The Protective Effect of Olive Leaf and Pomegranate Peel Extracts On Oxidative Stress And Liver Injury Induced By Oxytetracycline In Albino Rats.

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ABSTRACT: The present study aimed to investigate the possible protective effect of olive leaf extract (OLE) and pomegranate peel extract (PPE) against oxytetracycline (OTC)-induced hepatotoxicity in albino rats. The ethanolic extracts of olive leaf and pomegranate peel were screened for evaluating their free radical scavenging properties and total phenolic contents. They have a great antioxidant activity due to phenolic compounds. The protective role of the two examined extracts against OTC-induced alternation in blood biochemical and liver architecture was evaluated in male albino rats. OTC (200 mg/kg b.wt) was intraperitoneally (i.p.) injected for 15 days to assess the changes in biochemical parameters. OLE (80 mg / kg b.wt) or PPE (100 mg / kg b.wt) were administered by oral gavage into rats for 30 days to evaluate the potency of these extracts. The examined extracts were administered 15 days before and 15 days concomitantly with OTC. Blood samples were withdrawn at day 30 for determination of serum aminotransferases activity (ALT and AST), total protein (TP) albumin (Alb), total cholesterol (TC), triglycerides (TG), urea, creatinine, plasma malondialdehyde (MDA) and blood reduced glutathione (GSH). At the end of the experiment, the liver samples were taken for the histopathological examination. The obtained results revealed that the i.p. injection of OTC induced a significant increase in ALT & AST activity as well as TC, TG, urea, creatinine and plasma MDA, meanwhile a significant decrease in the levels of TP, Alb and blood GSH were obtained. These biochemical changes were associated with alternations in the architecture of liver tissue. The obtained results revealed that, the sole administration of OLE or PPE displayed no change in the examined parameters. The results also revealed the improving and protective effect of the pre and co-administration of the test extracts against the undue effects of OTC. 

Key words: Oxytetracycline, olive leaf extract, pomegranate peel extract, oxidative stress, transaminases, antioxidant markers, hepatic injury.

INTRODUCTION:
During the past half century, new information on drug-induced hepatic injury has filled an extensive database and the types of hepatic injury have been well–defined. Large number of drugs have been identified as potential causes of hepatic injury, a reasonable understanding of probable mechanisms of injury has emerged. The circumstance under which injury occurs is recognized; and the relative importance of drug induced injury is clear (Farrel, 1994).

Oxytetracycline (OTC) is a type of antibiotic called a tetracycline. It is commonly use as antibiotic for treatment of many diseases (Dollery, 1999). High dose of OTC is generally regarded as toxic and it has been reported that excessive dose of OTC produces hepatic damage (Saraswat et al., 1997). Also several lines of evidence show that OTC produces sever microvesicular steatosis of the liver in human (Pessayre et al., 2001). Freneaux et al. (1988) demonstrated the inhibition of mitochondrial β-oxidation of fatty acids by tetracycline in mice. Similarly, Amacher and Martin (1997) showed that the canine hepatocyte is susceptible to tetracycline-induced steatosis, when triglycerides (TG) accumulation were concomitant with the inhibition of mitochondrial lipid metabolism. Although other metabolic pathways could also be impaired, these additional mechanisms most probably play a secondary role in the pathophysiology and severity of microvesicular steatosis (Yin et al., 2006).

Some plants were firstly used by indigenous people in ecologically threatened areas may eventually become economically and medicinally important among industrialize people. This can take the form of a drug firm refining and marketing a pharmaceutical product or can occur more as phenomena with many individuals choosing to use raw or extracted plant products in their search for illness remedies or health maintenance (Barrett and Kieffer, 2001). Olea europea L. leaves are common medicinal plants. Benefits of the olive leaf (OLE) have been known for centuries and it has been traditionally used to prevent and treat some diseases (Dekanski et al., 2009). OLE is well known for its antioxidant properties (Bouaziz and Sayadi, 2005), antiatherogenic, anti-inflammatory, antitumoral (Grawish et al., 2010) and hepatoprotective properties (Poudyal et al., 2010). Olive leaf is the primary source of phenolic compounds. The major active components in olive leaf are known to be oleuropein and its derivatives such as hydroxytyrosol and tyrosol, as well as caffeic acid, p-coumaric acid, vanillic acid, vanillin,
leuteolin, dismetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside and diosmetin-7-glucoside (Farag et al., 2003). Oleuropein is one of the iridoid monoterpenes and the main phenolic constituent of olive leaves, which is thought to be responsible for their pharmacological effects. Furthermore, olive leaves contain triterpenes, flavonoids, and chalcones (Pereira et al., 2007). Its chemical content makes olive leaf as one of the most potent natural antioxidant (Dekanski et al., 2009).

