Protective effect of Aloe vera gel extract plant on liver diseases of experimental rats

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ABSTRACT
The present study was carried out to investigate the protective role of Aloe vera gel plant extract on carbon tetrachloride CCl₄ induced changes in liver enzymes of albino rats. Aloe vera is a medicinal plant belonging to the family Liliaceae, which has a wide range of therapeutic applications.

Sixty-four male Albino rats weighing about 130±5 g were taken and divided into eight groups, each with eight rats. The first group is the control (-) and fed on normal diet for 6 weeks. The second group received subcutaneous injection with CCl₄ in paraffin oil (50% v/v 2 ml/kg) twice per week for 2 weeks to induce chronic damage in the liver tissue and fed on normal diet (control +). The third, fourth and fifth groups were injected with CCl₄ in paraffin oil twice per week for 2 weeks then fed on 0.3, 0.6 and 0.9ml Aloe vera suspension gel (50 mg/mL), respectively by epi-gastric tube for remnant 4 weeks (injection groups). The sixth, seventh and eighth groups fed first on normal diet plus 0.3, 0.6 and 0.9ml Aloe vera suspension gel, respectively by epi-gastric tube for first 4 weeks then they were injected with CCl₄ in paraffin oil twice per week for remnant 2 weeks with continued fed on the same concentrations of Aloe vera suspension gel (protected groups).

The results indicated that Aloe vera powder increased the nutritional value of all treatments (protein, fat, carbohydrate and crude fiber), vitamin E (6.54µg/ml) and phenols (0.39 mg/100mg). Also, body weight gain (BWG) in protected group with 0.9 ml Aloe vera suspension gel (50 mg/mL) recorded the best result (58.90%) comparing with the control (-) (42.92%). Injected groups showed ratios of weight change or suffered a weight loss in liver comparing with control (+), ranged from -34.78 in 0.3ml to -11.37% in 0.9ml Aloe vera suspension gel after 6 weeks. Feeding rats on different ratios of Aloe vera gel decreased serum AST and ALT enzymes compared to the control (+) group. Also, decreased the mean values of uric acid, urea and creatinine in all tested groups was noticed compared to the control (+) group (2.43±0.17, 38.00±5.0 and 0.86±0.12, respectively). The best reduction in lipid profile was (89.60±12.41) for the triglycerides in high concentration of Aloe vera gel (0.9 ml) and follow by 0.6ml (90.60±18.57) and 0.3ml (91.80±8.81) comparing with control (+) in protected group.

Aloe vera improved liver and kidney histopathology in carbon tetrachloride induced hepatotoxicity in rats fed on different ratios of its extract (protective groups).

Key words: Aloe vera gel (Aloe Barbadensis miller), CCL₄, Albino rats, Liver enzymes, Kidney functions, Lipid Profile, Histopathology.

INTRODUCTION
Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test, and a substantial number of new antibiotics introduced on the market were obtained from natural or semi synthetic resources (Pankaj et al., 2013). Throughout the globe, many plants have been utilized for their medicinal properties. Aloe vera species has been used in folk medicine for over 2000 years and has
remained an important component in the traditional medicine of many countries. The ingredients responsible for the beneficial effects of this plant are present in leaves. It is commonly called "Guar patha" or Ghee-Kuar. (Jeyasakthy et al., 2017).

Aloe Barbadensis miller also known as Aloe vera is one of more than 400 species of Aloe vera and it belongs to family Liliaceae. Aloe vera’s prominent feature is its high water content, which ranges from 99.0–99.5%. The remaining 0.5–1.0% is reported to contain over 75 nutrients and 200 active compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids, and other different potentially active compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acid (López et al., 2017). Aloe vera has historically been used to treat skin wounds since it has antioxidant, anti-tumor and anti-inflammatory activities (Kanokporn and Kevin, 2015).

