total protein and albumin were determined according to the method described by Weichselbaum [12] and Bartholomev and Delany [13], respectively.
- Serum cholesterol, triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods described by Allain *et al.* [14], Buccolo and David [15] and Kostener [16], respectively.
- Livers of rats were perfused with 50 to 100 of ice cold 0.9%NaCL solution for estimation of liver cholesterol, total lipids, triglyceride and glycogen according to Abell *et al.* [17], Folch *et al.* [18], Young and Pestaner [19] and Rerup and Lundquist [20], respectively.
- Liver superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX) and malondialdehyde (MDA) levels were determined according to Beuchamp and Fridovich [21], Reed [22], Weiss *et al.* [23] and Uchiyama and Mihara [24], respectively.

Calculation of some Parameters: Food efficiency ratio (FER) was determined by Chapman *et al.* [25] as following: FER = weight gain (g)/ food intake (g). Serum globulin (G) value was determined by subtracting the albumin from the total proteins according to Coles [(1974). A/G ratio was calculated using albumin and globulin values for each individual sample. Very low density lipoprotein cholesterol (VLDL-c) was calculated as TG/5 while low density lipoprotein cholesterol (LDL-c) was calculated as following [$LDL\text{-}_C$ = Total cholesterol–$HDL\text{-}_C$ $VLDL\text{-}_C$] according to Fruchart [26]. Cholesterol /HDL_C value was calculated according to Castelli and levitar [27].

Statistical Analysis: Collected data were subjected to analysis according to SPSS program according to Snedecor and Cochran [28].

RESULTS

The nutritional results showed a significant increase in final body weight, body weight gain and food efficiency ratio ($p< 0.05$ and 0.01) in all treated rat groups compared with control (+ve) rat group but there were non significant difference in final body weight, body weight gain food intake and food efficiency ratio among treated groups as shown in Table 1. The values of serum ALT, AST, ALP and γGT were significantly decreased in all experimental rat groups($p< 0.01$&0.001) when compared with control (+ve) rat group and in the same time there were non significant difference in ALT, AST, ALP and γGT among all treated groups as shown in Table 2.

Table 1: Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of the experimental rat groups

Variables groups	Initial weight(g)	Final weight(g)	Weight gain (g)	Food intake(g/w)	FER
Control (+ve)	175.31±5.14^{a}	262.88±15.21^{b}	87.55+7.41^{b}	19.41±1.14^{a}	0.075±0.003^{b}
Marjoram extract	175.65±5.12^{a}	300.75±16.34^{a*}	125.14±12.12^{a**}	19.11±1.55^{a}	0.109±0.001^{a*}
Marjoram powder	173.14±5.19^{a}	285.25±18.21^{a*}	112.11±13.36^{a**}	18.75±1.71^{a}	0.099±0.005^{a*}
Choline	178.21±6.12^{a}	298.38±15.36^{a*}	120.17±10.14^{a**}	19.21±1.42^{a}	0.104±0.003^{a*}
Marjoram extract +choline	177.14±7.15^{a}	307.48±17.25^{a*}	130.34±12.16^{a**}	18.87±1.36^{a}	0.115+0.001^{a*}
Marjoram powder+ choline	175.33±6.31^{a}	302.50±17.43^{a*}	127.17±13.21^{a**}	19.31±1.31^{a}	0.109±0.002^{a*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.00

Mean values in each column having different superscript (a, b, c, d) are significant

Table 2: The Mean values ± SD of serum liver enzymes of the experimental rat groups

Variables groups	ALT (µ/ml)	AST (µ/ml)	ALP (µ/ml)	γGT (µ/ml)
Control(+ve)	71.39±8.12^{a}	122.14±13.13^{a}	112.15±15.24^{a}	10.33±1.19^{a}
Marjoram extract	45.87±5.11^{b**}	80.81±9.25^{b***}	65.11±6.05^{b***}	7.13±1.22^{b**}
Marjoram Powder	49.20±6.01^{b**}	79.36±9.36^{b***}	72.14±7.11^{b**}	8.22±1.24^{b**}
Choline	44.14±5.31^{b**}	82.11±8.26^{b***}	66.31±6.15^{b***}	7.55±1.33^{b**}
Marjoram extract + choline	38.71±4.20^{bc***}	71.14±9.34^{bc***}	62.27±7.18^{b***}	6.81±0.88^{b**}
Marjoram powder+ choline	46.67±5.16^{b**}	83.38±9.21^{b***}	70.08±8.16^{b**}	7.99±1.11^{b**}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Table 3: The Mean values ± SD of serum total protein, albumin, globulin and albumin/globulin (A/G) ratio of the experimental rat groups

Variables groups	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control(+ve)	5.33±0.87^{b}	3.11±0.15^{b}	2.22±0.41^{b}	1.40±0.11^{a}
Marjoram extract	6.51±1.06^{a*}	3.32±0.14^{ab}	3.29±0.33^{a*}	0.97±0.05^{b*}
Marjoram powder	7.11±1.24^{a*}	3.45±0.26^{a*}	3.66±0.52^{a*}	0.94±0.04^{b*}
Choline	7.32±1.55^{a*}	3.51±0.28^{a*}	3.81±0.54^{a*}	0.92±0.11^{b*}
Marjoram extract + choline	7.41±1.18^{a*}	3.60±0.33^{a*}	3.81±0.33^{a*}	0.94±0.12^{b*}
Marjoram powder+ choline	6.69±1.15^{a*}	3.12±0.44^{ab}	3.54±0.44^{a*}	0.87±0.03^{bc**}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Table 4: Mean values ± SD of serum lipid patterns of the experimental rat groups

Variables groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	Cholesterol/ HDLc
Control(+ve)	158.31±33.14^{a}	82.41±6.78^{a}	23.67±3.21^{b}	118.26±10.01^{a}	16.48±3.41^{a}	6.68±1.31^{a}
Marjoram extract	110.14±11.12^{b***}	64.45±7.19^{b**}	38.21±5.11^{a***}	59.11±6.31^{c**}	12.89±2.61^{b*}	2.88±0.57^{b**}
Marjoram powder	113.21±12.25^{b***}	61.17±6.01^{b**}	31.11±3.22^{a**}	69.90±7.21^{b**}	12.23±2.41^{b*}	3.63±0.73^{b**}
Choline	111.36±12.11^{b***}	63.21±7.12^{b**}	33.13±4.32^{b**}	65.60±6.14^{b**}	12.64±2.16^{b*}	3.36±0.44^{b**}
Marjoram extract + choline	98.61±9.11^{b***}	59.69±5.27^{b**}	37.36±3.21^{a**}	49.31±5.36^{d***}	11.99±1.29^{bc**}	2.63±0.23^{b**}
Marjoram powder+ choline	105.14±10.18^{b***}	62.31±6.67^{b**}	34.49±4.19^{a**}	58.20±6.24^{c**}	12.46±1.96^{b*}	3.04±0.12^{b**}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Table 5: Mean values ± SD of liver cholesterol, total lipids, triglyceride and glycogen of the experimental rat groups

Variables groups	Cholesterol (mg/g)	Total lipids (mg/g)	Triglyceride (mg/g)	Glycogen (mg/100g)
Control(+ve)	6.44±1.24^{a}	47.81±4.33^{a}	2.25±0.18^{b}	4.66±0.81^{c}
Marjoram extract	3.69±0.33^{b**}	35.32±5.61^{b**}	4.33±0.76^{a*}	7.25±1.11^{a***}
Marjoram powder	4.11±0.55^{b**}	35.66±5.26^{b**}	3.78±0.43^{a*}	7.66±1.24^{a***}
Choline	3.31±0.22^{b**}	34.36±5.13^{b**}	3.89±0.55^{a*}	6.91±1.31^{ab**}
Marjoram extract + choline	3.12±0.44^{b**}	32.14±4.18b^{c**}	4.45±0.56^{a*}	8.11±1.25^{a***}
Marjoram powder+ choline	4.01±0.64^{b**}	36.91±5.14^{b*}	3.77±0.61^{a*}	7.36±1.17^{a***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

