Hepatoprotective and Therapeutic Activity of *Origanum syriacum* Aqueous Extract in Paracetmol Induced cell Damage in Albino Mice

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Abstract: Ethnomedically genus *Origanum* L. is one of the most commonly used herb in many countries as a stimulant, analgesic, antitussive, expectorant, sedative, anti-inflammatory and antihelminthic agent. The hepatoprotective and therapeutic effects of *Origanum syriacum* aqueous methanolic extract on paracetamol induced liver cell damage in mice with respect to antioxidant status was investigated. Mice were treated with extract and sylimarin in recommended dose after or before paracetamol administration (400mg/ kg/ day). Lipid peroxides concentration was considerably decreased due to the elevation of reduced glutathione concentration(GSH) and enhancing of glutathione reductase(GR), glutathione transferase(GST), glutathione peroxidase (GPx)and superoxide dismutase(SOD) activities as compared to paracetamol or sylimarin treated mice. Liver function parameters are still in the normal levels in extract treated mice as compared to control. Using extract as a treating agent after subjecting mice to paracetamol gave better results, the liver tissue showing a nearly normal liver tissue except for a little cellular infiltrate around main blood vessels while sylimarin showing a noticeable dilatation of blood vessels that are surrounded by fibrosis and cellular infiltration. Liver tissue from mouse received *Origanum* extract and then paracetamol showing mild dilatation of blood sinusoids and cellular infiltration around main blood vessels while sylimarin treated mice showed marked dilatation of blood sinusoids, vacuolar degeneration in many of the hepatocytes and focal necrotic areas among the hepatocytes. In conclusion, *Origanum syriacum* extract has potent therapeutic activity than hepatoprotective activity and it is more effective than sylimarin in two cases. The plant extract was screened for its phytochemical constitutions.
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1. Introduction

As the etiology and pathogenesis for many diseases are unclear, there has been increasing interest in the potential therapeutic and protective agents. Antioxidant compounds are usually employed in the food industry to prevent undesirable changes due to oxidation reactions. In recent years, there is a wide interest in finding natural compounds that could replace synthetic antioxidants. Herbs and spices are employed as food ingredient to flavour sausages, meats and salads; some studies have reported that they contain a wide variety of compounds that have shown to have beneficial health effects. It has recently become clear that one of the values of many spices is that they contain natural antioxidants, which provide protection against harmful free radicals. Oregano is an herbaceous plant native to the Mediterranean regions (Baytop, 1999) used as a medicinal plant with healthy properties like its powerful anti-bacterial and anti-fungical properties (Elgayyar et al., 2001& Sokovic et al., 2002), antihelminthic agent (Khanna et al., 2007) and anti-inflammatory (Robledo et al., 2005). Cervato et al.(2000) have found some antiradical activity in aqueous and methanolic extracts of *Oregano* leaves also Bendini et al. (2002) reported that ethanolic extracts under selected conditions showed antioxidant activity. *Origanum* species are traditionally used as sedative, diuretic, degasifier, sweater and antiseptic also in the treatment of gastrointestinal diseases and constipation (Baytop, 1999). Drug-induced liver injury is a potential complication of virtually every prescribed medication, because the liver occupies a central role in the metabolic disposition of all drugs and foreign substances. Most of the hepatotoxic chemicals damage liver cells mainly by lipid peroxidation and other oxidative damages and this applies also to paracetamol which is a widely used analgesic/antipyretic agent regarded as generally safe when used at therapeutic levels (Danque et al., 1993) while representing the drug of choice in children. However, paracetamol hepatotoxicity is the leading cause of drug-induced liver failure in the western countries and an acute or cumulative overdose can

cause severe liver injury with the potential to progress to liver failure (Lee, 2004). The main toxicity mechanism advocated for include the Cyp2E1 metabolic activation of the reactive metabolite, N-acetyl-p-benzoquinone imine which depletes cellular glutathione and then covalently binds to critical cellular proteins and macromolecules (Cohen and Khairallah, 1997, Paglia and Valentine, 1967) followed by proteins alkylation, namely mitochondrial proteins on its turn, triggers (Park et al., 2005), then formation of reactive oxygen species into the mitochondria (Knight et al., 2001). These events are primarily based on the dysfunction of the cellular Ca^{2+} homeostasis, with enhancement of the cytosolic Ca^{2+} concentration, noxious translocation of Bax and Bid to the mitochondria and peroxynitrite formation too. Superoxide anions insofar generated can dismutate to form molecular oxygen and hydrogen peroxide, which then require electrons from GSH molecules to be reduced to water by glutathione peroxidase enzyme and brings about a significant increase of mitochondrial glutathione disulfide (GSSG) levels (Griffith, 1980, Habig et al., 1974). The present study aims to investigate hepatoprotective and therapeutic effects of *Origanum syriacum* aqueous methanolic extract on paracetamol induced liver cell damage in mice with respect to antioxidant status in liver tissue.

2. Material & Methods:

Plants extract preparation

The aerial parts of *Origanum syriacum* were defatted using petroleum ether then the defatted powder was extracted with 20% aqueous methanol. The crude methanolic extract was concentrated using rotary evaporator under reduced pressure then the concentrated extract was used in phytochemical screening and biological studies.