Aloe vera has immune modulatory activity and anti-inflammatory effects. These effects occur through the activation of macrophages and the suppression of mast cell migration to the injury and by inhibiting the enzyme cyclooxygenase, which controls the synthesis of prostaglandin E2, an important mediator in the occurrence of pain and inflammation (Muaz and Fatma, 2013). In addition, it shows a hepatoprotective effect of where its aqueous extract restores liver enzymes, triglycerides and improve liver histopathology in carbon tetrachloride induced hepatotoxicity (Suchittra and Sorrayut, 2014).

The liver is a very important organ with a lot of functions for the host to survive. Dietary components are essential for the healthy or diseased liver. Plants food plants can provide nutritional and medicinal support for liver diseases. (Guan and He, 2015). A vast majority of plants are now being used as phytomedicines, some of which have scientific proof, while others have purely holistic background. Two functionally and chemically distinct compositions can be prepared from Aloe vera leaves such as the latex and the gel. Latex is mainly composed of anthraquinone and its derivatives which are responsible for the purgative effects (Ishii et al., 1994). Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Several studies have shown that hepatoprotective effects are associated with phyto-extracts/phyto-compounds rich in natural antioxidants (Nayak et al., 2011). Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin – induced liver damage (Yousef et al., 2010). Therefore, this study was undertaken to evaluate the hepatoprotective effects of Aloe vera against hepatotoxin – induced liver damage.

**MATERIALS AND METHODS**

**Materials**

Aloe vera (L.) Burm, Family (Liliaceae) about (30kg) of leaves was obtained from the Orman-botanic-garden in Giza, Egypt. Samples were identified and authenticated at Cairo University Research Park (CURP). Sample collection was conducted during the months of November and December 2015.

Carbon tetrachloride CCl₄ (a colorless non–flammable liquid, of molecular weight 153: 84 and freshly diluted in paraffin oil (1:1) volume to a final concentration before use) was obtained from Sigma-Aldrich (St. Louis, MO, USA).
Methods

Preparation of Aloe vera gel

Fresh leaves of Aloe vera washed by water carefully then cut to species, the inner gel was obtained by clean sharp knife and then dried. Aloe vera gel was achieved by hot-air drying. For administration samples, the dried Aloe vera gel powder was suspended in distilled water and the dosage of homogenized suspension was adjusted to 50 mg/mL (Misawa et al., 2012). They were stored in tightly sealed dark containers in a freezer at −20°C for later use.

Analytical methods

Moisture, protein, lipid, ash and fiber was determined according the method of AOAC (2000). All determinations were done in triplicate.

Total carbohydrate contents were tested quantitatively according to Kostas et al., (2016). The absorbance was measured at a wavelength of 490 nm using UV-Vis Shimadzu Spectrophotometer (UV-1601 PC).

Vitamin E (total Tocopherols) was colorimetrically estimated by the method of Emmerie and Engel. (1939).

Total phenols were colorimetrically estimated by the method of Singleton and Rossi, (1965).

Biological experiment

Animal, housing and diets:

Sixty-four male Albino rats weighing about 130 ±5 g were obtained from the Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air and water and maintained at a temperature between 20-25°C with a 12 h light/dark cycle and light cycle (8-20 h) and relative humidity of 50%. The animals acclimatized for one week as an adaptation period. The animals were randomly divided into eight groups of eight rats each. The first group of rats, the control (-) fed on commercial diet (Table 1) for 6 weeks (total period of experimental). The second group was subcutaneous injected with CCL4 in paraffin oil (50% v/v 2 ml/kg) twice per week by subcutaneous injection for 2 weeks to induce chronic damage in their liver tissue (Jayasekhar et al., 1997) with fed on commercial diet (control +) as seen in Table (2). The third, fourth and fifth groups were injected with CCL4 in paraffin oil (50% v/v 2 ml/kg) twice per week by subcutaneous injection for 2 weeks like group 2, then fed on 0.3, 0.6 and 0.9 Aloe vera suspension gel (50 mg/mL), respectively by epi-gastric tube for remnant 4 weeks (Injection groups). The sixth, seventh and eighth groups fed first on commercial diet plus 0.3, 0.6 and 0.9 Aloe vera suspension gel (50 mg/mL), respectively by epi-gastric tube for first 4 weeks then injection with CCL4 in paraffin oil (50% v/v 2 ml/kg) twice per week for remnant 2 weeks with continued fed on the same concentrations of Aloe vera suspension gel (protected groups).

