The 6th international- 20th Arabic conference for Home Economics
Home Economics and Educational quality assurance December 23rd -24th, 2018
http://homeEcon.menofia.edu.eg

Journal of Home Economics

ISSN 1110-2578

Exploration The Hepatoprotective Activity Of Lemon Balm Leaves (Melissa Officinalis L.) In A Rat Model Of Oxytetracycline-Induced Fatty Liver

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Abstract

With growing burden of liver dysfunction, using natural plant products is increasing because of their attributes of sturdy antioxidant contents, negligible side effects and economical features. Thirty two rats weighing 120-130 g were haphazardly divided into four groups (n=8). Oral administration of oxytetracycline (120 mg/kg body weight/day) for three days produced hepatic damage as manifested by a significant elevation in serum hepatic markers namely aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferse (GGT) and lactate dehydrogenase (LDH), also increased hepatic lipid peroxidation (MDA). The oral administration of lemon balm (10 and 20 g/kg body weight) attenuated the oxytetracycline induced hepatotoxicity by significantly ($p \leq 0.05$) reducing levels of serum AST, ALT, GGT, LDH, glucose, TG, TC, LDL-C, urea, creatininie and malondialdehyde (MDA) and significantly ($p \leq 0.05$) increased in serum total protein, albumin, globulin, HDL-C in lemon balm treated rats comparing with untreated fatty liver group. As well as increase in superoxide dismutase (SOD) and total antioxidant capacity in lemon balm treated groups especially the dose of 10 g comparing with untreated fatty liver rats. In conclusion, lemon balm treatment had remarkable effects on hepatic

biomarker enzymes level and lipid peroxidation in rats. It is stipulated that administration of lemon balm leaves to treated groups were partially protected from hepatocellular damage caused by oxytetracycline.
Key words: Mellissa officinalis; oxytetracycline; hepatoprotective; liver; lipid profile; lactate dehydrogenase; superoxide dismutase.

Introduction

The liver is the most important organ of the body, contributing about 2% of the total body weight in the average human. It is linked with most of the physiological processes and serves vital function in human body. **(Dey *et al.*, 2013).** Several human diseases particularly, obesity, insulin resistance and type 2 diabetes were shown to be linked with accumulation of triglyceride within the cytoplasm of hepatocytes which collectively known as fatty liver, or steatosis. (**Ratziu *et al.*, 2010).** "In these types of diseases, more than 5%–10% of the increase in the body weight was from fat accumulation in the liver. **(Szczepaniak *et al.*, 2005).** "When hepatosteatosis is present in the absence of excessive alcohol consumption, it is termed non-alcoholic fatty liver disease (NAFLD). **(Ratziu *et al.*, 2010 & Marchesini *et al.*, 2001),** which is theorized to be the hepatic manifestation concerning the metabolic syndrome" **(Shulman and Mangelsdorf, 2005).** Fatty liver disease comprises a wide spectrum of hepatic damage, from simple steatosis alone, to inflammatory changes found in nonalcoholic steatohepatitis (NASH) and advanced fibrosis and cirrhosis of the liver. The prevalence of fatty liver disease has apparently increased in proportion to the increasing incidence of obesity in different ages. **(Sathya *et al.* 2002 and Clark, 2006).** Presently, there is no effective drug available that can stimulate liver function or regeneration of liver cell in spite of their adverse effect. Therefore it is necessary to find out some alternative medication for liver damage **(Mishra *et al.*, 2014).**

"Over the last few years the importance of medicinal plant based substances have increased greatly all over the world for management and treatment of various diseases" **(Nigam and Nambiar, 2015).**

Interestedly, More trend were turned to herbal medicine due to their widely uses and functional health impacts, although, the legal regulations and food organizations still need more evidences to confirm its effectiveness on regulating liver diseases. **(Rajaratnam *et al.*, 2014).** Medical plants being important sources of natural antioxidants, their importance for use as nutritional supplements or food additives have already been established **(Kaur and Kapoor, 2000).** Searching for naturally occurring antioxidants from safe and effective edible plants especially herbs is now focused on **(Miliauskas *et al.*, 2004).**

Lemon balm (*Melissa officinalis*) is one of these known herbs from Lamiaceae family and mainly grows and cultivated worldwide for its edible properties. " Long time ago, Lemon balm was used in managing many diseases, such as gastrointestinal disorders, headaches, migraine, toothache, neurological diseases, rheumatoid, hypertension as a mean for treatment and protection, as well as for relieving of menstrual cramps and fever caused by cold" **(Wichtl, 2004; Jun *et al.*, 2012 and Ondrejovic *et al.*, 2012**).