Table 6: Mean values ± SD of liver SOD, GSH, GPX and MDA of the experimental rat groups

Variables groups	SOD (µg /mg)	GSH (µg /mg)	GPX (µg /mg)	MDA (nmol/g)
Control(+ve)	22.35±2.14^{b}	31.14±3.21^{c}	40.31±5.16^{c}	18.11±2.33^{a}
Marjoram extract	48.36±4.29^{a***}	62.14±7.19^{a***}	79.16±7.21^{a**}	7.14±1.78^{b***}
Marjoram powder	42.49±4.36^{a***}	55.36±5.22^{b**}	69.11±6.26^{b**}	9.21±1.88^{b**}
Choline	43.57±3.99^{a***}	54.34±5.30^{b**}	70.11±8.31^{b**}	9.16±2.18^{b**}
Marjoram extract + choline	49.36±5.21^{a***}	65.34±6.16^{a***}	79.96±7.91^{a**}	8.69±2.11^{b***}
Marjoram powder+ choline	48.91±4.33 ^{a***}	53.30±5.11^{b**}	71.71±7.81^{b**}	7.99±1.11^{b***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant

All treated rat groups showed a significant increase in the values of serum total protein and globulin (p< 0.05) but showed a significant decrease in the value of A/G ratio (p< 0.05) in comparing with control (+ve) rat group. The value of albumin was significantly increased in marjoram powder, choline and marjoram extract with choline rat groups in comparing with control (+ve) rat group. There were non significant differences among in total protein, albumin, globulin and A/G ratio among treated groups as represented in Table 3.

Data in Table 4 presented that all treated rat groups showed a significant decrease in the values of serum cholesterol, triglyceride, LDLc, VLDLc and cholesterol /HDLc ratio (p<0.05, 0.01and 0.001) but showed a significant increase in the values of serum in HDLc (p< 0.01) in comparing with control (+ve) rat group. There were non significant differences in serum cholesterol, triglyceride, HDLc, VLDLc and cholesterol /HDLc ratio among treated groups. There were non significant differences in serum LDLc between marjoram extract group and marjoram powder with choline group and also between marjoram powder group and choline group. Data in Table 5 showed that all treated rat groups showed a significant decrease in the values of liver cholesterol (P<0.01) and liver total lipids (P<0.05 and 0.01) but a significant increase in the values of liver triglyceride (P<0.05) and liver glycogen (P<0.01 and 0.001) in comparing with control (+ve) rat group. There were non significant differences in liver cholesterol, total lipids, triglyceride and glycogen among treated groups. Data in Table 6 showed that all treated rat groups showed a significant increase in the values of liver SOD (P<0.001), GSH (P<0.01&0.001) and GPX (P<0.01) but showed a significant decrease in the value of liver MDA (P<0.001) in comparing with control (+ve) rat group. There were non significant differences in liver SOD, GSH, GPX and MDA among treated groups.

DISSCUSION

Several studies have suggested that higher dose of acetaminophen produces a centrilobular hepatic necrosis. Paracetamol increases oxidative stress that depletes glutathione and covalently binds to proteins. The loss of glutathione with an increased formation of reactive oxygen and nitrogen species in hepatocytes is undergoing necrotic changes [29]. Groups of male and female inbred Leeds strain rats were fed diets containing either 0.5% or 1.0% paracetamol by weight for up to 18 months. At the 1.0% dosage level, 20% of rats of both sexes developed neoplastic nodules of the liver, a statistically significant incidence. These rats also showed gross enlargement of their livers and an increase in foci of cellular alteration, the latter also being observed in the low dosage male rats [30]. Previous studies have also shown that marjoram contains phenolic terpenoids, flavonoids, tannins, phenolic glycosides and sitosterol. Along with the essential oil, tannin, bitter and sistosterine elements, marjoram is also rich in vitamins A and D. These compounds in marjoram stimulates digestion, increases diuresis, absorbs gases, increases food appetite Also, it contains ursolic acid and essential oil and in particular to thymol and carvacrol which have bactericidal, antiseptic and antifungal. The antioxidant and antitumour activities of marjoram have recently been determined. Marjoram contains some components that activate chief and parietal cells and increase basal acid and pepsin secretion [31, 32]. Marjoram has high antioxidant capacity due to its high polyphenolic content. Natural antioxidants can protect the human body from free radicals and could retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [33]. It has been shown that water extracts, which isolate a range of different compounds, are rich in phenolic acids and flavonoids also have strong antioxidant activity [34].

Consumption of *Origanum onites* distillate had beneficial effects on lipid profiles, antioxidant status and endothelial function in patients with mild hyperlipidaemia. A significantly greater increase in high density lipoprotein-cholesterol and significantly greater decreases in low density lipoprotein-cholesterol, apolipoprotein B, lipoprotein(a) and high sensitivity C-reactive protein occurred in all participants prescribed 25 ml of aqueous distillate of Origanum onites over the 3-month compared with the control group [35]. The flavonoids may also promote healthy arteries and heart by preventing cholesterol buildup and improving blood circulation. The flavonoids in marjoram are supposedly good for cardiac health and are known to boost healthy arteries and heart by enhancing blood circulation and preventing cholesterol buildup [32]. The effect of administration of *O. majorana* (volatile oil, alcoholic and aqueous) induced a significant decrease in serum activities of aminotransferase enzymes (AST & ALT), ALP, urea and creatinine and improved the liver and kidney histology in comparison with lead acetate treated group [36, 37]. The treated group with marjoram leaves showed a significant decrease in serum creatinine, uric acid and total bilirubin and also decrease in liver triglycerides, cholesterol and total lipids in compared to non treated group [38]. Activities of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and the level of reduced glutathione in the plasma, erythrocytes, liver and kidney decreased in the hepatotoxic rats [39]. Dry marjoram and wild marjoram extracts effectively inhibited lipid peroxidation in chicken meat. Water extracts, which isolate a range of different compounds, are rich in phenolic acids and flavonoids also have strong antioxidant activity [2, 34].

Numerous reports have indicated that choline is an essential nutrient required by the body to make several important compounds necessary for healthy cell membranes. This nutrient helps form phosphatidylcholine, the primary phospholipids of cell membranes [40]. Choline is an essential precursor of acetylcholine, a stimulatory neurotransmitter. It also helps in the production of lipotropic agents which converts fats into useful products and aids in the production of HDL cholesterol. Choline intake may reduce cardiovascular disease risk in humans. The mechanism may be the ability of choline to be transformed into betaine. Higher intakes of dietary choline and betaine are related to lower homocysteine concentrations associated with reducing cardiovascular disease [41]. Choline has been shown to protect the liver from certain types of damage and can help reverse damage that has already occurred. Fat and cholesterol consumed in the diet are transported to the liver by lipoproteins called chylomicrons. In the liver, fat and cholesterol are packaged into lipoproteins called very low density lipoproteins (VLDL) for transport through the blood to tissues that require them. Phosphatidylcholine is a required component of VLDL particles and prevents fat and cholesterol accumulation in the liver. Choline deficiency is an elevated level of the liver enzyme ALT [42]. Choline also stops fats from being deposited in the liver and help move fats into the cells. Deficiency of choline can lead to cirrhosis with associated conditions such as bleeding; kidney damage hypertension, cholesterolemia and atherosclerosis [41, 43].

It is concluded that the consumption of marjoram and choline have ability to improve liver function and healthy status in rats.

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