Punica granatum L. (pomegranate, Family Lythraceae) is a deciduous tree distributed throughout the world. Pomegranate fruit is used in the traditional medicine of different Asian cultures for the treatment of a variety of ailments (Jurenka, 2008).

The beneficial effects of pomegranate include the cardiovascular protective role, neuroprotective activity, hypoglycemic effect and anticancer properties in particular against prostate, colon and breast cancer (Johanningsmeier and Harris, 2011). Recently, the interest in the antioxidant properties of phenolic constituents from pomegranate fruits (i.e., arils and peels) has emerged (Madrigal-Carballo et al., 2009).

Both flavonoids and tannins are more abundant in the peels. The presence of alkaloids (e.g., pelletierine) in the peel is equivocal (Lansky and Newman 2007).

The aim of this study is to investigate the potential hepatoprotective effect of olive leaf and pomegranate peel extracts as natural source antioxidants against liver injury in albino rats.

**MATERIALS AND METHODS:**

**Materials:**

**Plants**

The olive leaf (Olea europea) was collected from the Center of Agriculture Research, Giza, Egypt, meanwhile pomegranate fruits (Punica Granatum) were carried out from the local market.

**Chemicals**

Oxytetracycline, all chemicals and reagents used in this study were pure analytical grade and purchased from Sigma Company.

**Animals**

Thirty six adult male albino rats weighting 180 ± 20 g were provided from NODCAR, Egypt. The animals were housed and left for 2 weeks under standard laboratory conditions of light/dark cycle (12/12h) and temperature 25±1°C before starting the experiment for acclimatization and allowed to food standard diet of commercial pellets and tap water ad libitum. All animals received care in compliance with the Egyptian rules for animal protection.

**Methods**

**Preparation of olive leaf and pomegranate peel extracts (OLE and PPE)**

Pomegranate fruits were washed then peel were carefully separated. The washed and cleaned olive leaf and pomegranate peel were air dried in a ventilated oven at 40°C for 48 h and ground to fine powder. Five hundreds gram of both fine powders of plants were extracted twice, on each occasion with 2.5 L of 80% ethanol (Tavafi et al., 2012; Ahmed and Ali, 2010). The both filtrates were concentrated to dryness under reduced pressure in a rotatory evaporator then dried in freeze drier and stored at 4°C for further analysis.

**Total Phenolic Contents Measurement**

Total phenolic contents (TPC) in OLE and PPE were determined as mg gallic acid equivalent per g extract (mg GAE/g extract) according to Temraz and EI-Tantawy (2008) method.

**DPPH Radical Scavenging assay**

The ability of the OLE and PPE to scavange 1,1-diphenyl-2-picrylhydrazyl radical ( DPPH) free radicals were determined by the method described by Blois (2002).

**Biochemical evaluation**

The experiment was conducted on thirty six adult male wistar albino rats to study the protective effect of olive leaf and pomegranate peel extracts against oxytetracycline induced hepatotoxicity. The experiment was lasted for 30 days and rats were divided randomly into equal six groups (6 rats each).

Group (1): animals were received distilled water daily by oral gavage and served as negative control. Groups (2) and (3): rats were orally received a daily dose of OLE at 80 mg/kg b.w (Dekanski et al., 2011) or PPE at 100 mg/kg b.w (Ahmed and Enas, 2010) until the end of experiment. Group (4): Rats were intraperitonealy (i.p) injected with OTC (200mg/kg b.w daily) for 15 days. Groups (5) and (6): Rats were received OLE and PPE daily for 30 days and on 15th day the rats were i.p injected with OTC along the extract until the end of experiment. The change in body weigh was recorded. At the end of the experimental period blood samples from each animal were withdrawn in two separated tubes, one with anticoagulant for the determination of blood GSH and plasma separation for MDA, the 2nd tube without anticoagulant for serum separation. Rats were killed by cervical decapitation, the liver and kidneys were removed by dissection, washed with ice cold saline and blotted between two filter papers and then weighed.

**Biochemical analysis**

The activity of serum transaminases were determined by the method of Reitman and Frankel (1957), total protein by Doumas (1975), albumin was determined according to the method of Doumas et al. (1971), total cholesterol by Allain et al.(1974), triglycerides by Bucolo and David (1973), urea by Tabacco et al. (1979), creatinine according to Allston (1993), blood glutathione by Beutler et al. (1963) and malondialdehyde (MDA) by Buege and Aust (1978) with a slight modification in the incubation period according to the method described by Erdincler et al. (1997). The weight of liver and kidneys were calculated relative to body weight as liver index according to Peng et al. (2009).

**Statistical analysis**

The results were expressed as the mean values ± standard deviation of six animals in each group. Variance between groups was assessed by one-way analysis of variance (ANOVA). Subsequent multiple comparisons between the different groups were analysed by Duncan s multiple comparison test. Data were statistically analysed using the statistical package.
RESULTS:
Total phenolic content: TPC of the examined extracts was determined by Folin-Ciocalteu (F-C) assay using Gallic acid as a standard. TPC of the examined extracts are demonstrated in Table (1).
Free radial scavenging activity: The olive leaves and pomegranate peel extracts exhibited proton-donating ability by DPPH assay. The antioxidant potential is inversely proportional to IC50 which calculated from the liner regression of the % antioxidant activity versus extracts concentration is shown also in Table (1).
Biochemical assays: Data presented in Table (2) revealed that, the administration of OLE or PPE extracts during the time course of the experimental period elicited no effect on the activities of the examined hepatic marker enzymes (ALT and AST) as well as the levels of TP and Alb with respect to normal control group. Meanwhile, the i.p injection of OTC for 15 days caused a significant increase in ALT and AST activities compared to normal control. Significant reduction in the total protein and albumin levels was also recorded. The obtained data also revealed that the 15 days pre and 15 days co-administration of OLE or PPE with OTC caused a significant reduction in the activities of ALT and AST and a well marked increase in serum total protein and albumin levels compared with OTC-treated group. The depicted data also revealed that, the two examined extracts displayed the same effect on liver cells with restoring the enzymatic activities to be near the control values.
The continuous administration of OLE or PPE for 30 days induced a significant decrease in total cholesterol (TC) levels with no effect on TG levels as well as urea and creatinine in comparison with normal control group. The obtained data also revealed that, the i.p injection of OTC for 15 days caused a significant increase in TC, TG, urea and creatinine levels with respect to the control value. Meanwhile, the pre- and co-administration of OLE or PPE with OTC displayed a reducing effect on TC, TG, urea and creatinine levels (Table 3).
The administration of OLE or PPE extracts for 30 days exerted no effect on lipid peroxidative index as evaluated in the term of MDA and GSH compared with the normal control group (Table 4). Meanwhile, the i.p injection of OTC for 15 days induced a significant increase in plasma MDA level and a significant reduction in blood GSH compared to normal control. The pre and co-administration of OLE or PPE extracts caused a significant reduction in MDA levels and a significant increase in blood GSH with respect to OTC treated group.
The OTC treatment induced a significant increase in relative liver and kidney weight meanwhile, the pre and co-administration of OLE or PPE with OTC displayed a reducing effect of the relative liver and kidney weight and also the administration of OLE or PPE extracts did not cause any effect on liver or kidneys index marker (Fig 1).

Histopathological examinations
Liver sections obtained from the normal control animals, showed no histopathological alternations and the normal hepatocyte structure of the central vein and surrounding hepatocytes Fig. (2). Also liver sections obtained from animals treated with OLE or PPE extract alone did not show any significant alternations in the hepatic structure (Fig.3 and 4). However, histopathological examination shows characteristic pathological changes in OTC-treated group. These include fatty degenerative changes, inflammatory aggregations with dilation in the central vein (Fig.5 and 6). In contrast, liver sections obtained from rats pre and co- administered OLE or PPE with OTC showed ameliorative effect (Fig.7 and 8).

DISCUSSION:
Phenolics or polyphenols are secondary plant metabolities that are ubiquitously present in plants and plant products. Phenolic compounds contribute to the overall antioxidant activities of plants mainly due to their redox properties. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Li et al., 2009).
In the current study the ethanolic extract of PPE revealed the highest total phenolic content approximately 2 fold more than the ethanolic extract of OLE. The measurement of the scavenging of DPPH radical allows one to determine exclusively the intrinsic ability of substance to donate hydrogen atom or electrons to this reactive species in a homogenous system. The DPPH radical-scavenging capacity in the studies was reported after 30 min reaction time for all samples evaluated. The parameter used to measure the radical scavenging activity of extracts and fractions evaluated is IC50 value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC50 value, the higher antioxidant activity of the plant extracts (Maisuthisakul et al., 2007). PEE revealed the higher activity compared to that of OLE. The phytochemicals which might be responsible for the scavenging activity in the examined extracts is phenolic and flavonoid constituents. It has been reported that, PPE was rich in gallic acid, ellagic acid, flavonols, flavones, flavanones and anthocyanidines (Aslam et al., 2006). OLE contains a mixture of polyphenolic compound mainly oluropein and hydrotyrosol (Bouaziz and Sayadi, 2005; Dragana et al., 2009). The biological activity of OLE are derived from these compounds (kim et al., 2014). The obtained data revealed that, the i.p injection of OTC increased indices of liver function including ALT and AST. These elevations are attributed to hepatocellular damage and decreased liver function. This elevation of serum indices for hepatocellular damage has been previously reported in OTC-induced hepatotoxic model (Eman et al., 2011). The increased activities of serum ALT and AST are the most sensitive markers employed in the diagnosis of hepatic damage.

because these are cytoplasmic in location and are released into the circulation after cellular damage (Janbaz et al., 2004). Elevated levels of these enzymes in the serum are presumptive markers of drug-induced alternations in the hepatocytes (Shabana et al., 2012). The obtained increase in serum ALT and AST activities after 15 days of OTC administration is accompanied by a significant decrease in serum total protein and albumin levels as well as a significant increase in triglycerides and total cholesterol levels. The decrease in serum total protein and albumin recorded in the current work agrees with the results reported by Rafiq et al. (2010). The reduction of total protein and albumin levels indicate that the administration of drug caused impairment in the capacity of liver function to synthesize albumin from the hepatic parenchyma. The obtained increase in cholesterol in the current study confirm the finding of Santhosh et al. (2006) who reported that the damage in hepatocytes might cause the release of the cholesterol from the hepatocytes into the blood stream.

Urea and creatinine are waste products eliminated from the blood through the kidneys by blood filtration process. The significant increase in the level of urea in serum of OTC-treated rats is in the harmony with the results of earlier studies done by Miller and McGarity (2009). Shabana et al. (2012) reported that the increased levels of serum urea and creatinine in OTC-treated rats reflect the renal damage that tetracycline caused by the inhibition of the corporation of amino acids into protein causing an increase in the urea level. Many studies have demonstrated the ability of antibiotics to facilitate the generation of oxygen radicals both in vivo and in-vitro, and this process plays an important role in antibiotic induced tissue injuries. OTC is also known to cause hepatic dysfunction in man by inducing steatosis with the accumulation fat droplets in the hepatocytes (Begriche et al., 2011). In the present study, the increased fat accumulation occurred in the liver of rats treated with the higher dose of antibiotic could apparently provide more substrate for lipid peroxidation and in turn lead to enhanced generation of reactive oxygen species (ROS). The increase in plasma MDA that accompanied by a significant decline in blood GSH reflect the generation of ROS after OTC administration. The formed free radicals seemed to initiate lipid peroxidation in OTC-treated rats suggesting that, the increase in lipid peroxidative index as evaluated in term of plasma MDA and blood GSH might be associated with cellular damage.

The protective effect of OLE or PPE in recovering the induced reduction in hepatic function in the current study is indicated by the significant reduction in ALT and AST activities as well as TP, Alb, TC, TG, urea and creatinine levels. Zari and Al-Attar (2011) demonstrated the effectiveness of pretreatment with olive leaf extract in improving the hematological, biochemical and histopathological alternation of liver and kidney that induced by carbendazin intoxication in mice. The authors referred the improving effect of OLE to its direct free radical scavenging activity by the polyphenolic compounds present in OLE. According to this finding, it could be postulated that OLE preserved the structural integrity of liver tissue from the toxic effect of OTC. The obtained results are in accordance with that of Khalil (2004) who demonstrated the protective effect of OLE against liver injury induced by overdose of paracetamol administration. The hepatoprotective effect of PPE on liver function that obtained in the current study is in accordance with the studies of Osman et al. (2011) and Ashoush et al. (2013), who examined the effectiveness of PPE in prevention of CCl4 induced liver injury.

The co-administration of OLE or PPE with OTC decreased TC and TG levels; this finding reflects the capability of the test materials to prevent the increases in TC and TG through the improvement of liver functions. Hossin (2009) evaluated the pomegranate peel powder as a dietary fiber source and reported that dietary supplementation with peel powder at different concentrations significantly reduced serum total cholesterol and triglycerides levels in hypercholesteremic rats. The co-administration of OLE or PPE with OTC decreased also blood urea and creatinine levels. This findings reflects the nephro-protective effect of the examined extracts and the beneficial role of these extracts against the nephrotoxicity and metabolic disorders that induced by OTC. The obtained results are in consistent with the results of Ahmed and Enas (2010).

Also, the pre and co-administration of OLE with OTC significantly reduced the plasma and hepatic MDA levels. The recorded decreasing effect of OLE is displayed by the phenolic compounds present in OLE that scavenge OH* groups and inhibit lipid peroxidation. This lowering effect of PPE may be attributed to the effect of the phenolic compounds such as punicalgin, gallic acid, ellagittamins, anthocyanins and tannin (Neyrinck et al., 2013). The recorded decline in MDA levels during the pre and co-administration of the examined extracts with OTC demonstrate that, the test materials share a common mechanism through stabilizing the plasma membrane of liver tissue through the polyphenol and flavonoids in each extract.

The reduced GSH plays a vital role in cellular function (Gerush et al., 2006). GSH effectively scavenge free radicals and other reactive oxygen species (hydroxyl radical, lipid peroxyl radical, peroxynitrite and H2O2) directly or indirectly through enzymatic reactions (Cetinkaya et al., 2006). The increase in blood GSH contents during the pre and concomitant administration of the examined extracts may attribute to the presence of polyphenols such as oleuropein and its derivatives such as hydroxytyrosol and tyrosol as well as caffeic acid, p-coumaric acid, vanillic acid, vanillin, leuteolin, dismetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside and diosmetin-7-glucoside in OLE (Farag et al., 2003) and also punicalgin, gallic acid, ellagittamins, anthocyanins and tannin in PPE (Neyrinck, 2013).

The histopathological examination confirmed the biochemical results obtained in the current study and proved the hepatoprotective properties of OLE and PPE.
Conclusion: the potential of OLE or PEE to maintain the hepatic architecture along with significant decreases in biochemical parameters suggest the usage of these extracts as an efficient strategy for the prophylactic management of drugs induced liver damage.

Table (1): The Antioxidant activity and total penolic content of olive leaves and pomegranate peel ethanolic extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>Antioxidant activity (IC₅₀ µg/ml)</th>
<th>Total Phenol content (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLE</td>
<td>39.4</td>
<td>222.2 ± 22.0</td>
</tr>
<tr>
<td>PPE</td>
<td>11.5</td>
<td>447.2±72.5</td>
</tr>
</tbody>
</table>

Fig. (1) Effect of OLE, PPE and OTC treatments as well as co-administration with OTC on relative percentage of liver and kidney weight of albino rats.

The presence of different capital letters at the same organ means significant differences between group at P < 0.05

Relative Liver weight = liver weight/body weight × 100
Relative Kidneys weight = kidneys weight/body weight × 100

Table (2): Effect of OLE and PPE on serum ALT and AST activities (U/ml) as well as T.P (g/dl) and Alb contents (g/dl) on OTC co-administration in albino rats

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>ALT U/ml %</th>
<th>AST U/ml %</th>
<th>TP g/dl %</th>
<th>Alb g/dL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>23.33 ± 2.73B</td>
<td>38.83 ± 2.48A</td>
<td>8.46 ± 0.20C</td>
<td>4.22 ± 0.26C</td>
</tr>
<tr>
<td>OLE</td>
<td>20.35 ± 3.62A</td>
<td>37.33 ± 6.25A</td>
<td>8.15 ± 0.56C</td>
<td>3.66 ± 0.27C</td>
</tr>
<tr>
<td>PPE</td>
<td>20.09 ± 4.39A</td>
<td>38.66 ± 2.33A</td>
<td>8.46 ± 0.31C</td>
<td>-----------</td>
</tr>
<tr>
<td>OTC</td>
<td>55.5 ± 4.88C</td>
<td>137.8↑</td>
<td>6.4 ± 0.28A</td>
<td>24.35↑</td>
</tr>
<tr>
<td>OLE+OTC</td>
<td>26.33 ± 1.96C</td>
<td>53.16 ± 4.62B</td>
<td>7.31 ± 0.23B</td>
<td>13.59↑</td>
</tr>
<tr>
<td>PPE+OTC</td>
<td>27.08 ± 2.78B</td>
<td>55.5 ± 4.27B</td>
<td>8.08 ± 0.29C</td>
<td>4.49↑</td>
</tr>
</tbody>
</table>

Results were expressed as mean of six values ± SD.
The presence of different capital letters means significant differences between groups in the same column using ANOVA test followed by Duncan's multiple comparisons between groups at P < 0.05 were employed.
% Change corresponding to normal group.
Table (3): Effect of OLE and PPE on serum total cholesterol (TCH), triglycerides (TG), urea and creatinine contents on OTC co-administration in albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TCH</th>
<th>TG</th>
<th>Urea</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg / dl</td>
<td>%</td>
<td>mg / dl</td>
<td>%</td>
</tr>
<tr>
<td>CONTROL</td>
<td>73.25 ± 2.40&lt;sup&gt;A&lt;/sup&gt;</td>
<td>49.66±1.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>22.68±1.07&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.57±0.02&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>OLE</td>
<td>61.2 ± 6.55&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.45↓</td>
<td>50.00±1.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.68†</td>
</tr>
<tr>
<td>PPE</td>
<td>65.83± 3.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.13↓</td>
<td>51.32±2.13&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.34†</td>
</tr>
<tr>
<td>OTC</td>
<td>88.7 ± 6.25&lt;sup&gt;C&lt;/sup&gt;</td>
<td>21.09↑</td>
<td>126.92±3.4&lt;sup&gt;D&lt;/sup&gt;</td>
<td>155.58↑</td>
</tr>
<tr>
<td>OLE+OTC</td>
<td>71.28 ± 2.50&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.69↓</td>
<td>70.30±3.11&lt;sup&gt;B&lt;/sup&gt;</td>
<td>41.56↑</td>
</tr>
<tr>
<td>PPE+OTC</td>
<td>73.56 ± 1.93&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.42↑</td>
<td>73.56 ± 1.93&lt;sup&gt;B&lt;/sup&gt;</td>
<td>48.13↑</td>
</tr>
</tbody>
</table>

Results were expressed as mean of six values ± SD.
The presence of different capital letters means significant differences between groups in the same column using ANOVA test followed by Duncan’s multiple comparisons between groups at P < 0.05 were employed.
% Change corresponding to normal group

Table (4): Effect of OLE and PPE on blood glutathione (mg/dl) and MDA (nm/ml) levels on OTC co-administration in albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg / dl</td>
<td>%</td>
</tr>
<tr>
<td>CONTROL</td>
<td>28.18 ± 2.16&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.35 ± 0.23&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>OLE</td>
<td>28.33 ± 3.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.53†</td>
</tr>
<tr>
<td>PPE</td>
<td>27.58 ± 2.28&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.13↓</td>
</tr>
<tr>
<td>OTC</td>
<td>18.93 ± 0.95&lt;sup&gt;A&lt;/sup&gt;</td>
<td>32.82↓</td>
</tr>
<tr>
<td>OLE+OTC</td>
<td>25.83 ± 1.83&lt;sup&gt;B&lt;/sup&gt;</td>
<td>8.34↓</td>
</tr>
<tr>
<td>PPE+OTC</td>
<td>26.26 ± 2.3&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.81↓</td>
</tr>
</tbody>
</table>

Results were expressed as mean of six values ± SD.
The presence of different capital letters means significant differences between groups in the same column using ANOVA test followed by Duncan’s multiple comparisons between groups at P < 0.05 were employed.
% Change corresponding to normal group

Fig. (2): The normal histological structure of the hepatocytes
Fig. (3): Photomicrograph of liver tissue of animal (OLE) showing: intact liver architecture intact hepatocytes (H&E) (X:400)
Fig. (4): Photomicrograph of liver tissue of animal (PPE) showing: intact liver architecture intact hepatocytes (H&E) (X:400)
Fig. (5): Photomicrograph of liver tissue of positive induced animal showing: hepatocytes with fatty degenerative changes (arrow head), dilated sinusoids (double arrow) (H&E) (X :200).

Fig. (6): Photomicrograph of liver tissue of positive induced animals showing: inflammatory aggregates (arrow) central vein (cv) (double arrow) (H&E) (X :100).

Fig. (7): Photomicrograph of liver tissue of animal OLE+OTC showing: hydropic degeneration of hepatocytes. (H&E) (X : 400).

Fig. (8): Photomicrograph of liver tissue of animal treated with the pre and co-administration PPE and OTC showing: slight dilation in hepatic sinusoids. (H&E) (X : 400).

REFERENCES:


التأثير الوقائي لمستخلصات ورق الزيتون وقشر الرمان ضد الإجهاد التأكسدي والاعتلال الكبدى المستحدث بواسطة أكي تتراسيكلين في الجرذان البيضاء

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قسم الكيمياء الحيوية - كلية العلوم - جامعة القاهرة

شعبة الكيمياء الحيوية - الهيئة القومية للرقابة والبحوث الدوائية

هجريت هذه الدراسة ليقييم التأثير الوقائي للمستخلص الكحولي لكل من ورق الزيتون وقشر الرمان ضد الاعتلال الكبدى المحدث نتيجة استخدام جرعة عالية متكررة من الأكي تتراسيكلين.

وتم كذلك قياس قدرة كل من المستخلصي لمواد مضادة للا كيدة ومتبلطة للشوارد الحرة وأيضا تقييم المحتوى الكلي للفينولات في كل من المستخلصي وقد تم استخدم عدد 36 من الفئران كدراسه كملايين ى مستخلص ورق الزيتون وقشر الرمان بجرعات متغيرها. كل الفئران تم تقسيمها إلى 6 مجموعات كما تمت تقدير الوزن النبضى لكل الفئران وتم تقدير البناء الكبدى مع استخدام فحص مجهرى للنسيج الكبدى للفئران المستخدمة في هذه الدراسة.

وقد أوضح نتائج الدراسة أن المستخلصي لورق الزيتون وقشر الرمان له قدرة أعلى على تحسين وظائف الكبد وتثبيط الشوارد الحرة وهو أعزى إلى وجود كمية أعلى من الفينولات في قشر الرمان.