Since more than 2,000 years ago, Lemon balm was extensively used in traditional medicine. Different biological activities were greatly reported for Lemon balm, which vary from reducing the heart rate, antivirus, antibacterial, antiinflammatory, anti-cancer, sedative, antioxidant, antispasmodic, a neurotherapeutic agent, to peripheral analgesic, besides its activity as a binding agent to cholinergic receptors. **(Naghibi *et al.*, 2005; Ghayoor *et al.*, 2010 ; Yosofi *et al.*, 2011 and Zarei *et al.*, 2015**).

Today lemon balm is commonly used in food industries **(Birdane *et al.*, 2007),** due to their properties as antioxidant agents (**Rostami *et al.*, 2010)**. Phenolic compounds were the most important components present in lemon balm such as caffeic acid, rosmarinic acid, metrilic acid, cholinergic acid, and flavonoids like luteolin7-oxide-glucoside, apigenin and monoterpene derivatives such as beta-caryophyllene, germacrene, oleanolic, volatile oil, and tannins **(Rasmussen *et al.*, 2011).** Furthermore a study by **Adelifar *et al.*, *(*2016)**

reported that supplementation of melissa officinalis to athletes can increase total antioxidant capacity and prevent the enhancement of malondialdehyed level.

Fatty liver can induce by certain drugs or toxins as oxytetracycline, which consider as a type of antibiotic called a tetracycline. It is commonly used antibiotic for the treatment of many diseases such as, Lyme disease, Relapsing Fever, Tularaemia, Syphilis, Plaque, Respiratory infection, Acne and Streptococcal infection. Oxytetracycline is generally considered as toxic at high doses, because it produce a fairly large number of opposite effects like, severe microvesicular steatosis of the liver in patients and finally produce hepatic damage" **(Jayanthi and Subash, 2010)**

In this study, we are trying to evaluate the probable hepatoprotective and antioxidant effects of lemon balm leaves against changes occurs in albino wistar rats intoxicated with oxytetracycline.

Material and Methods

Plant material: Lemon balm (*Melissa officinalis L.*) leaves were acquired from the International Herbals Company, Cairo, Egypt.

Chemicals: Oxytetracycline was acquired from Sigma Company for Pharmaceutical Industries Cairo, Egypt. Other chemicals and reagents used for the experiments were of analytical grade obtained from El-Gomhoria Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Animal Model: Thirty-two male albino rats of Wistar strain with average weight of 120-130 g. They were purchased from Laboratory of Animal Colony, Helwan, Egypt. The animals were housed in plastic cages with metallic stainless covers maintained in controlled temperature. Rats were fed with basal diet which formulated according to **NRC (1995)** and were kept to acclimatize for 1 week before the study was commencement as adaptation period. Water was provided *ad libitum*, animals were subjected to a 12 hours light and 12 hours dark schedule.

Experimental Design: The experiment was performed in Animal House in the Faculty of Home Economics Cairo, Egypt. The rats were randomly divided into four groups of 8 animals per group and treated as follow:

Group I served as normal control group, and was fed on the basal diet and *ad libitum*

Group II served as untreated fatty liver group. The rats injected intraperitonealy with oxytetracycline (120 mg/kg) for 3 days for fatty liver induction (**Nicola *et al*., 1996).**

Group III injected intraperitonealy with oxytetracycline (120 mg/kg body weight) for first 3 days and followed by administration of lemon balm at dose 10g/kg daily by gastric tube for next 30 days.

Group IV injected intraperitonealy with oxytetracycline (120 mg/kg body weight) for first 3 days and followed by administration of lemon balm at dose 20g/kg daily by gastric tube for next 30 days.

Daily food intake (FI) and body weight gain were calculated weekly. Food efficiency ratio (FER) was determined according to the method of **(Chapman *et al.*, 1959).** At the end of the experimental period, animals were anesthetized under light ether anesthesia and then sacrificed. Blood samples were collected and kept for 30 minutes without disturbance then centrifuged at a rate of 3000 (rpm) for 15–20 minutes to yield the serum, which collected into sterilized tubes and stored at -20 °C **(Helal *et al.*, 2012)**

Biochemical analysis

Serum glucose: was detected according to colorimetric enzymatic method described by **Tietz (1994).**

Liver enzymes assay: Aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined by the method of **Breuer (1996).** Serum γ-glutamyl transferase (GGT) was performed by kinetic method according to **Persijn *et al.*, (1976).** Lactate dehydrogenase (LDH) was assayed by the method of **Wrebleski and La Due (1975)**. Serum total protein and albumin levels were determined according to the method of **Doumas *et al.*, (1975) and Dumas *et al.*, (1997).** The

globulin value for each sample was obtained by subtracting the albumin value from the corresponding total protein value. The A/G ratio for each sample was obtained by dividing the albumin level to globulin level.

Serum lipids profile assay: Total lipids were assayed by the method of **Kaplan (1984).** Serum triglycerides (TG) were determined according to the method of **Fossati and Prencie (1982).** Serum total cholesterol (TC) was performed according to **Henry *et al*., (1974).** Serum high density lipoproteins cholesterol (HDL-cholesterol) was assayed according to **Burstein (1970)**. The concentration of low density lipoproteins cholesterol (LDL-cholesterol) in serum was estimated by the equation used by **Friedewald *et al*., (1972**) as follow:

LDL- cholesterol (mg/dl) = Total cholesterol - HDL cholesterol - (TG/5).

Determination of serum urea and creatinine: Measurement of serum urea was done according to the method of **Patton and Crouch (1977)**. Serum creatinine was evaluated according to the method of **Jaffe (1980).**

Determination of serum antioxidant and oxidant parameters: Superoxide dismutase (SOD) activity, total antioxidants capacity (TAC), and malondialdehyde (MDA) were determined according to **Nishikimi *et al*., (1972); Cao *et al*., (1993) and Ohkawa *et al*., (1979)**, respectively.

Data analysis: All data were expressed as mean ± standard deviation. Data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups**. (Snedecor and Cochran, 1967).**

Results

Effect of Lemon balm on nutritional parameters of fatty liver rats.

After a 30 day consumption of two doses lemon balm leaves (10 and 20 g/kg) the body weight was significantly increased of treated groups with two doses of lemon balm (10 and 20 g/kg) as compared with fatty liver (untreated) group (Table 1). The level of body weight in treated group received 20g/kg of lemon balm leaves was lower when compared to normal control and also with treated group that received 10g/kg of lemon balm. Likewise the feed intake readings and food efficiency ratio were significantly ($p \leq 0.05$) higher in treated groups with lemon balm leaves as compared with untreated group.

Table 1: Effect of Lemon balm administration on nutritional parameters in rats with fatty livers

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
Body weight gain (g)	113.76 ±8.11 [a]	66.59± 6.11 [b]	105.77±9 .17 [a]	102.13± 9.13 [a]
Food intake (g/day)	15.94± 2.20 [a]	13.58± 2.03 [b]	15.85±2. 32 [a]	15.16±2.21 [a]
Food efficiency ratio	0. 255±0. 03 [a]	0.175± 0.02 [b]	0.257±0. 04 [a]	0.246±0.03 [a]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different ($P \leq 0.05$);
LBL: Lemon Balm Leaves.

Effect of Lemon balm on hepatic marker enzymes and serum glucose of fatty liver rats.

Data in table (2) indicated that, the liver marker enzymes (ALT, AST, GGT and LDH) were significantly ($p \leq 0.05$) increased in fatty liver

group (untreated) as compared to normal as well as treated groups with lemon balm at the two doses (10 and 20 g). Levels of AST and ALT in untreated fatty liver group were elevated as (128.23, 95.66 U/ml). Likewise, the elevation of GGT and LDH readings were 22.38 iu/ L and 433 U/L.

Table 2: Effect of Lemon balm administration on serum glucose and liver enzymes of fatty liver rats

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
AST (U/ml)	94.78±1.12 [d]	128.23±1.38 [a]	96.89±1.16 [c]	107.49±1.22 [b]
ALT (U/ml)	49.14±1.74 [d]	95.66±1.58 [a]	50.90±1.72 [c]	55.76±1.65 [b]
GGT(iu/L)	10.24±0.43 [d]	22.38±0.83 [a]	11.16±0.77 [c]	13.87±0. 22 [b]
LDH (U/L)	189±2.95 [d]	433±3.48 [a]	197±2.89 [c]	201±2.77 [b]
Glucose (mg/dl)	72.63±1.48 [c]	96.0±1.22 [a]	73.4±1.36 [c]	79.94±1.22 [b]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different (P≤0.05);

AST: aspirate amino transferase, ALT: alanine amino transferase, GGT: gamma glutamyl transferse , LDH : lactate dehydrogenase.

Data also demonstrated a significant (p≤0.05) elevation of serum glucose level in fatty liver untreated rats compared to normal control (Table 2). Rats treated with lemon balm for 30 days resulted in significant decrease when compared to untreated fatty liver rats.

Effect of Lemon balm on serum total proteins, albumin, globulin and A/G ratio in of fatty liver rats.

There was a significant ($p \leq 0.05$) increase in serum total protein of lemon balm treated groups compared to the untreated fatty liver group (Table 3). Results showed that groups treated with two doses of 10 and 20g/kg lemon balm leaves, had significantly ($p \leq 0.05$) increasing levels of serum albumin compared to fatty liver (untreated) group. Likewise the rats treated with lemon balm showed significant ($p \leq 0.05$) elevation in the serum globulin when compared with untreated fatty liver group. While A/G ratio showed significant change in 10 g lemon balm treated group that produced value similarly to normal control group in comparison with untreated fatty liver group.

Table 3: Effect of Lemon balm administration on serum total proteins, albumin, globulin and A/G ratio of fatty liver rats

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
Total Protein (g/dl)	7.59±0.08 [a]	5.87±0.09 [d]	7.35±0.11 [b]	6.14±0.22 [c]
Albumin (A) (g/dl)	4.43±1.74 [a]	3.73±0. 08 [d]	4.44±0.09 [b]	4.19±1.54 [c]
Globulin (G) (g/dl)	3.16±0.08 [a]	1.90±0.08 [d]	3.15±0.07 [b]	3.02±0.09 [c]
A/G ratio	1.42±0.07 [c]	2.18±0.01 [a]	1.46±0.06 [b]	2.09±0.03 [a]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different ($P \leq 0.05$).

Effect of Lemon balm on lipid profile in rats with fatty livers

As presented in Table (4), fatty liver rats of untreated group exhibited remarkable increase in total lipids, TG, TC and LDL-C levels compared to normal control ($p \leq 0.05$). Decline in the untreated fatty liver group was cleared regarding serum HDL-C levels, but the difference was not statistically significant. After treatment with lemon balm at the

two doses of 10 and 20g/kg, TG, TC and LDL-C levels were declined significantly compared to the untreated fatty liver group ($p \leq 0.05$).

Table 4: Effect of Lemon balm administration on serum total lipids, Triglycerides, cholesterol, LDL and HDL in rats with fatty liver

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
Total Lipids (mg/dl)	309.11±1.6 [d]	411.42±2.1 [a]	314.07±1.7 [c]	323.22±1.8 [b]
TG (mg/dl)	42.14±1.9 [c]	60.31±1.4 [a]	43.87±1.8b [c]	45.57±1.7 [b]
TC (mg/dl)	95.80±1.5 [c]	104.34±1.5 [a]	95.40±3.1 [c]	99.18±2.1 [b]
LDL-C (mg/dl)	40.58±2.88 [c]	53.86±1.4 [a]	46.55±1.4 [c]	48.97±1.4 [b]
HDL-C (mg/dl)	46.79±1.6 [a]	38.42±1.2 [c]	40.08±1.6 [a]	41.10±1.8 [b]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different ($P \leq 0.05$);
TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein cholesterol, LDL: Low density Lipoprotein cholesterol.

Effect of Lemon balm on serum urea and creatinine in rats with fatty livers

Data in Table (5) showed that, untreated fatty liver group recorded high significant ($p \leq 0.05$) increase in serum urea and creatinine compared to normal control group. While, administration of two doses lemon balm to the end of experiment tended to ameliorate the effect of oxytetracycline on serum urea and creatinine levels.

Table 5: Effect of Lemon balm administration on serum urea and creatinine in rats with fatty liver

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
Urea (mg/dl)	37.12± 2.6 [c]	86.54± 8.15 [a]	40 .72± 1.9 [bc]	44.21± 1.7 [b]
Creatinine (mg/dl)	0.71± 0.02 [c]	2.04± 0.01 [a]	0.79± 0.08 [c]	1.93± 0.06 [b]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different (P≤0.05);

Effect of Lemon balm on SOD, total antioxidant capacity and MDA levels in rats with fatty livers

As shown in Table (6), injection of oxytetracycline caused an elevation of hepatic MDA, but a decline of total antioxidant and SOD levels compared to normal control group. The elevated MDA level was reduced by 47% and the total antioxidant and SOD levels increased by 29 and 23% in lemon balm group that treated with 10g/kg. Similarly, MDA decreased by 53% and total antioxidant and SOD increased by 41and 30% in lemon balm group that treated with 20g/kg. Compared to fatty liver (untreated) group, the group that treated with lemon balm at dose of 20g/kg was more effective in elevating the content of hepatic SOD (119 vs 154.73, p≤0.05).

Table 6: Effect of lemon balm administration on serum antioxidant parameters in rats with fatty liver

Parameters	Normal control	Fatty liver (Untreated)	Fatty liver + LBL (10g/kg)	Fatty liver + LBL (20g/kg)
SOD (U/mL)	179.70 ±9.95 [a]	119.00± 7.00 [d]	146.37±8.55 [c]	154.73±9.07 [b]
Total antioxidants (mmol/L)	2.35±0.22 [a]	1.37±0.15 [d]	1.77±0.15 [c]	1.94±0.06 [b]
MDA (mmol/L)	4.52±0.33 [c]	10.34±1.09 [a]	5.48±0.26 [b]	4.83±0.21 [bc]

Values are expressed as mean ± SD, Means with different superscript litters within a row are significantly different (P≤0.05);
SOD: Superoxide dismutase, MDA: Malondialdehyde **.**

Discussion

One of the common liver disease distributed in Egypt is fatty liver, and possibly worldwide. Moreover, patients seem to be rapidly affected, so, increasing the disease has reached epidemical rates. Nowadays, fatty liver demonstrated as reference to insulin resistance and metabolic syndrome and seem to be an independent predictor of cardiovascular morbidity and death-rate **(Helal *et al.*, 2012).** Medicinal plants being important sources of natural antioxidants, and the search for safe and effective naturally edible plants especially herbs are now focused on **(Miliauskas *et al.*, 2004).** Lemon balm (*Melissa officinalis L*) plant is known as one of the oldest herbaceous aromatic plants, with therapeutic effects that ascribed to a variety of bioactive components in lemon balm leaves which make therapeutic properties possible **(Zarei *et al.*, 2015).**

When liver gets damaged after oxytetracycline induced fatty liver, it leads to leakage of cellular enzymes into plasma **(Ozougwu *et al.*, 2014).** The increased levels of serum enzymes such as ALT, AST, and LDH observed in fatty liver group (untreated) compared to the normal rats , that could be due to hepatocellular damage because these enzymes are normally located in the cytoplasm and released into the circulation after cellular damage **(Hassan and El-Gendy, 2003).** Furthermore, elevated serum levels of AST, ALT and LDH were indicative of poor hepatic function in untreated fatty liver rats **(Moss and Handeson, 1999)**. On the other hand, the significant decrease in the serum levels of the ALT, AST, GGT and LDH in lemon balm administered animals might be due to decreased leakage from the liver cells. This suggests that the leaves of lemon balm were able to repair the probable hepatic injury and restore the cellular permeability; thus reducing the toxic effect of oxytetracycline on the liver cells. It seems that, the effect of lemon balm on reducing liver enzymes is known to be due to its powerful antioxidant properties. This plant contains phenolic compounds, which are among the most important antioxidant agents **(Zarei *et al.*, 2014).** Serum total protein, albumin and globulin were decreased in untreated fatty liver group, while A/G was increased. This decrease could be due to hepatic dysfunction and decreased protein synthesis. Also it may be related to the damage of vital biological processes or to changes in permeability of liver, kidney and other tissue cells leading to leakage of protein via the kidney **(Helal *et al.*, 2012).**

Oxytetracycline induced hyperglycemia in fatty liver rats and treating with lemon balm leaves with 10 and 20 g turning glucose levels back to normal values. The improvement in glycemic status may be due to essential oil of lemon balm that has anti diabetic properties and improves glucose tolerance and adjusts the expression of the genes involved in hepatic gluconeogenesis **(Chung *et al.*, 2010).** As well as oxytetracycline caused kidney dysfunction which appeared through high increase in serum urea and creatinine levels, while after treatment with

lemon balm leaves, renal function indicators back to normal values, which related to the presence of effective and bioactive antioxidants, especial ability to inhibit the production of free radicals, that has given a unique feature to this plant. These results are in correlation with researches of **Namjoo *et al.*, (2013)** showed that serum activity of urea and creatinine levels decreased significantly after treating with lemon balm extract.

In the present study, the ameliorative effects of lemon balm on lipid profile are in agreement with **Bolkent *et al.* (2005)** who indicated that, lemon balm improved lipid profile of hypercholesterolemic rats by reducing serum lipid concentrations and lipid peroxidation in the liver of rats. Furthermore, **Jun *et al.* (2012)** revealed that, "lemon balm extract produced significant decrease in serum triglycerides levels and the extract exhibit a significant lipid reducing activity and protect tissues from lipid peroxidation". In addition **Changizi-Ashtiyani *et al.* (2013)** reported that the hypolipidemic properties of lemon balm were related to the antioxidant properties.

Lipidperoxidation is considering as one of the fundamental mechanism of cellular damage, caused by free radicals. Free radical reacts with lipid causing peroxidation, resulting in the release of product such as malanodialdehyde (MDA). Lipidperoxidation is one among these and it is a process, which is formed by means of the oxidation of polyunsaturated fatty acids, thus MDA is one of the final products of lipidperoxidation **(Ramesh and Dhanaraj, 2016)**. An increase in lipid-peroxides produce serious damage to cell membranes, inhibition of several important enzymes, reduced cellular function and cell death. The level of lipidperoxidation of untreated fatty liver group was significantly higher in the serum (10.34 mmol/L/) as compared with normal control group. But in treated groups with lemon balm leaves the level of lipidperoxidation were significantly lower (5.48, 4.83 mmol/L) as compared with fatty liver group (untreated). Such results are in

agreement with **Zarei *et al.* (2015)** who stated that lemon balm can inhibit the production of chemical active species in their early stages or later and that may block lipid peroxidation through various processes.

Administration of lemon balm showed significant hepatoprotective activity at 10g/kg and 20g/kg, which were comparable to the standard control group. The hepatoprotective effects were more pronounced with a lower dose of 10g/kg of lemon balm. The increased serum levels of ALT and AST levels in oxytetracycline treated animals might be due to the leakage of enzymes into the serum. Furthermore results revealed that the GGT increment in experimental period was higher like other values. These results are in parallel with that obtained by **Ramesh and Dhanaraj (2016)** who indicated that, the high level of GGT is an indicator to the liver damage which induced by chemicals substances. The increased level of AST, ALT and LDH were indicative of cellular leakage and loss of functional integrity of liver cell membrane **(Drotman and Lowhorn, 1978).** Also the elevation in total serum protein that, observed in oxytetracycline hepatotoxic rats suggested abnormal conjugation of total portein by the liver due to generalized hepatocellular damage (**El-Sherbiny et al., 2003).** Total serum protein was decreased in oxytetracycline hepatotoxic rats after treatment with lemon balm leaves. The possible mechanism of action of administration lemon balm leaves may be through their antioxidative effects. That because lemon balm has active ingredients that are capable of free radical scavenging in living system **(Dastmalchi *et al.*, 2008 ; Rostami *et al.*, 2010 and Zarei *et al.*, 2015).** These results were supported with levels of SOD and total antioxidant capacity, which were significantly decreased in untreated fatty liver group as compared with normal group. While after treatment with lemon balm the value of SOD significantly increased than untreated fatty liver group. The higher value was presented in 10g lemon balm treated rats. These results are in correlation with the finding of **Adelifar *et al.* (2016)** stated that administration of

lemon balm to athletes increase total antioxidant capacity and enhanced Malondialdehyed level.

Conclusion

Based on the above results, it can be concluded that lemon balm exert significant hepatoprotection against oxytetracycline induced fatty liver in experimental animals. Lemon balm leaves have demonstrated hepatoprotective activity based on reducing AST, ALT, GGT, LDH, glucose, TG, TC, LDL-C, urea, creatinine and MDA levels, while there are increase in total protein, albumin, globulin and HDL-C levels in groups treated with lemon balm leaves at two doses (10 and 20 g/kg) in compared with untreated fatty liver group . As well as increase in antioxidant parameters such as SOD and total antioxidant capacity in lemon balm treated groups comparing with untreated fatty liver rats. These encouraging results may have future clinical importance because of the increased use of natural herbs worldwide.

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Journal of Home Economics

المؤتمر الدولى السادس – العربى العشرون للإقتصاد المنزلى
" الاقتصاد المنزلى وجودة التعليم"
٢٣-٢٤ ديسمبر ٢٠١٨ م

http://homeEcon.menofia.edu.eg ISSN 1110-2578

استكشاف النشاط االواقي للكبد لبلسم الليمون (*Melissa officinalis* L.) ضد الأوكسي تتراسيكلين المسبب للكبد الدهني في فئران الألبينو

مع تزايد الأعباء على الكبد وإختلال وظائفه ، يزداد استخدام المنتجات النباتية الطبيعية بسبب خصائص محتواها القوي المضاد للأكسدة ، والآثار الجانبية القليله والميزات الاقتصادية.

خلال ٣٠ يوما هي فترة التجربة، تم تقسيم مجموعه ٣٢ من الفئران التي تزن ١٢٠-١٣٠ جم بشكل عشوائي إلى أربع مجموعات من ثمانية فئران في كل مجموعة. تناول عن طريق الفم (١٢٠ ملجم أوكسي تتراسيكلين/ كجم من وزن الجسم / يوم) لمدة ثلاثة أيام لإحداث الضرر الكبدي كما تم تحديدها من خلال حدوث زيادة معنوية في قياسات الكبد كالأسبرتات أمينوترانسفيراس (AST)، والألانين أمينوترانسفيراس (ALT) ، وجاما جلوتاميل ترانسفيراز (GGT) و لاكتات ديهيدروجينس (LDH) في السيرم، أيضا زيادة معنوية لمؤشر بيروكسيد الكبد الدهني (MDA).

أوضحت النتائج أن إعطاء بلسم الليمون بنسب (١٠ و ٢٠ جم / كيلوجرام من وزن الجسم) عن طريق الفم قد خفف من الأضرار الكبدية الناجمة عن تناول الأوكسي تتراسيكلين عن طريق خفض مستويات AST، ALT، GGT، LDH ، الجلوكوز ، TG، TC، LDL-C ، اليوريا ، creatininie و MDA في السيرم مع زيادة كبيرة في إجمالي البروتين في الدم ، الألبومين ، الجلوبيولين ، HDL-C في الفئران المعالجة بجرعات بلسم الليمون مقارنة مع المجموعة الغير معالجة. وكذلك زيادة في أنزيم سوبر أوكسيد دسميوتيز والقدرة على مضادات الأكسدة الكلية في المجموعات المعالجة ببلسم الليمون خاصة جرعة ١٠ جرامات مقارنة مع الفئران الغير معالجة.

تخلص الدراسة إلى أن تناول جرعات بلسم الليمون لها آثار تحسينية ملحوظة على مستوى إنزيمات الكبد و بيروكسيد الدهون في الفئران. ولذا نوصي بتناول أوراق بلسم الليمون للمجموعات المعرضة للإصابة بالكبد الدهنى لقدرة بلسم الليمون على الحماية الجزئية لخلايا الكبد من التلف الناجم عن تناول الأوكسي تتراسيكلين.

الكلمات المفتاحية: بلسم الليمون؛ الأوكسي تتراسيكلين؛ وقاية الكبد؛ مستوى الدهون؛ لاكتات ديهيدروجينيز؛ سوبر أوكسيد ديسموتاز.