The following steps by Schermer (1967) were done in rats after six weeks of treatment in each group.

* The animals were fasted for 12 h.
* Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes.
* Blood samples were collected into plain tubes without anticoagulant and allowed to clot.
* Blood samples were centrifuged at 3000 rpm for 10 min at 4°C, to obtain clear serum.
* Serum was frozen at -18°C until analyzed.
* The animals were anesthetized with ether and sacrificed.
* They were quickly dissected to excise the liver, kidney, spleen and heart.
* These organs were weighed and then kept until histological investigations.
Table (1): Composition of commercial diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, [soy flour meal + sun flower meal + gluten]</td>
<td>21.00</td>
</tr>
<tr>
<td>Fat</td>
<td>03.26</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>03.29</td>
</tr>
<tr>
<td>Dl. Methionine</td>
<td>00.40</td>
</tr>
<tr>
<td>Vitamins mixed</td>
<td>01.00</td>
</tr>
<tr>
<td>Minerals mixed</td>
<td>04.00</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>67.05</td>
</tr>
</tbody>
</table>

Table (2): Experimental diets.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Commercial diet (control (-) group)</td>
</tr>
<tr>
<td>Second</td>
<td>CCL₄ + Commercial diet (control (+) group)</td>
</tr>
<tr>
<td>Injection groups</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>(CCL₄ + Commercial diet) + (0.3 ml Aloe vera by epi gastric tube).</td>
</tr>
<tr>
<td>Fourth</td>
<td>(CCL₄ + Commercial diet) + (0.6 ml Aloe vera by epi gastric tube).</td>
</tr>
<tr>
<td>Fifth</td>
<td>(CCL₄ + Commercial diet) + (0.9 ml Aloe vera by epi gastric tube).</td>
</tr>
<tr>
<td>Protected groups</td>
<td></td>
</tr>
<tr>
<td>Sixth</td>
<td>(Commercial diet + 0.3 ml Aloe vera by epi gastric tube) + CCL₄</td>
</tr>
<tr>
<td>Seventh</td>
<td>(Commercial diet + 0.6 ml Aloe vera by epi gastric tube) + CCL₄</td>
</tr>
<tr>
<td>Eighth</td>
<td>(Commercial diet + 0.9 ml Aloe vera by epi gastric tube) + CCL₄</td>
</tr>
</tbody>
</table>

Histopathology Technique

The tissue sample from liver and kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydrated using ascending grades of alcohol, cleaned in xyline and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (Banchroft et al., 1996). Then examined by the light microscope for detection of any histopathological alteration.

Biological Determination

Biological evaluation of the different tested diets was carried by determination of food intake (FI), body weight gain% (BWG %) and organs weight/body weight% according to Chapman et al. (1959).

BWG% = [(Final weight - Initial weight) / (Initial weight)] X 100
Organ weight/ body weight % = (Organ weight / Final weight) X 100

Biochemical analysis

Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by biodiagnostic kits.

Serum (uric acid, urea nitrogen and creatinine) were measured colorimetrically using spectrophotometer (model DU 4700) adjusted at 510 nm, 550 nm and 510 nm, respectively by Vassault et al. (1986), Chaney et al. (1962) and Young et al. (1975), respectively.
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Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined colorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of Henry et al. (1960).

Serum cholesterol and triglycerides concentrations were determined by Tietz, (1975) and Vassalt et al. (1986), respectively using spectrophotometer (model DU 4700) at 546 nm and 500-550 nm, respectively.

Serum lipoprotein (HDL-C and LDL-C) was determined by Fossati and Principe (1982) and Watson (1960), respectively using spectrophotometer (model DU 4700).

Statistical Analysis

The obtained data were exposed to analysis of variance. Duncan’s multiple range test at 5% level of significance was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 2006).

RESULTS AND DISCUSSION

Physicochemical analyses of Aloe vera gel, powder and solution

The proximate composition of Aloe vera (A.V) gel, powder and solution are shown in Table (3). The results revealed that moisture; carbohydrate, protein, fat, fiber and ash were 90.66, 66.82, 6.39, 0.93, 10.51, and 27.67 %, respectively in Aloe vera gel. While its powder showed an increase in protein, carbohydrate, crude fiber, ash and fat and decrease in moisture content (17.87, 76.48, 6.50, 10.56, 1.58 and 26.46%), respectively. Antherton (1998) mentioned that Aloe gel is often commercialized as powdered concentrate and it is used to prevent progressive intra-arterial drug abuse.

The obtained results showed a significant (P≤0.05) increase in protein content that exhibited 114.35% in Aloe vera gel as compared to solution. This increase may be due to the presence of high concentration in Aloe Vera gel with low moisture content (Madukwe et al., 2013). Also, data showed a significant (P≤0.05) increase in ash content 988.0%, carbohydrate 233.04% and fiber 101.94% between Aloe vera gel and solution, this data agreed with Yaron, (1993). Pandey (2016) reported that Aloe vera gel is mainly composed of water (90%), sugars, amino acids, vitamins A, C, and E, minerals (Zinc, Selenium) as well as the enzymes glutathione peroxidase, superoxide dismutase.

The solution with Aloe vera gel did not have any significant effect on pH of prepared Aloe vera gel. This indicated that pH didn’t sensation by changed form of Aloe vera (gel, powder and solution).

Table (3): Chemical constituent of Aloe Vera (A.V) Gel, Powder and solution

<table>
<thead>
<tr>
<th>Constituents (%)</th>
<th>Gel ± SDM</th>
<th>Powder ± SDM</th>
<th>Solution ± SDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>90.66±0.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>17.87±0.73&lt;sup&gt;C&lt;/sup&gt;</td>
<td>98.58±0.48&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>66.82±0.26&lt;sup&gt;B&lt;/sup&gt;</td>
<td>76.48±0.48&lt;sup&gt;A&lt;/sup&gt;</td>
<td>24.12±0.19&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>6.39±0.15&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.50±0.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.58±0.27&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>0.93±0.06&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.58±0.22&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.62±0.03&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibre</td>
<td>10.51±0.22&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.56±0.28&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.31±0.29&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>2.47±0.47&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.65±0.31&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.25±0.01&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>5.49±0.17&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.52±0.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.38±0.43&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Data are presented as means ± SDM (n=3).

A, B, C: Means with different letter among treatments in the same rows are significantly different.
Antioxidants:
Antioxidants are component of plant foods play an important role in the treatment of diseases and as a major. The type and amount of various antioxidants in Aloe vera gel, powder and solution are presented in Figure (1). The obtained data showed that Aloe vera powder is a rich source of phenols 0.39 mg/100 mg. While, Aloe vera solution showed a significant (P≤ 0.05) decrease (0.31 mg/100mg) comparing with gel and powder forms. On the other hand, Aloe vera powder was the higher in vitamin E (6.54µg/ml) than Aloe Vera gel and solution (5.83 and 5.75 µg/ml, respectively). This increase may be due to the presence of high concentration of vitamin E in Aloe vera powder with low moisture content (Madukwe et al., 2013). Suchittra and Sorrayut (2014) found that, the level of total phenolic contents was 4.19 mg gallic acid equivalents per 1 g of Aloe vera gel powder.

![Fig (1): Comparison between Aloe vera gel, powder and solution in vitamin E and phenols](image)

Langmead et al. (2004) reported that different fractions of Aloe vera as well as unfractionated whole gel have antioxidant effects. Phenolic anti-oxidant was found in Aloe vera gel, which may be responsible for these anti-oxidant effects. Chen et al. (2009) mentioned that the beneficial effects of Aloe vera could be attributed to its antioxidant activity and could be related to the presence of phenolic compounds and antioxidant vitamins, which have the ability to open tight junctions to allow paracellular transport. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity. Several studies have shown that hepatoprotective effects are associated with phyto-extracts/phyto-compounds rich in natural antioxidants (Nayak et al., 2011). Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin – induced liver damage (Yousef et al., 2010).

**Biological evaluation of Aloe vera gel on experimental rats**
**General signs in the rats**
No rats among groups died during the experimental period (6 weeks) and all the rats in groups exhibited no abnormal signs throughout the test period.
Protective effect of *Aloe vera* gel extract plant on liver diseases of experimental rats

Body and organs weight gain of experimental rats fed on different ratios of *Aloe vera* gel

The final body weights (FBW) of rats for different groups are given in Table (4) and Figure (2). There were significant differences (P≤0.05) in the final body weights of rats in the control (-) (175.8±22.0) and the remaining treatment groups (protected groups) (166.1±7.1 in suspension gel 0.3ml and 197.2±6.2 in 0.9ml). While in the ratio 0.6ml there was no significant difference comparing with control (-) group. The same trend was noticed in the BWG in protected group.

**Table (4): Mean body weight gain (g) of rats fed on different ratios of *Aloe vera* suspension gel**

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Protected groups</th>
<th>Injected groups with CCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW</td>
<td>123.0±2.12</td>
<td>125.0±3.1</td>
<td>123.9±5.87</td>
<td>123.3±4.0</td>
</tr>
<tr>
<td>FBW</td>
<td>175.8±22.0</td>
<td>155.0±21.7</td>
<td>166.1±7.1</td>
<td>176.3±5.3</td>
</tr>
<tr>
<td>BWG</td>
<td>42.9±9.37</td>
<td>24.0±6.05</td>
<td>34.05±4.20</td>
<td>42.98±4.19</td>
</tr>
</tbody>
</table>

*Data are presented as means ± SDM (n=8). Data in a row with different superscript letters are statistically different (P ≤ 0.05)*

IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain

**Fig (2): Body weight gain of rats fed on basal diet and different ratios of *Aloe vera* suspension gel in protected and injected groups**

Body weight gain (BWG) in protected group with 0.9 ml *Aloe vera* suspension gel (50 mg/mL) recorded the best result (58.90%) comparing with the control (-) group (42.92%), while values for the remaining treated groups with *Aloe vera* suspension gel of 0.3 and 0.6ml ranged between 34.05 and 42.98%,
respectively. The lowest rate of body weight gain occurred in control (+) group was 24.0%, while the best increase in injected groups was 36.37% fed on 0.9ml Aloe vera suspension gel. Nna et al. (2013) reported that Aloe vera gel increased body weight in normal animals without increasing food intake. Also, they noticed that increased serum AST and ALT observed in test 3 in their study may be related to the effect of Aloe vera gel on body weight, rather than liver damage.

The weights of various organs/body weight % of rats are shown in Table (5). The weights of the organs (liver, kidney, spleen and heart) of rats maintained on experimental diets + suspension gel 0.3ml (protected group) were (2.73±0.23, 0.71±0.08, 0.41±0.09 and 0.41±0.09), while for 0.6ml were (2.85±0.33, 0.76±0.08, 0.45±0.08 and 0.30±0.04) and for 0.9ml were (3.02±0.25, 0.79±0.26, 0.48±0.16 and 0.34±0.03), respectively. In injected groups with CCl4, there was almost significant difference in the weight of liver, kidney, spleen and heart of rats from control (+) groups. The remaining 3 treatments (injected group) were either show ratio of weight change or suffered a weight loss in liver comparing with control (+) ranged from -34.78 in 0.3ml to -11.37% in 0.9ml Aloe vera suspension gel after 6 weeks. Also, experimental groups (injected group) with 0.3, 0.6 and 0.9ml Aloe vera suspension gel of rats showed weight improvement, being (1.56, 8.57 and 12.5%) in kidney, (3.03, 6.06 and 12.12%) in spleen and (0.0, 3.33 and 3.33%) in heart respectively.

Table (5): Mean organs weight / body weight % of experimental rats treated by different ratios of Aloe Vera suspension gel

<table>
<thead>
<tr>
<th>Organs weight (%)</th>
<th>Aloe vera suspension gel (50 mg/mL)</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Protected groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Liver</td>
<td>2.70±0.33ab</td>
<td>2.99±0.09a</td>
<td>2.73±0.23b</td>
<td>2.85±0.33b</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.79±0.25a</td>
<td>0.64±0.15b</td>
<td>0.71±0.08ab</td>
<td>0.76±0.08ab</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.37±0.06b</td>
<td>0.33±0.06b</td>
<td>0.41±0.09ab</td>
<td>0.45±0.08ab</td>
</tr>
<tr>
<td>Heart</td>
<td>0.32±0.03ab</td>
<td>0.30±0.02b</td>
<td>0.41±0.09b</td>
<td>0.30±0.04b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Injected groups with CCl4</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Protected groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.70±0.33ab</td>
<td>2.99±0.09a</td>
<td>1.95±0.24b</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.79±0.25a</td>
<td>0.64±0.15b</td>
<td>0.65±0.08b</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.37±0.06a</td>
<td>0.33±0.06b</td>
<td>0.34±0.1ab</td>
</tr>
<tr>
<td>Heart</td>
<td>0.32±0.03a</td>
<td>0.30±0.02b</td>
<td>0.30±0.04a</td>
</tr>
</tbody>
</table>

* Data are presented as means ± SDM(n=8).

Data in a row with different superscript letters are statistically different (P ≤ 0.05)

Biochemical analysis

Results of biochemical analysis for all tested groups are presented in Table (6). Alterations in the liver enzyme (ALT and AST) were statistically significant (P ≤ 0.05) in all tested groups. Kidney function (urea, uric acid and creatinine) was statistically different from control (-) group (fed on experimental diets). Amounts of uric acid and creatinine were statistically different from control (-). The mean values of serum uric acid, urea nitrogen and creatinine increased gradually with increasing the level of protein in the diet. In this respect, Frey (2007) mentioned that serum urea nitrogen is a substance that is formed in the liver when the body breaks down protein.

The positive control group (Fig. 3) recorded a significant increase (P ≤ 0.05) in the activities of serum ALT, as compared to negative control group or the protected samples (different ratio of Aloe vera suspension gel 0.3, 0.6 and 0.9 ml,
Protective effect of Aloe vera gel extract plant on liver diseases of experimental rats respectively). Feeding rats on different ratios of Aloe vera gel decreased serum AST enzyme compared to the control (+) group. It was also noticed that there was a decrease in the mean values of uric acid, urea and creatinine in all tested groups, compared to the control positive group (2.43±0.17, 38.00±5.0 and 0.86±0.12). Paoulomi et al., (2012) reported that there are evidences on elevated serum creatinine and decrease in urea level produced on using Aloe vera in combination with standard anti diabetic drugs, and enables to reduce arsenic induced oxidative stress, keeping renal biochemical parameters remained unchanged but there is no profound evidence of its nephroprotective activity.

Table (6): Liver and kidney function of experimental rats treated by different ratios of Aloe vera gel.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aloe vera suspension gel (50 mg/mL)</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Protected group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver functions (U/I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>8.32±1.62c</td>
<td>15.47±1.58a</td>
<td>12.33±0.58a</td>
<td>12.30±0.88a</td>
</tr>
<tr>
<td>AST</td>
<td>15.72±2.37c</td>
<td>31.47±3.11a</td>
<td>28.65±2.89a</td>
<td>26.50±3.79b</td>
</tr>
<tr>
<td>Kidney function (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric Acid</td>
<td>2.07±0.13b</td>
<td>2.43±0.17a</td>
<td>2.33±0.25ab</td>
<td>2.21±0.23ab</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.66±0.11b</td>
<td>0.86±0.12a</td>
<td>0.59±0.05a</td>
<td>0.58±0.03b</td>
</tr>
<tr>
<td>Urea</td>
<td>30.20±4.21b</td>
<td>38.00±5.0a</td>
<td>33.4±4.93ab</td>
<td>31.80±6.76b</td>
</tr>
</tbody>
</table>

* Data are presented as means ± SDM (n=8).

Data in a row with different superscript letters are statistically different (P ≤ 0.05).

AST: aspartate amino transferase
ALT: alanine amino transferase
Fig. (3): Effect of different ratios of Aloe vera suspension gel on ALT and AST liver enzyme of experimental rats

The results demonstrated that ALT and AST levels were significantly lower in groups injected with CCl₄ compared with control + group. Increased serum creatinine above normal levels may reflect destroy of 50% of renal nephron (Lee and Nieman, 1996). Wafay et al., (2012) stated that the CCl₄ caused an increase in serum levels of the diagnostic enzymes (ALT, AST and GGT) in rats that received CCl₄ as compared to the control (-) group. Etim et al. (2006) mentioned that due to the structural damage of liver, the level of liver enzymes are increased in serum because liver enzymes are located in cell cytoplasm after damaging or injury they are released into the blood circulation and raises the level of enzymes in serum. Aloe vera possesses hepatoprotective activity and reduces the level of ALT (Nayak et al., 2011). In current study Aloe vera also decreased the level of ALT in all ratios 0.3, 0.6 and 0.9 ml and the best one was 0.9 ml in both of protected and injected groups.

ALT is a biomarker to evaluate the liver disease and detection of liver damage (Girish et al., 2009). Reduction of ALT indicates the restoration of normal functioning of liver (Sudheer et al., 2011). Aloe vera protects the liver from oxidative stress and inhibits the excessive free radicals accumulation (Parmar et al., 2010). Some bioactive compounds of Aloe vera are very effective such as tinnins, steroids and alkaloids. Specific steroids and flavonoids are responsible to protect the liver from oxidative stress and play a key role in hepatoprotection (Joseph, and Raj, 2010).

Lipid profile of rats fed on different ratios of Aloe vera suspension gel.

Results of lipid profile analysis for all tested groups are presented in Table (7). The results demonstrated significant increase (p<0.05) in the values of cholesterol, triglycerides, HDL-c and LDL-c in control positive group comparing with other treatments in protected and injected groups.
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### Table (7): Lipid profile of experimental rats treated by different ratios of *Aloe vera* gel.

<table>
<thead>
<tr>
<th>lipid profile (mg/dl)</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Protected group 0.3</th>
<th>Protected group 0.6</th>
<th>Protected group 0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>76.8±14.34&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>88.6±10.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.6±14.93&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>84.4±4.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>82.4±11.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>79.40±7.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.60±10.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.80±8.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.60±18.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.60±12.41&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-c</td>
<td>26.60±5.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.60±3.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>45.60±4.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.00±2.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.40±16.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-c</td>
<td>10.72±15.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.08±10.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.64±12.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.76±5.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.28±10.64&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data are presented as means ± SDM (n=6).*

The best reduction in lipid profile was recorded for the triglycerides 89.60±12.41 in high concentration of *Aloe vera* gel (0.9 ml) and followed by 0.6 ml (90.60±18.57) and 0.3 ml (91.80±8.81) comparing with control (+) 97.60±10.70 in protected group. The same trend was noticed in triglycerides in injected group. Also, the best reduction in LDL-c was noticed in protected group 19.28±10.64 (0.9 ml) comparing with control (+) group, the same trend was also noticed in injected group.

Dixit and Joshi, (1983) found that orally administered *Aloe* gel lowered total cholesterol by 61% and increased the proportion in the high density lipoprotein (HDL). Taslima *et al.* (2013) reported that pre-treatment of rats with *Aloe vera* gel significantly reduced the CCl<sub>4</sub> induced lipid peroxidation in liver and biochemical changes associated with CCl<sub>4</sub> induced hepatotoxicity and evaluated the effects of *Aloe vera* gel pre-treatment on it.

Yokozawa *et al.*, (2003) reported that elevated urea concentration can serve as an indicator of renal dysfunction and in patients with renal failure, the correlation between the severity of the pathological condition and the concentration of blood urea nitrogen is actually relatively good. It is well known that the elevated serum creatinine, uric acid and urea nitrogen are considered as indicator for a defect in renal nephrons. Increased serum creatinine above normal levels may reflect a destroy of 50 % of renal nephron.

#### Histopathological examination

Organs such as liver and kidney were examined by a histological approach and the photomicrographs of hematoxylin – eosin stained liver and kidney are illustrated in Figures (4 and 5).

#### Liver

The liver sections from control (-) group (normal rats fed on commercial diet only) showed normal histological structure of the central vein and surrounding hepatocytes in the hepatic parenchyma (Fig.4-1). While in control (+) there was sewer congestion in the central veins and sinusoids (Fig. 4-2). Also, congestion in portal vein with edema and few inflammatory cells infiltration and multiple newly formed bile ductless in the portal area were observed (Fig. 4-3).

Animals fed on *Aloe vera* 0.3 ml then affected by CCl<sub>4</sub> (protected group) showing inflammatory cells infiltration and multiple newly formed bile ductless in the portal area. In contrast, congestion in central vein was observed in the animals affected by CCl<sub>4</sub> and treated with 0.3 ml *Aloe vera* (injected group) as seen in Figure (4 A and D). Rats fed on 0.6 ml *Aloe vera* then affected by CCl<sub>4</sub> (protected
group) showing inflammatory cells infiltration in portal area while, injected group with 0.6 ml *Aloe vera* after affected by CCl₄ showing dilatation portal vein with oedema in the portal area (Fig. 4 B and E). Mild dilatation in central vein was observed in protected group (0.9 ml) (Fig.4 C). While, injected group (0.9 ml *Aloe vera*) showing mild congestion in portal vein with oedema in portal area (Fig.4 F).

Taslina *et al.*, (2013) postulate that *Aloe vera* gel exert its hepatoprotective effects against oxidative stress by three possible mechanisms – i) Interfering with the generation of free radicals from the toxic chemical, ii) Scavenging the generated free radicals by its own antioxidantive enzymes (glutathione peroxidase, superoxide dismutase), and vitamins (A, C, and E), iii) Stimulating the expression and/or activation of endogenous antioxidative enzymes. All these possible mechanisms might work independently or synergistically to protect liver against oxidative stress induced damages. The first and the second mechanisms may play the vital role during the short period of in vitro incubation, whereas, all three mechanisms may act in the in vivo scenario. However, expression and activation of endogenous antioxidative enzymes may be the key mechanism here.

**Kidney**

The control (-) group showed normal histological structure of the glomeruli and tubules or the cortea Fig. (5-1). While, control (+) affected by CCl₄ showed congestion in the blood vessels and glomeruli (Fig. 5-2) and necrosis in the lining epithelium of some tubules at the cortex (Fig. 5-3).

In protected group affected by CCl₄ plus fed on *Aloe vera* (0.3, 0.6 and 0.9 ml) showed congestion in cortical blood vessels and glameruli as seen in Figure (5 A, B and C), respectively. The same changes were observed in injected group affected by CCl₄ then fed on *Aloe vera* (0.3ml) (Fig. 5 D 0.3ml). While, there was no histopathological alteration recorded in the injected group and fed on (0.6 and 0.9 ml) *Aloe vera* as seen in Figure 5 (E and F), respectively.

Shubha Priyanvada, *et al*. (2008) reported that several approaches, utilizing different mechanisms have been attempted to reduce gentamicin (GM) nephrotoxicity and related aminoglycoside antibiotics. Many different agents and strategies have been reported to ameliorate GM nephrotoxicity in experimental animals (Nagai, and Takano, 2004). Among them protection against GM nephrotoxicity was mainly focused on the use of various antioxidant agents including the extracts from medicinal plants with antioxidant properties. However, none of these approaches have been found safe/suitable for clinical practice.
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Fig (4): Histological changes in the liver stained with (H&E X40) on using different doses of *Aloe vera* in protected and injected groups

**Fig. 4-1** Control (-) showing normal histological structure of the central vein and surrounding hepatocytes (h) in the hepatic parenchyma.

**Fig. 4-2** Control (+) showing congestion in central veins (CV) and sinusoids.

**Fig. 4-3** Control (+) showing sewer congestion in portal vein (PV) with edema (O) and few inflammatory cells infiltration and multiple newly formed bile ductules in the portal area.

**Fig. 4-A** