Effect of thyme powder, extract and oil on carbon tetrachloride-induced liver injury

Nawal .A. Al Badr

Food Sciences and Nutrition Dep, Faculty of Food Sciences and Agriculture, King Saud
nawalbdr@yahoo.com

Abstract: Forty -two albino male rats, Sprague Dawley strain were randomly classified into six groups (7 rats each). One served as control (-ve) group while the other groups were administered CCL4 to induce liver injury which were control (+ve), silymarin, thyme powder, thyme extract and thyme oil rat groups. The results showed that control (+ve) rat group showed a significant decrease in final body weight , body weight gain ,food efficiency ratio (FER) , blood hemoglobin, packed cell volume & glutathione (GSH) , serum total protein & globulin and liver GSH , superoxide dismutase (SOD), glutathione peroxidase (GPX) ,glycogen and triglyceride. Moreover , showed a significant increase in blood malondialdehyde (MDA) ,serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST**,** ALP &γGT) enzymes activity ,total bilirubin ,A/G ratio and liver MDA, cholesterol and total lipid compared with control (-ve) group. Silymarin showed a significant decrease in final weight, hemoglobin, blood GSH, liver GSH, SOD & glycogen and a significant increase in serum AST& MDA and liver cholesterol. Thyme powder showed a significant decrease in final weight, blood GSH & MDA and liver GSH ,SOD and glycogen and a significant increase in serum ALT, AST**,** ALP and γGT enzymes activity but thyme oil showed a significant decrease in hemoglobin ,liver glycogen and significant increase in the values of liver cholesterol compared with control (-ve) group. The all treated rat groups showed a significant increase in serum total bilirubin, A/G ratio and liver MDA, triglyceride & total lipid and a significant decrease in body weight gain ,FER, serum globulin and liver GPX compared with control (-ve) group.
[Nawal .A. Al Badr. **Effect of thyme powder, extract and oil on carbon tetrachloride-induced liver injury.** Journal of American Science 2011;7(3):221-227]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: thyme leaves; oil ,extract; liver ;rats.

1. Introduction

The liver is the most important organ in terms of biochemical activity in the human body. The liver has a great capacity to detoxify and synthesize useful substances. There are several characteristic pathologies in the livers of patients with liver disease including fatty liver, hepatitis, hepatocirrhosis and liver cancer. Liver fibrosis is the common end stage of most chronic liver diseases regardless of the etiology the early stage of liver fibrosis can be reversed, while liver cirrhosis cannot (Achliya et al., 2004 and Bataller and Brenner, 2005).

Herbs treatments are safe because they are natural and fit into the image of a gentle harmless alternative to conventional medicine. Silymarin is known as milk thistle or Silybum marinum and is a member of the aster family that has been used as a medicinal plant since ancient times. Silymarin might exert beneficial effects in chronic liver diseases through antifibrotic properties. Silymarin interferes with leukotriene formation in Kupffer cell cultures and may thereby inhibit hepatic stellate cell activation, which is a crucial event in fibrogenesis (Stickel and Schuppan 2007 and Cecilia et al., 2009).The valuable medicinal properties of different plants are due to presence of several constituents such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters. Among them some are act as synergistic and enhance the bioactivity of other (Kaefer and Milner 2008). Essential oils are natural products extracted from vegetable materials, which can be used as antibacterial, antifungal, antioxidants, and anti-carcinogenic agents or to preserve and give specific flavors to foods (Kruma et al., 2008).

Thyme (*Thymus vulgaris L.*) is belonging to the Lamiacea family and is aromatic native herbs in the Mediterranean region. The leafy parts of thyme and its essential oil have been used in foods for the flavour, aroma and preservation so added to meat, fish and food products and also used as herbal medicinal products. Thymol and carvacrol displayed a concentration dependent antioxidant capacity (Undeger. et al., 2009). The main constituents of essential oil extracted from thyme were borneol, thymol , carvacrol methyl ether , camphene , α-humulene and carvacrol (Bounatirou et al., 2007 and Amarowicz et al., 2008). The present investigation was undertaken to study the effect of thyme leaves in form of powder, extract and oil on carbon tetrachloride-induced liver injury.

2. Material and Methods

I-Materials:

1-Carbon tetrachloride (CCL4) and silymarin:

Carbon tetrachloride was obtained from SIGMA Company for Pharmaceutical Industries. The calculated dose for inducing rats liver injuriy was 0.5 ml/rat, administered by back subcutaneous injection according to Moritz and Pankow ,(1989).Silymarin drug was obtained from (CID) Chemical Industries Development. Each capsule contains 140 mg of silymarin and given to rats in dose 50 mg/kg body weight of rats mixed in standard diet (Mourelle et al., 1989).

2- Thyme plant:

Thyme plant *(Thymus Vulgaris*) was obtained from local market. Then dried in dry freezer and crushed into powder then added to standards diet in 5% in substitution of fiber.

3-Experimental rats

Forty two Sprague Dawley strain male rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 110±8 g. The standard diet was performed according to Nelson (2000).

II-Methods:

1- Preparation of thyme extract and thyme oil:

Thyme extract and thyme oil were prepared from thyme powder according to Charles et al., (1993) and Radwan, (1978), respectively. Thyme extract was given to rats at dose 200 mg/kg b.w rat and thyme oil at dose 0.5 ml/kg b.w rat by stomach tube daily.

2-Experimental design:

After adaptation period, rats were divided into one group served as contol (-ve) rat group and five CCL4 induced liver injury groups which were control (+ve) and four treated groups which were silymarine, thyme powder, thyme extract and thyme oil rat groups. During the study period (8 weeks), the daily food intake and weekly body weight gain were recorded. Rats were sacrificed to otain blood and liver. Heparenized blood was used for estimation of hemoglobin, packed cell volume, malondialdehyde (MDA) and glutathione (GSH) according to Drabkin (1949), Mc Inory (1954), Yagi (1987) and Ellman (1958), respectively. Serum aminotransferase (ALT, AST), alkaline phosphates enzymes activity (ALP), gamma glutamyle peptidase (γGT), total protein and albumin, were estimated according to Reitman and Frankel (1957), Kind and King (1954), Henry, (1974),Weichselbaum (1946) and Bartholomev and Delany (1966) ,respectively. 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.Livers of rats were analyzed for estimation of GSH, MDA , superoxide dismutase (SOD), glutathione peroxidase (GPX) , glycogen, cholesterol , total lipids and triglyceride according to Reed (1999), Ohkawa et al .,1979 , Beuchamp and Fridovich (1971), Flohe and Gunzler.(1984), Rerup and Lundquist, (1967) ,Abell et al., (1952)., Folch et al.,(1957) and Young and Pestaner ,(1975) ,respectively.

III -Statistical analysis:

Collected data were subjected to analysis according to SPSS program according to Snedecor and Cochran (1967).

3. Results

The statistical data in table (1) showed a significant decrease in final weight, body weight gain and food efficiency ratio ($p<0.001$, 0.01&0.05) in control (+ve), silymarin and thyme powder rat groups but showed a significant decrease in body weight gain and food efficiency ratio ($p<0.05$) in thyme extract and thyme oil rat groups compared with control (-ve) group. Final weight, body weight gain and food efficiency ratio were significantly increase in the all treated rat groups compared control (+ve) rat group.

Table (1): Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of control and CCL4 treated rats.

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Initial. weight(g)	110.36± 4.22 ^{a}	112.41± 4.41 ^{a}	112.55± 5.31 ^{a}	110.33± 5.21 ^{a}	114.20± 4.32 ^{a}	115.31± 4.28 ^{a}
Final weight(g)	205.50± 11.12 ^{a}	157.62± 12.17 ^{c**}	188.86± 10.21 ^{b*}	183.54± 10.34 ^{b*}	196.65± 11.24 ^{ab}	197.62± 11.28 ^{ab}
Weight gain (g)	95.14± 5.44 ^{a}	45.21± 3.24 ^{d***}	76.31± 6.11 ^{c**}	73.21± 5.28 ^{c**}	82.45± 6.27 ^{bc*}	85.31± 6.60 ^{b*}
Food intake(g/w)	16.25± 1.71 ^{a}	14.31± 1.35 ^{a}	15.33± 1.21 ^{a}	15.11± 1.40 ^{a}	15.67± 1.22 ^{a}	15.11± 1.41 ^{a}
FER	0.097± 0.002 ^{a}	0.052± 0.001 ^{c***}	0.082± 0.003 ^{b*}	0.080± 0.001 ^{b*}	0.087± 0.004 ^{b*}	0.088± 0.002 ^{b*}

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

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

Table (2) showed a significant decrease in hemoglobin, packed cell volume and GSH ($p<0.01$&0.001) and a significant increase in MDA ($p<0.001$) the control (+ve) rat group and also showed a significant decrease in hemoglobin and GSH ($p<0.05$) and a significant increase in MDA ($p<0.05$) in silymarin rat group compared with control (-ve) group. The value of MDA was significantly increased but GSH was significantly decreased ($p<0.05$) in thyme powder rat group while the value of hemoglobin was significantly decreased ($p<0.05$) in thyme oil rat group compared with control (-ve) group. Silymarin and thyme oil rat groups showed a significant increase in GSH and a significant decrease in MDA while thyme powder and extract rat groups showed a significant increase in hemoglobin, packed cell volume and GSH and a significant decrease in MDA compared with control (+ve) rat group.

Table (2): The Mean values ± SD of hemoglobin packed cell volume, glutathione (GSH) and malondialdehyde (MDA) in control and CCL4 treated rats.

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Hemoglobin (g/dl)	14.10± 1.67 ^{a}	11.36± 1.51 ^{c**}	12.30± 1.14 ^{c*}	13.55± 1.38 ^{ab}	14.21± 1.11 ^{ab}	12.55± 1.35 ^{c*}
packed cell volume (%)	38.60± 5.40 ^{a}	31.82± 3.81 ^{b**}	34.21± 4.25 ^{ab}	37.17± 5.66 ^{a}	40.15± 6.20 ^{a}	33. 19± 3.33 ^{ab}
GSH (n mol/l cells)	7.30± 1.60 ^{a}	1.72± 0.13 ^{d***}	5.69± 1.03 ^{bc*}	5.40± 1.10 ^{bc*}	6.50± 1.08 ^{ab}	6.36± 1.19 ^{ab}
MDA (nmol/ml cells)	2.11± 0.15 ^{c}	6.20± 1.22 ^{a***}	3.61± 0.45 ^{b*}	4.14± 0.77 ^{b*}	2.51± 0.15 ^{c}	2.66± 0.44 ^{c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

Table (3) showed a significant increase in serum ALT, AST**,** ALP and γGT enzymes activity ($P<0.001$&0.01) in control (+ve) and thyme powder ($P<0.05$) while AST was significantly increased ($P<0.05$) in silymarin rat group compared with control (-ve) group. Serum ALT, AST**,** ALP and γGT enzymes activity were significantly decreased in all treated rat group compared with control (+ve) rat group.

Table (3): The Mean values ± SD of serum amino transferase (ALT & AST), gamma glutamyle peptidase (γGT) and alkaline phosphatase enzymes (ALP), of control and CCL4 treated rats groups

Groups Variables	Control(-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
ALT (µ /ml)	22.71± 3.77 ^{cd}	67.61± 9.11 ^{a**}	31.19± 5.60 ^{bc}	38.33± 6.21 ^{b*}	28.40± 4.11 ^{c}	27.37± 4.31 ^{c}
AST (µ /ml)	36.36± 4.22 ^{c}	78.99± 9.62 ^{a***}	43.87± 4.40 ^{b*}	44.30± 6.01 ^{b*}	40.17± 5.55 ^{bc}	41.31± 6.24 ^{bc}
ALP (µ /ml)	40.21± 4.14 ^{c}	83.11± 8.71 ^{a***}	38.67± 5.61 ^{c}	45.11± 4.21 ^{b*}	42.71± 3.21 ^{c}	39.99± 4.01 ^{c}
γGT (µ /ml)	5.14± 1.16 ^{c}	12.14± 2.18 ^{a***}	7.15± 1.24 ^{bc}	8.22± 1.55 ^{b*}	6.96± 1.65 ^{c}	6.59± 1.71 ^{c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (4) showed a significant increase in serum total bilirubin and A/G ratio ($P<0.001$) and a significant decrease in serum total protein and globulin ($P< 0.01$) in control (+ve) rat group. The all treated rat groups showed a significant increase in serum total bilirubin and A/G ratio and a significant decrease in serum globulin ($P<0.05$) compared with control (-ve) group but showed a significant decrease in serum total bilirubin and A/G ratio and a significant increase in serum total protein and globulin compared with control (+ve) group .

Table (4): The Mean values ± SD of serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) of control and CCL4 treated rats groups

Groups Variables	Control (-ve)	Control (+ve)	Silymarin drug	Thyme powder	Thyme extract	Thyme oil
Bilirubin (mg/dl)	0.55± 0.61 ^{c}	1.87± 0.18 ^{a***}	0.95± 0.05 ^{b*}	1.01± 0.12 ^{b*}	0.89± 0.02 ^{b*}	0.99± 0.01 ^{b*}
T.protein (g/dl)	7.57± 1.31 ^{a}	5.17± 0.88 ^{b**}	6.67± 1.12 ^{a}	6.11± 1.19 ^{a}	6.50± 1.05 ^{a}	6.49± 0.88 ^{a}
Albumin (g/dl)	3.49± 0.40 ^{a}	3.02± 0.33 ^{a}	3.21± 0.44 ^{a}	2.95± 0.28 ^{ab}	3.21± 0.48 ^{a}	3.13± 0.57 ^{a}
Globulin (g/dl)	4.04± 0.57 ^{a}	2.15± 0.23 ^{c**}	3.46± 0.36 ^{b*}	3.16± 0.38 ^{b*}	3.29± 0.55 ^{b*}	3.36± 0.44 ^{b*}
A/G ratio	0.86± 0.07 ^{c}	1.40± 0.13 ^{a***}	0.92± 0.05 ^{b*}	0.93± 0.03 ^{b*}	0.97± 0.02 ^{b*}	0.93± 0.01 ^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

The data in table (5) showed a significant decrease in the values of liver GSH SOD and GPX (P<0.001) and a significant increase in MDA in control (+ve), silymarin and thyme powder rat groups (P<0.05 & 0.01) but showed a significant decrease in the values of liver MDA and a significant decrease in GPX in thyme extract and oil rat groups (P<0.05) compared with control (-ve) group. Liver GSH SOD and GPX were significantly increased but liver MDA was significantly decreased in all treated rat groups compared with control (+ve) rat group.

Table (5): The Mean values ± SD of some liver glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in control and CCL4 treated rats

Groups Variables	**Control (-ve)**	**Control (+ve)**	**Silymarin drug**	**Thyme powder**	**Thyme extract**	**Thyme oil**
GSH(mg/g)	9.11± 2.14 ^{a}	3.17± 0.41 ^{d***}	6.71± 1.11 ^{b*}	5.99± 0.78 ^{bc*}	7.17± 1.20 ^{ab}	7.11± 1.14 ^{ab}
MDA(mmol/g)	30.21± 4.41 ^{d}	100.14± 20.14 ^{a***}	55.35± 6.17 ^{b**}	52.16± 5.20 ^{b**}	48.17± 7.11 ^{bc*}	45.33± 5.21 ^{bc*}
SOD(µ /mg)	41.87± 5.11 ^{a}	20.14± 3.21 ^{d***}	33.21± 4.55 ^{c*}	31.81± 3.29 ^{c*}	37.14± 6.36 ^{ab}	35.22± 4.33 ^{abc}
GPX(µ /mg)	60.61± 7.67 ^{a}	25.67± 3.14 ^{d***}	35.47± 4.11 ^{c**}	40.21± 4.57 ^{b*}	45.41± 5.12 ^{b*}	46.32± 6.14 ^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

The data in table (6) showed a significant decrease in the values of liver glycogen and triglyceride and a significant increase in the values of liver cholesterol and total lipid in control (+ve) at P<0.01 & 0.001 , silymarin and thyme oil at P<0.01 while showed a significant decrease in the values of liver glycogen and triglyceride and a significant increase in the value of liver total lipid (P<0.05) in thyme powder rat group but a significant decrease in the values of liver triglyceride(P<0.01) and a significant increase in the values of liver in thyme extract total lipid (P<0.05) when compared with control (-ve) group. All treated rat groups showed a significant increase in the values of liver glycogen and triglyceride and a significant decrease in the values of liver cholesterol and total lipid compared with control (+ve) rat group.

Table (6): The Mean values ± SD of liver glycogen, cholesterol, total lipids and triglyceride in control and CCL4 treated rats

Groups Variables	Control (-ve)	Control (+ve)	Silymarine drug	Thyme powder	Thyme extract	Thyme oil
Glycogen (mg/100g)	5.40± 0.44[a]	2.11± 0.14[c**]	4.33± 0.69[b*]	4.81± 0.87 [b*]	5.18± 0.68 [ab]	4.21± 0.77 [b*]
Cholesterol (mg/g)	4.11± 0.48[c]	6.98± 1.11[a***]	5.02± 0.13[b*]	4.55± 0.22 [c]	4.36± 0.44[c]	5.11± 0.38[b*]
Total lipids (mg/g)	35.81± 3.01[c]	52.78± 6.01[a***]	45.31± 5.21[b*]	42.16± 5.19 [b*]	39.71± 4.29[b*]	44.11± 5.21[b*]
Triglyceride (mg/g)	4.11± 0.58[a]	2.35± 0.11[c**]	3.21± 0.22[b*]	3.17± 0.37 [b*]	2.98± 0.18[b**]	3.11± 0.45[b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c, d) are significant.

4-Disscusion

It is well known that carbon tetrachloride (CCl4) has been widely used in animal models to investigate chemical toxin-induced liver injury. The most remarkable pathological characteristics of CCl4-induced hepatotoxicity are steatosis, fatty liver, cirrhosis and necrosis **(**Lee. et al 2005). The CCl4 produced damage to liver cells and was followed by the significant increase in serum alanine aminotransferase ALT activity and hepatic lipid peroxidation after 24 h. Increased lipid peroxidation is a mechanism which is commonly suggested to explain the progression of liver damage and the development of fibrosis, and eventually cirrhosis in experimental animals and in alcoholic liver disease (Goldani et al., 2007).Experimental studies demonstrated antioxidant and free radical scavenging properties, improvement of the antioxidative defence by prevention of glutathione depletion, and antifibrotic activity (Basu 2003).

According to several early studies, silymarin has hepatoprotective properties. Silymarin prevents of free-radical damage, stabilization of plasma membranes, and stimulation of new liver cell production (Jacobs et al., 2002). Silymarin acts as an antioxidant and free-radical scavenger that is many times more potent than vitamin E and has also been shown to inhibit lipid peroxidation and to prevent glutathione depletion induced by liver toxins, even increasing total glutathione levels in the liver by 35% over controls. Silymarin has the ability to stimulate protein synthesis resulting in production of new liver cells to replace older and damaged ones (Valenzuela et al. 1985 and Cecilia et al., 2009).

Thymus vulgaris L. has a large number of flavonoids and vitamin E. The main phenolic compounds in thyme are glycuronids of apigenin, luteolin, eriodyctiol, luteolin glycosides, rosmarinic acid and quercitine. These extracts from different parts of leaves and flowers of this plant are of interest as flavourings, as well as being natural antioxidants for the food industry (Justesen, 2000) Serum hemoglobin concentration was taken as response parameters for the bioavailability of iron. Dry thyme was particularly rich in iron. Iron intake and total iron absorption were highest for the rats fed the dry thyme diet (Abu Jadayil et al., 1999).

The essential oil of thyme plant had harmless effect on liver and kidneys tissues because of inducing little changes in aminotransferase activity in rat plasma and desirable changes in total cholesterol (Hazzit et al., 2006 and Nadia and Nadia 2008). Dietary supplementation of thyme extract to rats was able to reduce the acute hepatotoxicity caused by carbon tetrachloride. In particular, rats fed with thyme essential oil showed higher activities of liver endogenous enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase , as well as an increase of total antioxidant status compared to the control group (Vitaglione et al., 2004). There were significant declines in the superoxide dismutase activities and liver glutathione peroxidase increased significantly in the liver of old rats. There were also significant declines in the total antioxidant status in each tissue examined. A general feature of these various antioxidant parameters measured was that their activities remained higher in rats whose diets were supplemented with thyme oil because of a more favourable antioxidant capacity during their life span (Kuresh et al., 1999 and Kruma et al., 2008).

Thymol inhibited the non-enzymatic lipid peroxidation of normal mice liver homogenate. thymol protects the liver against CCl4 -induced toxicity and the protection may be mediated through its ability to inhibit lipid peroxidation. Thymol acted as a free

radical scavenger of lipid peroxidation in vitro (Aeschobach et al., 1989 and Alam et al., 1999). Volatile oil from thyme was overall the most effective in this protective capacity by reversing the normal trend in polyunsaturated fatty acid metabolism during aging wherein a decrease in levels is concomitant with a reduction in tissue function and integrity (Deans et al., 1993, Youdim and Deans 2000 and Dapkevicius et al., 2002).

Finally the aforementioned results recommended thyme leaves can protect the injury of liver.

References

1. Abell L.L., Levy, B.B., Brodie, B.B and Kendal, R. (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J .Biol .Chem, 357-366.
2. Abu Jadayil, S.; Tukan, S. K. H. and Takruri, H. R. (1999): Bioavailability of iron from four different local food plants in Jordan. Plant Foods for Human Nutrition, 54: 285-294.
3. Achliya, G.S., Wadodkar, S.G. and Dorle, A.K. (2004): Evaluation of hepatoprotective effect of *Amalkadi Ghrita* against carbon tetrachloride-induced hepatic damage in rats.Journal of Ethnopharmacology 90, 229–232.
4. Aeschobach R.; Loliger J.; Scotte B. C.; Murcia A.; Butler J. and Halliwell B. (1994): Antioxidant actions of thymol, carvacrol , 6- gingerol , zinzerone and hydroxytyrosol . Food Chem Toxicol; 32: 31 – 6.
5. Alam, K.; Nagi, M. N.; Badary, O. A.; Al-Shabanah, O. A. and Al-Rikaby, A. C. (1999): The protective action of thymol against carbon tetrachloride hepatotoxicity in mice. Pharmacological research, 40 (2) : 159 – 163.
6. Amarowicz, R.; Zegarska, Z.; Rafałowski, R.; Pegg, R. B.; Karamac, M. and Kosin, A. (2008): Antioxidant activity and free radical-scavenging capacity of ethanolic extracts of thyme, oregano, and marjoram. Eur. J. Lipid Sci. Technol., 110: 1–7.
7. Bartholomev, R.J. and Delany, A. (1966): Proc Aust. Assoc. Biochemists. 1, 214.
8. Basu, S. (2003): Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicology 189, 113–127.
9. Bataller, R.and Brenner, D.A. (2005): Liver fibrosis. Journal of Clinical Investigation 115,209–218.
10. Beuchamp, C an Fridovich, J. (1971): Superoxide dismutase. Improved an assay applicable to acrylamide gels. Anal Biochem.44:276-287.
11. Bounatirou ,S.; Smiti, S.; Miguel, M. G.; Faleiro, L.; Rejeb, M. N.; Neffati, M.; Costa, M. M.; Figueiredo, A. C.; Barroso, J. G. and Pedro, L. G. (2007): Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus*. *Food Chem*., 105: 146-155.
12. Cecilia, L. B., Enrique, J. S., Aldo, D. M .and Marcelo, G. R. (2009): Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. Chemico-Biological Interactions, 179, 297–303.
13. Charles, D.J.; Morales, R. and Simon, E. (1993): Essential oil content and chemical composition of hydroalcoholic extract of fennel. New crops,570-3.
14. Coles, E. H. (1974): Veterinary Clinical Pathology. Saunders Company, Philadelphia and London.
15. Dapkevicius, A.; Beek T. A. V.; Lelyveld, G. P.; Veldhuizen, A. V.; De Groot, A.; Linssen J. P. H. and Venskutonis R. (2002): Isolation and Structure Elucidation of Radical Scavengers from Thymus vulgaris Leaves. J. Nat. Prod., 65(6): 892-896.
16. Deans, S. G.; Noble, R. C.; Penzes, L. and Imre, S. G. (1993): Promotional effects of plant volatile oils on the polyunsaturared fatty acid status during aging. Age, 16: 71-74.
17. Drabkin, D.I. (1949): The standardization of hemoglobin measurements. Am. J. Med. Sci 21 (7): 710.
18. Ellman GL (1958): Liver glutathione. A colorimetric method for determining low concentration of glutathione. Arch Biochem Biophys 78: 443–450.
19. Flohe, L.and Gunzler, W.A. (1984): Analysis of glutathione peroxidase. Methods Enzymol 105:114–21.
20. Folch J; lees, M. Yloane, and Stanley, G.H. (1957): A simple method for isolation and purification of total lipid from animal tissue. J Biol Chem 266: 497-509.
21. Goldani, H. A. S.; Matte, U. S.; Ramos, A. R. L.; Costa, T. G.; Winkelmann, L. V.; Meurerc, L.; Vieiraa, S. M. G.; Kielinga, C. O. and Silveira, T. R. (2007): The role of food restriction on CCl4-induced cirrhosis model in rats. Experimental and Toxicologic Pathology, 58: 331–337.
22. Hazzit, M.; Baaliouamer, A.; Faleiro, M.L. and Miguel, M.G. (2006): Composition of the essential oils of Thymus and Origanum species from Algeria and their antioxidant and antimicrobial activities. *J. Agric. Food Chem.,* 54: 6314-6321.
23. Henry, R. J. (1974): Clinical Chemistry, Principles and techniques 2nd Edition Harper and Row P. 525.
24. Jacobs, B., Dennehy, C., Ramirez, G., Sapp, J. and Lawrence, V.A. (2002): Milk thistle for the

treatment of liver diseases: a systematic review and metaanalysis. Am J Med;113:506–15.
25. Justesen, U. (2000): Negative atmospheric pressure chemical ionization low-energy collision activation mass spectrometry for the characterization of flavonoids in extracys of fresh herbs. Journal of Chromatography A, Vol. 902, pp. 369-379.
26. Kaefer, C. M. and Milner, J. A. (2008): The role of herbs and spices in cancer prevention ournal of Nutritional Biochemistry 19: 347-361.
27. Kind, P.R and King, E.J. (1954): Estimation of alkaline phosphatase activity by determination of hydrolyzed phenol with aminoantipyrene. J. Clin.Path.7, 322.
28. Kruma, Z.; Andjelkovic, M.; Verhe, R. 2. and Kreicbergs, V. (2008): Phenolic compounds in basil, oregano and thyme. Foodbalt, 99-103.
29. Kuresh, A.; Youdim, S. G. and Deans, S. (1999): Dietary supplementation of thyme (*Thymus vulgaris L.*) essential oil during the lifetime of the rat: its effects on the antioxidant status in liver, kidney and heart tissues. Mechanisms of Ageing and Development, 109: 163-175.
30. Lee, G.P., Jeong, W.I., Jeong, D.H., Do, S.H., Kim, T.H and Jeong, K.S.(2005): Diagnostic evaluation of carbon tetrachloride-induced rat hepatic cirrhosis model. Anticancer Res. Mar-Apr; 25(2A):1029-38.
31. Mc Inory, R.A. (1954): A micro heamatocrit for determining the packed cell and hemoglobin concentration on capillary blood. J. Clin. Path. (7): 32.
32. Moritz, R.P. and Pankow, D. (1989): Effect of carbon tetrachloride and chloroform on hematologic parameters in rats: Folia Haematol Int Mag Klin Morphol Blutforsch. ; 116(2):283-7.
33. Mourelle, M., Muriel, P., Favari, L. and Franco, T. (1989): Prevention of CC14-induced liver cirrhosis by silymarin. Fundam Clin Pharmacol; 3:183–91.
34. Nadia, M. 1 and Nadia, M. (2008): Safety evaluation of thyme essential oil feeding on liver and kidneys functions of normal healthy rats. *J. Agric. Sci., Mansoura Univ.*, 33: 887-898.
35. Nelson, F. (2000): A Periodic Table of the Element from Mineral Institute, Google.
36. Nelson, F. (2000): A Periodic Table of the Element from Mineral Institute, Google.
37. Ohkawa, H., Ohishi, N. and Yagi, K.(1979): Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–8.
38. Radwan, S.S. (1978): Coupling of two dimension thin layer chromatograph with gas chromatography for the quantitative analysis of lipids classes and their constituent fatty acid .J. Chromatog. Sci., 16:538-542.
39. Reed, D.J. (1999): Glutathione: toxicological implications. Ann Rev Pharm Toxicol 30:603–631.
40. Reitman S. and Frankel S. (1957): Clin .Path. Am. J. 28: 57-63.
41. Rerup,E. and Lundquist, S. (1967): Precipitation and purification of liver glycogen in rats .Acta Pharmmic. Tox. 25:47-51.
42. Snedecor,G.W. and Cochran,W.G. (1967):Statistical methods .6th ed. Iowa State Univ.,Press Iowa Ames USA.
43. Stickel, F. and Schuppan, D. (2007): Herbal medicine in the treatment of liver diseases. Digestive and Liver Disease 39 293–304.
44. Undeger, Ü.; Basaran, A.; Degen, G.H. and Basaran, N. (2009): Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 Chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. Food and Chemical Toxicology, 47: 2037–2043.
45. Valenzuela, A., Lagos, C., Schmidt, K. and Videla, L. (1985): Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. Biochem Pharmacol; 34:22.
46. Vitaglione, P.; Morisco, F.; Caporaso, N. and Fogliano, V. (2004): Dietary Antioxidant Compounds and Liver Health. Critical Reviews in Food Science and Nutrition, 44: 575–586.
47. Weichselbaum, T.F (1946): An accurate and rapid method for the determination of protein in small amount of blood serum and plasma .Am .J. Clin .Path. (16):40.
48. Yagi K. (1987): Lipid peroxides and human diseases. *Chem Phys Lipids*45: 337-351.
49. Youdim, K. A. and Deans, S. G. (2000): Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *Br. J. Nutr.* 83 (1): 87 – 93.
50. Young, A. and Pestaner, D.L. (1975): Determination of triglycerides in serum .Clin.Chem. 21:5-7.

2/18/2011