Animals

Male albino mice weighing 30± 35g were housed in polypropylene cages, each cage was contained ten mice in case of LD_{50} assessment while it was contained eight mice in case of hepatoprotective study. Animals were fed on standard diet, temperature through the housing was controlled at 24ºC, relative humidity 65±5% and light/dark cycles (12/12hrs). This study was approved by Medical Research Ethics Committee, National Research Center, Egypt, under registration no. 10 033

Experimental design

Male albino mice (25-30g) were obtained from animal house of national Research Centre. Animals were kept for one day under the condition of experiment then they were intraperitoneally injected with 0.5ml of different solution used in experiment using infantile syringe. Animals were divided into three main groups includes negative control group, normal group treated with extract or sylimarin and treated group. Each subgroup and control group was contained eight mice. The control group was injected with saline solution (0.5ml/ day/ 5 days). The second main group is healthy normal group which was divided into three subgroups, the first one was injected with extract of medicinal plant prepared in saline solution (0.5ml of 1/10 extract LD_{50}/ day/ 5days), the second was injected with silymarin in a recommended dose (25mg/kg) for 5days prepared in 0.5ml saline solution while the third group was injected with paracetamol as a super saturated solution in 0.9 % saline at the dose of 500mg/ kg body weight. The third main group was treated group which contain four subgroups, the first one was treated with medicinal plant extract(0.5ml of 1/10 extract LD_{50}/day/5days) then was injected with paracetamol at a dose as mentioned above, blood samples, livers were collected after 48hrs of paracetamol injection. The second subgroup was treated with paracetamol then was injected with plant extract (0.5ml of 1/10 extract LD_{50}/ day/ 5days) after 48hrs of paracetamol injection blood samples and livers were collected after 24hr of last injection. The third subgroup was treated with silymarin (0.5ml of 25mg/ kg/ day/ 5days) then was injected with paracetamol in a dose of 500mg/ kg b.wt. while blood samples and livers were collected after 48hrs of paracetamol injection. The fourth subgroup was treated with paracetamol then was injected with silymarin (0.5ml contain dose of 25mg/ kg/ day/ 5days) after 48hrs of paracetamol injection, blood samples and livers were collected after 24hr of last injection.

Acute toxicity assay

The acute toxicity test for *Origanum* extract was carried out to evaluate any possible toxicity. Swiss albino mice (n = 10) of either ten mice were subjected to a 24 hour fast with water before initiating the test then was administered with different doses of the extract by increasing or decreasing the dose according to the response of animal (Bruce, 1985). The dosing patron was 500, 1000, 1500, 2000, 2500 and 3000mg/ kg by intraperitoneally injection while the control group received only the normal saline. All groups were observed for any gross effect or mortality during 48h.

Biochemical assessment

At the end of the experimental period, animals were fasted for 12 h and blood samples were obtained from the experimental and control mice by puncturing retro-orbital plexus. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were measured with kits (Reitman and Frankel, 1957). After collection of blood samples mice's livers were, collected then immediately excised, rinsed in ice cold normal saline. Liver homogenate(5%) was prepared in bidistilled water using potter-Elvehjem homogenizer with Teflon pestle. Protein concentration was measured as described**,** by Sedlack and Lindsay (1968). GPx activity was assayed spectrophotometrically at 340nm (Paglia and Valentine, 1967) and the amount of the

enzyme converting 1μmol GSH per min per mg protein was taken as 1 activity unit. GR activity was measured spectrophotometrically at 340nm (Goldberg and Spooner, 1983) and the amount of the enzyme reducing 1μmol GSSG per min per mg protein was regarded 1 activity unit as elsewhere described. GST activity was measured spectrophotometrically at 340nm (Habig et al., 1974) and the amount of the enzyme that conjugate 1, chloro-2, 4- dinitrobenzene with reduced glutathione per min per mg protein was regarded 1 activity unit. SOD was measured at 560nm (Fridovich , 1974) as the reduction suppression rate of nitrotetrazolium blue and for 1 unit of activity, the amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions. GSH concentration was measured spectrophotometry at 405nm (Griffith, 1980). and the unit of concentration was mg/g tissue using Ellman's reagent (5,5'-dithiobis 2-nitrobenzoic acid; DTNB), which was reduced by thiol groups to form 1 mol 2-nitro 5-mercaptobenzoic acid/mol thiol and with maximal absorption at 412 nm. Malondyaldehyde (MDA) determination in liver was assayed by spectrophotometric method at 534nm (Ohkawa et al., 1979). and the unit of concentration was μmol/ g tissue.

Statistical analysis

Data were analyzed by one-way ANOVA test for comparisons among means at $p \leq 0.05$.

Histopathological assessment:

Specimens of liver and kidney from all animals were dissected immediately after death. All the specimens were fixed in 10% neutral-buffered formal saline for 72 hours at least, washed in distilled water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 6 μm thick were cut and stained with Haematoxylin and eosin (Drury and Walligton, 1980) for histopathological investigation.

Histochemical investigation by using Periodic acid Schiff's reagent (Mac-Manus and Cason, 1950) was performed to evaluate the mucopolysaccharide content in these tissues.

Images were captured and processed using Adobe Photoshop version 8.0.

3. Results

1) Effect of OSE on antioxidant parameters:

Administration of paracetamol significantly decreased glutathione concentration also considerably reduces GR, GST, GPx and SOD activities while it elevated protein concentration and lipid peroxides concentration in liver tissue as compared to control group.

Data presented in table (1) shows the elevation of glutathione production in mice treated with extract and sylimarin as compared to paracetamol induced mice or healthy group administered saline, the healthy group administered OSE or sylimarin has the same trend of results. GR was significantly enhanced by administration of OSE and sylimarin but it was magnified by OSE administration more than sylimarin administration, sylimarin administration has approximately the same activity when administered as hepatoprotective or therapeutic agent but OSE is superior to sylimarin in two cases.

Paracetamol administration significantly decreased GST by 68% as compared to healthy group. Administration of OSE or sylimarin significantly enhanced GST and there was no significant difference between GST activities when OSE was administrated without liver injury or used as therapeutic drug after paracetamol administration, it means that GST was magnified to be in a healthy level. Also there was no significant difference in GST when sylimarin was administered as hepatoprotective or therapeutic agent. The best recorded activity was in case of OSE administration as therapeutic drug.

Data presented in table (1) shows that, paracetamol decreased GPx by 72% while OSE and sylimarin induced GPx in healthy animal treated with OSE or sylimarin also GPx was induced to be nearly ve- control group in paracetamol induced liver injury mice when treated with OSE. Sylimarin as protective agent gave results nearly the same to OSE as therapeutic agent, while it gave the least GPx activity when administered as therapeutic agent.

Injection of paracetamol highly decreased SOD by about 82% while OSE and sylimarin administration significantly enhanced SOD to be more than healthy group in healthy mice treated with OSE and sylimarin also SOD was elevated in liver injured mice when they were treated with OSE as hepatoprotective or therapeutic agent. Sylimarin also induced SOD and the hepatoprotective effect was more sufficient than therapeutic effect but OSE is superior to sylimarin in enhancing SOD activity either protective or therapeutic agent.

The obtained results indicates that injection of paracetamol significantly induced LPC by 90.5% while treating mice with extract or sylimanin didn't enhance liquid peroxide production so LPC is still nearly to control level in healthy treated mice also treating mice with extract or sylimarin protect liver from increasing of lipid peroxide as a response of liver injury with paracetamol in both methods of treatments, as hepatoprotective or therapeutic. *Origanum syriacum* extract significantly decrease LPC as compared to peracetamol group and there is no significant difference between ve- control group (injected with saline) and group treating with extract as hepatoprotective agent, these results are true with sylimarin. Treating mice with extract as therapaeutic agent showed the best result in decreasing LPC as compared to paracetamol group.

The obvious mentioned results shows that the extract significantly increase all determined antioxidant enzyme activities and glutathione concentration so it significantly reduces lipid peroxide concentration in both cases, hepatoprotective or therapeutic agent, and it is superior than sylimarin and the best result was

Table (1): Antioxidant activity of *Origanum syriacum* extract in paracetamol induced - liver injury in mice

Parameter / Groups	**Glutathione concentration mg/g tissue Mean± SD**	**Protein concentration mg/ g tissue**	**Glutathione reductase activity µmol/mg protein/min**	**Glutathione transferase activity µmol/mg protein/min**	**Glutathione peroxidase activity µmol/mg protein/min**	**Superoxide dismutase activity U/mg protein**
Control (ve$^-$)	2.84±0.063 [a]	182.19±1.41 [a]	3.5±0.14 [a]	2.66±0.09 [a]	1.43±0.067 [a]	12.14±0.72 [a]
Paracetamol group (ve+control)	0.55±0.05	275.32±1.44	1.18±0.13	0.84±0.04	0.39±0.02	2.15± 0.11
Origanum extract (ve+control)	4.09±0.001 [a]	185.26±2.46 [a]	12.21±0.2 [a]	4.34±0.16 [a]	2.66±0.08 [a]	40.04±1.18 [a]
Sylimarine (ve+)	3.73±0.002 [a]	182.19±2.02 [b]	8.22±0.19 [a]	4.96±0.09 [a]	3.08±0.09 [a]	29.17±0.66 [a]
Extract as hepatoprotective agent	3.19±0.001 [a]	177.4±1.08 [a]	9.93±0.21 [a]	3.26±0.18 [a]	1.09±0.14 [a]	64.27±0.76 [a]
Sylimarin as hepatoprotective agent	2.92±0.06 [c]	175.33±0.99 [a]	8.67±0.22 [a]	4.63±0.07 [a]	1.33±0.05 [ac]	39.37±0.82 [a]
Extract as therapeutic agent	3.65±0.08 [a]	191.33±1.64 [a]	11.53±0.5 [a]	6.58±0.26 [a]	1.62±0.07 [a]	50.58±0.46 [a]
Sylimarin as therapeutic agent	2.48±0.09 [a]	158.43±1.68 [a]	8.49±0.22 [a]	3.94±0.05 [a]	0.92±0.06 [a]	46.78±0.89 [a]

Data are presented as the means±S.D a $P<0.001$, compared to paracetamol group b $P<0.05$, compared to control c n.s. , compared to control

recorded in case of extract as therapeutic agent.

Injection of paracetamol in an over dose significantly increased protein production in liver tissue while it was decreased when these animals were treated with extract or sylimarin as hepatoprotective agents and sylimarin as therapeutic agent although it was significantly increased when extract used as therapeutic agent. There was no significant difference between ve$^-$ control group and healthy groups treated with OSE and sylimarin also between OSE and sylimarin as hepatoprptective agents, this means that both of OSE and sylimarin have the same effect on protein production in liver tissue.

2) Effect of OSE on Liver function

Glutamic-oxalocetic transaminase was significantly enhanced by paracetamol as mentioned in table (2) while it was still at the control level when extract was administered as therapeutic agent also GOT was around the recorded values of healthy group when it was administered as hepatoprotective agent. Administration of sylimarin increased GOT when it was administered as hepatoprotective or as therapeutic drug, as compared to healthy group administered saline. The obtained results indicate that *Origanum* extract reduced the danger effect of paracetamol on liver by 72.9% as hepatoprotective and by 74.8% as therapeutic agent. *Origanum* extract showed the same effect on glutamic- pyruvate transaminase so there was no significant difference between healthy group treated with saline or healthy group treated with extract, treating liver injured

animals with extract prevent elevation of GPT by paracetamol administration. It is clear from the recorded activities that treating animals with sylimarin was less sufficient than *Origanum* extract when used as hepatoprotective or therapeutic agent.

Table (2): Liver function of *Origanum syriacum* extract in paracetamol induced - liver injury in mice

Groups / Parameter	Control (ve-) Mean±SD	Paracetamol treated group	*Origanum* extract	Sylimarin	Extract as hepato-protective agent	Sylimarin as hepato-protective agent	Extract as therapeutic agent	Sylimarin as therapeutic agent
Glutamic-oxalacetic transaminase	50.83 ±0.79[a]	206.85 ±0.79	50.98 ±0.96[c]	53.96 ±0.75[a]	55.85 ±1.07[a]	60.05 ±0.83[a]	52.4 ±0.6[a]	65.62 ±1.27[a]
Glutamic-pyruvic transaminase	44.33 ±0.63[a]	179.97 ±2.00	43.62 ±1.19[c]	45.45 ±0.56[c]	46.27 ±0.89[b]	47.36 ±0.68[a]	45.36 ±0.75[c]	49.31 ±0.63[a]

Data are presented as the means±S.D a $P<0.001$, compared to paracetamol group
b $P<0.05$, compared to control c n.s. , compared to control

Histopathological Results:

Using paracetamol had a marked damaging effect on many organs in the body. In liver tissue its effect appears in the form of marked dilatation of the main blood vessels with fibrosis around and multiple foci of cellular infiltration, proliferation of kuppfer cells in blood sinusoids and acidification of some hepatocyte cytoplasm (Fig. 1,b).

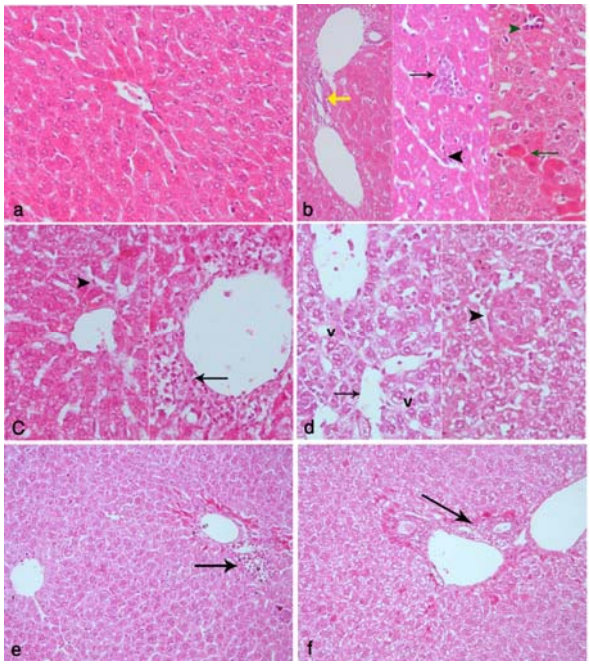

Figure 1: (a) is a section of liver tissue from a control rat showing the normal structure of it, where the hepatocytes are arranged in the form of plates radiating from the central vein. (b) is a section of liver tissue from a rat received paracetaml showing marked dilatation of blood vessels with fibrosis around (yellow arrow), focal cellular infiltration (black arrow), proliferation of kuppfer cells in blood sinusoids (black arrow head) and acidification of some hepatocyte cytoplasm (green arrow). Signs of apoptosis were observed in the form of DNA fragmentation (yellow arrow head) and karyolysis (white arrows). (c) is a section of liver tissue from a rat received origanum extract and then paracetamol showing mild dilatation of blood sinusoids (arrow head) and cellular infiltration around main blood vessels (arrow). (d) is a section of liver tissue from a rat received sylimarin and then paracetamol showing marked dilatation of blood sinusoids (arrow), vacuolar degeneration in many of the hepatocytes (v) and focal necrotic areas among the hepatocytes (arrow head). (e) is a section of liver tissue from a rat received paracetamol and then origanum extract showing a nearly normal liver tissue except for a little cellular infiltrate around main blood vessels (arrow). (f) is a section of liver tissue from a rat received paracetamol and then sylimarin showing a noticeable dilatation of blood vessels that are surrounded by fibrosis and cellular infiltration (arrow).
(Hx. & E. X 50, 100)

In our work, examination of renal tissue from mice exposed to paracetamol revealed the presence of deformity of glomeruli and vacuolar degeneration in

tubular lining epithelium in a good number of tubules (Fig. 2,b).

Using origanum extract as a protecting agent before subjecting mice to paracetamol gave moderate results as mild cellular infiltration specially around blood vessels in liver tissue (Fig. 1,C) and some deformed glomeruli in renal tissue (Fig. 2,C) were still noticed.

Using this extract as a treating agent after subjecting mice to paracetamol gave better results. Liver tissue showed quite normal liver tissue except for only very mild cellular infiltrate around blood vessels (Fig. 1,e) and renal tissue showed normal appearance except for a few glomeruli that appeared atrophied (Fig. 2,e).

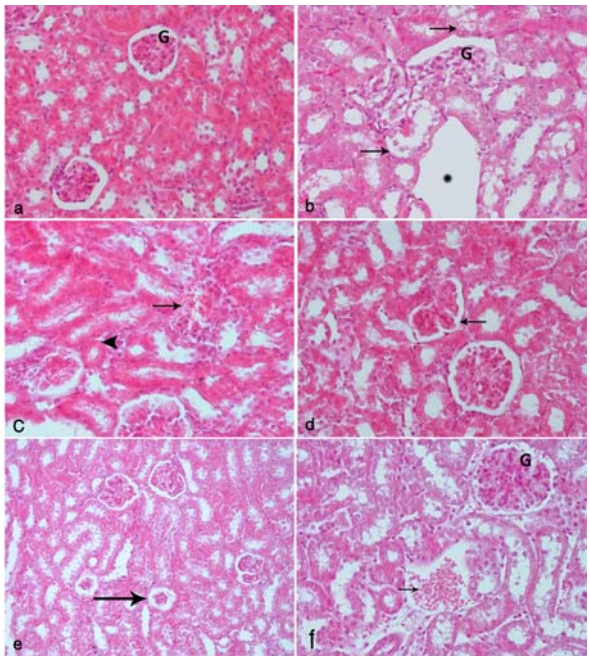

Figure 2: (a) is a section of renal tissue of a control rat showing the normal structure of this tissue composed of glomeruli (G) and different types of tubules. (b) is a section of renal tissue of a rat received paracetamol showing deformation of glomeruli (G), vacuolar degeneration of epithelial lining of the tubules and large gaps denoting edema. (c) is a section of renal tissue of a rat received origanum extract and then paracetamol showing that most of the tubules appear normal (arrow head), while glomeruli show some deformity and/or hemorrhage (arrow). (d) is a section of renal tissue of a rat received sylimarin and then paracetamol showing lobulation of some glomeruli (arrow). (e) is a section of renal tissue of a rat received paracetamol and then origanum extract showing a nearly normal renal tissue except for atrophy of a few glomeruli. (f) is a section of renal tissue of a rat received paracetamol and then sylimarin showing hemorrhage (arrow) in interstitial tissue. Some of the epithelial lining of tubules show vacuolar degeneration.

(Hx. & E X 50, 100)

On the other hand, using sylimarin as a protective agent gave less effective results than *Origanum* as in liver tissue marked dilatation of blood sinusoids, vacuolar degeneration in many of the hepatocytes, focal necrotic areas among the cells (Fig. 1,d) and lobulation of some glomeruli in renal tissue (Fig. 2,d) were still present.

Using sylimarin as a therapeutic agent gave better results than those obtained from using it as a protective agent, although still less than those obtained from *Origanum* extract as dilatation of blood vessels with fibrosis and cellular infiltration in liver tissue (Fig. 1,f) hemorrhage and vacuolar degeneration of the epithelial lining of some tubules in renal tissue (Fig. 2,f) was observed.

Results obtained from *Origanum syriacum* extract as a protecting and a treating agent were much better than those obtained from sylimarin used as a protecting and a treating agent respectively.

Histochemical results:

The histochemical results of the present work confirmed those of the histopathological investigations as Periodic acid Schiff reagent showed that injection of mice with paracetamol caused marked depletion of mucopolysaccharide content of cells both in liver tissue (Fig. 3, b) and in renal tissue (Fig. 4, b).

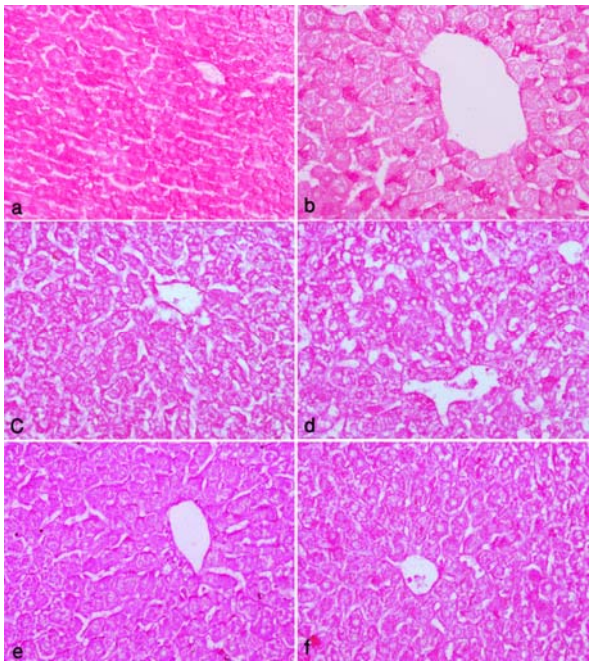

Fig. 3: (a) is a section of liver tissue from a control rat showing the normal component of mucopolysaccharides in hepatocytes. (b) is a section of

liver tissue from a rat received paracetaml showing marked depletion of mucopolysaccharides in most of the hepatocytes. (c) is a section of liver tissue from a rat received origanum extract and then paracetamol showing a mild decrease in mucopolysaccharide content in hepatocytes (d) is a section of liver tissue from a rat received sylimarin and then paracetamol showing a moderate decrease in mucopolysaccharide content in hepatocytes if compared with control group. (e) is a section of liver tissue from a rat received paracetamol and then origanum extract showing restoration of the normal mucopolysaccharide content in liver tissue. (f) is a section of liver tissue from a rat received paracetamol and then sylimarin showing a more or less same result as the previous group.

(Periodic acid Schiff X 100)

Using origanum extract before subjecting animals to paracetamol led to moderate amelioration in the mucopoltsaccharide content in hepatocytes (Fig. 3, C) and renal cells (Fig. 4, C), while using it after injection of paracetamol led to restoration of the normal content of mucopolysaccharides in both liver tissue (Fig. 3, e) and renal tissue (Fig. 4,e).

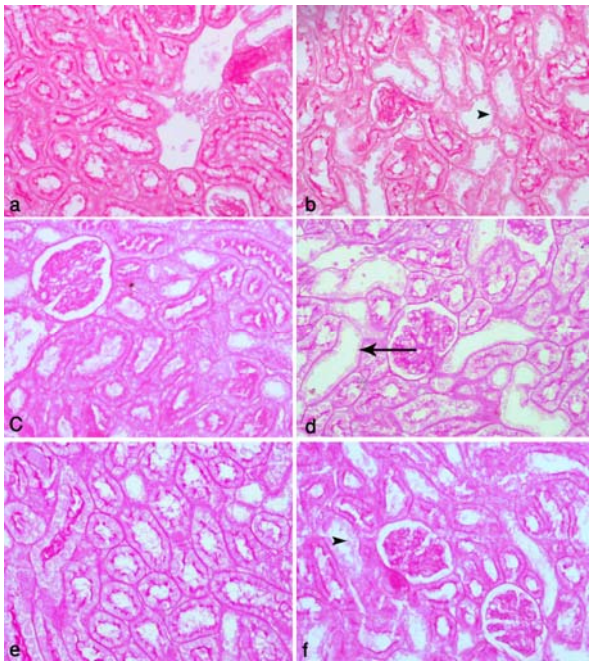

Fig. 4: (a) is a section of renal tissue of a control rat showing the normal content of mucopolysaccharides in renal tissue, being localized at the basement membranes of renal tubules and Bowmen's capsules and also in the brush borders of proximal convoluted tubules. (b) is a section of renal tissue of a rat received paracetamol showing loss of positive reaction of the stain at the site of brush borders of many tubules (arrow head). (c) is a section of renal tissue of a rat received origanum extract and then paracetamol showing a positive reaction of the stain at the normal places but with less density of the stain if compared with the control group. (d) is a section of renal tissue of a rat received sylimarin and then paracetamol showing a weak positive reaction of the stain and complete loss of it at the site of brush border of some tubules (arrow). (e) is a section of renal tissue of a rat received paracetamol and then origanum extract showing restoration of the normal content of mucopolysaccharide in renal tissue. (f) is a section of renal tissue of a rat received paracetamol and then sylimarin showing a loss of positive reaction of the stain at the site of brush borders of some tubules (arrow head).

(Periodic acid Schiff X 100)

Using sylimarin as a protective agent led to slight amelioration of mucopolysaccharide content in hepatocytes (Fig. 3, d) and in cells of renal tissue (Fig. 4, d). Using the same drug as a therapeutic agent gave better results, although they are less than those obtained from origanum extract in both liver tissue (Fig. 3, f) and in renal tissue (Fig. 4, f).

4. Discussion

Drug-induced liver injury (DILI) is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure Study Group (Rumack, 2004), DILI accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%). Because of the significant patient morbidity and mortality associated with DILI, the U.S. Food and Drug Administration (FDA) has removed several drugs from the market (Elgayyar et al., 2001). Paracetamol is widely used as an analgesic and antipyretic agent. However, accidental or intentional intake of high doses often causes acute hepatocellular necrosis with high morbidity and mortality (Park et al., 2005& Qiu et al., 2001). It was reported that many mechanisms are involved in paracetamol hepatotoxicity, Jaeschke et al. (2003) showing that the toxicity is mediated by CYP450 metabolism of paracetamol to N-acetyl- p-benzoquinone imine which covalently binds to critical proteins leading to inactivation of these proteins, especially after GSH depletion. CYP2E1 is usually assumed to be the most active CYP450 in catalyzing the metabolism of paracetamol to hepatotoxic NAPQI (Xia Chen et al., 2009).

The histopathological results of the present work go in coincidence with these reported findings as multiple foci of cellular infiltration, proliferation of kuppfer cells in blood sinusoids and acidification of some hepatocyte cytoplasm can be explained by the fact that the toxicity of paracetamol is mediated by generation of rather toxic

metabolite, N-acetyl- p-benzoquinone imine, whose detoxification may lead to a dramatic depletion of hepatic GSH (Yokozawa and Dong, 2001).

Deformity of glomeruli and vacuolar degeneration in tubular lining epithelium in a good number of tubules observed in sections of renal tissue from animals subjected to paracetamol are in coincidence with Ortiz *et al*. (2000) who stated that an acute paracetamol overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals and in severe cases to death. Paracetamol is a phenacetin metabolite. Phenacetin was considered one of the most nephrotoxic analgesics. Tubular cell loss is a characteristic feature of both acute renal failure and chronic renal disease and is observed when cell death predominates over mitosis. Apoptosis is an active form of cell death that offers the opportunity for therapeutic intervention.

There is increasing evidence to suggest that the endoplasmic reticulum stress apoptotic pathway is important in the kidney, specifically in tubular epithelial cells. It has been found that the expression of GADD153, a marker of endoplasmic reticulum stress, is increased in tubular epithelial cells during paracetamol-induced apoptosis. (Corinal *et al*., 2004).

The Importance of antioxidant constituents of plant materials in maintaining health and in protecting against many diseases and cancer is raising interest among scientists, food manufacturers and consumers, as the trend of the future is moving towards functional food with specific health effects (Kahkonen et al., 1999). In our present study, we evaluate *Origanum syriacum*, aqueous methanolic extract, hepatoprotective and therapeutic activities as compared to sylimarin which recommended as hepatoprotective drug. The antioxidant activity of extract in liver tissue was used as indicator for amelioration of liver to be healthier as compared to ve$^-$ control group and paracetamol induced liver injury group. It increased GST, GR, SOD, GSH and decreased lipid peroxides.

Glutathione-S-transferases are multifunctional enzymes, which play a key role in cellular detoxification. The enzyme protect cells against toxicant by conjugating them to glutathione, thereby neutralizing their electrophillic sites, and rendering the products more water soluble, the glutathione conjugates are metabolized further to therapeutic acid and then excreted. The enzyme is comprised of both cytosolic and microsomal enzyme.

Cellular glutathione peroxidase is a member of peroxidases enzyme whose function is to detoxify peroxides in cell because peroxides can decompose to form highly reactive radicals, the GP_X play a critical role in protecting cell from free radical damage. It catalyzes the reduction of H_2O_2 to organic peroxides using glutathione as a source of reducing equivalents (Paglia and Valentine, 1967).

Superoxide dismutase is metalloenzyme that catalyzes oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism that prevent body from disease linked to oxidative stress. Lipid peroxide concentration was determined as indicator for oxidative status of liver tissue.

Using *Origanum* extract as a protecting agent gave moderate results in both liver and renal tissue as mild cellular infiltration specially around blood vessels in liver tissue and some deformed glomeruli in renal tissue were still noticed, while using it as a therapeutic agent gave better results because liver tissue showed quite normal appearance except for only very mild cellular infiltrate around blood vessels and renal tissue appeared normal except for a few atrophied glomeruli.

Origanum effect on liver status may due to the high content of polyphenol in *Origanum* extract. It has a high content of total phenols and flavonoids also *Oregano* extracts (aqueous and methanol extracts) have very high polyphenol content while anthocyanins and catechins represent a smaller amount (Cervato et al., 2000). Rosmarinic acid is a dominant component detected in Oregano aqueous tea also the Oregano aqueous extract contains eriocitrin, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, Caffeic acid, quercetin, luteolin and apigenin so the aqueous tea infusions of *Oregano* represented a good source of the compounds with significant antioxidant activity (Kulisic et al., 2006)

In conclusion, the results indicate that the sufficient activity of *Origanum* extract in hepatic protection against administration of paracetamol in an over dose as liver injury drug. All results shows the role of *Origanum* extract in liver amelioration to be in a healthy status and it is more effective than sylimarin as hepatoprotective or therapeutic drug but it is superior as therapeutic than hepatoprotective also it is sufficient to decrease the oxidative stress on liver as mentioned in magnification of glutathione-antioxidant system and detoxification in liver with glutathione path way.

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References

1. Baytop T. Therapy with Medicinal Plants in Turkey: Today and in Future. Istanbul University Press, Istanbul, 1999. pp.166–167.

2. Balbaa SI. Chemistry of crude drugs, laboratory manual. Al- Shaab printing house, Cairo, Egypt, 1974. pp 1-194.
3. Bendini A, Toschi TG and Lercker G. Antioxidant activity of oregano (*Origanum vulgare*) leaves. Ital. J. Food Sci. 2002. 14: 17.
4. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundamental and Applied Toxicology 1985. 5: 151–157.
5. Cervato G, Carabelli M, Gervasio S, Cittera A, Cazzola R and Cestaro A. Antioxidant properties of extracts of oregano (*Origanum vulgare*) leaf extracts. J. Food Biochem. 2000. 24: 453-465.
6. Cohen SD and Khairallah EA. Selective protein arylation and acetaminophen- induced hepatotoxicity. Drug Metab Rev. 1997. 29: 59- 77.
7. Corina Lorz, Pilar Justo, Ana Sanz, Dolores Subirá, Jesús Egido and Alberto Ortiz. Paracetamol-Induced Renal Tubular Injury. A Role for ER Stress. J Am. Soc. Nephrol. 2004. 15: 380- 389.
8. Danque PD, Chen HB and Patil J. Image analysis versus flow cytometry for DNA plody Quantitation of solid tumors: A comparison of six methods of sample preparation. Mod. Pathol. 1993. 6: 270-275.
9. Drury RAB and Wallington FA. Carleton's Histological Technique 4th Ed. Oxford, New York, Toronto, Oxford University press 1980.
10. Elgayyar M, Draughon FA, Golden DA and Mount JR. Antimicrobial activity of essentials oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot. 2001. 64:10.
11. Feulgen R and Rosenbeck HC. Manual of Histological Demonstration Technique. Butter worth & Co (publishers) Ltd. London, Therford, havrhill 1942.
12. Fridovich I. Superoxide dismutases. Adv. Enzymol Relat Areas. Mol. Biol. 1974. 41: 35- 97.
13. Goldberg DM and Spooner RJ. Methods of enzymatic analysis(Bergmeyen H.V. Ed.) 3rd ed 1983. V. 3, pp. 258-265, Verlog Chemie, Deerfield beach, Fl.
14. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinyl pyridine. Anal. Biochem. 1980. 106: 207- 212.
15. Habig WH, Pabst MI and Jacoby WB. Glutathione-S- transferase. J. of Biol. Chem. 1974. 249(22): 7130-7139.
16. Hemant R and Pooja C. Prevention of Acetaminophen-Induced Mitodepression with Myrobalan (Fruit of *Terminalia chebula*) in *Allium cepa* Model. Iranian Journal of Pharmacology &Therapeutic (IJPT) 2005. 4: 100- 104**.**
17. Jaeschke H, Knight TR, Bajt ML.. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxicol Lett. 2003. 144: 279- 288.
18. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen, M. antioxidant activity of plant extracts containing phenolic compounds. Journal of Agriculture and Food Chemistry 1999. 47: 3954- 3962.
19. Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B. Natural product as a gold mine for arthiritis treatment. Current Opinion in Pharmacology. 2007. 7: 344– 351.
20. Knight TR, Kurtz A, Bajt ML, Hinson JA and Jaeschke H. Vascular and hepatocellular peroxynitrite formation during acetaminophen-induced liver injury: role of mitochondrial oxidant stress. Toxicol Sci., 2001. 62: 212- 220.
21. KULIsic T, Verica D and Malden M. Antioxidant Activity of Herbal Tea Infusions prepared from oregano, thyme and wild thyme. Food Technol. Biotechnol. 2006. 44 (4): 485– 492.
22. Lee WM. Acetaminophen and the US Acute Liver Failure Study Group: Lowering the risks of hepatic failure. Hepatology 2004. 40: 6- 9.
23. Lee KH, Lee JS, Lee JH, Kim *et al.* Prognostic value of DNA flow cytometry in stomach cancer: a 5-year prospective study. Br. J. Cancer 1999. 79 (11-12): 1727 – 1735
24. Lee WM, Squires RH Jr, Nyberg SL, Doo E and Hoofnagle JH. Acute liver failure: Summary of a workshop. Hepatology 2008. 47: 1401- 1415.
25. Mac-Manus JPA and Cason JE. Carbohydrate histochemistry studies by acetylation technique. Periodic acid method. J. Exp. Med. 1950. 91: 651.
26. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979. 95: 351- 358.
27. Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase.. J. Lab. Clin. Med. 1967. 70, 158-169.
28. Park BK, Kitteringham NR, Maggs JL, Pirmohamed M and Williams DP. The role of metabolic activation in drug-induced hepatotoxicity. Annu Rev Pharmacol Toxicol 2005. 45: 177- 202.
29. Qiu Y, Benet LZ, Burlingame AL. Identification of hepatic protein targets of the reactive metabolites of the non-hepatotoxic regioisomer of acetaminophen, 3'-hydroxyacetanilide, in the mouse *in vivo* using two-dimensional gel electrophoresis and mass spectrometry. Adv Exp Med Biol, 2001. 500: 663-673.
30. Reitman S and Frankel S. A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., Chicago 1957. 28: 56- 61.

31. Robledo S , Viviana MT, Diana LM, David LC, Ivan DV and Margorie AJ .. *Leishmania tarentolae*: utility as an in vitro model for screening of anti-Lishmanian agents. Antimicrobial Agents and Chemother. 2005. 49: 1652– 1655.

32. Rumack BH. Acetaminophen misconceptions. Hepatology 2004. 40: 10- 15.

33. Sedlack J and Lindsay RH. (1968). Estimation of total, protein bound and non sulphydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 192-205.

34. Sokovic M, Tzakou O, Pitarokili D and Couladis Mb. Antifungal activities of selected aromatic plants growing wild in Greece. Nahrung. 2002. 46: 317.

35. Yokozawa T and Dong E. Role of ginsenoside-Rd in cisplatin-induced renal injury: Special reference to DNA fragmentation. Nephron, 2001. 89: 433-438.

36. Xia Chen, Chang-Kai Sun, Guo-Zhu Han, Jin-Yong Peng, Ying Li, Yan-Xia Liu, Yuan-Yuan Lv, Ke-Xin Liu, Qin Zhou, Hui-Jun Sun. Protective effect of tea polyphenols against paracetamol-induced hepatotoxicity in mice is significanly correlated with cytochrome P450 suppression. World J Gastroenterol 2009. 15(15): 1829-1835.

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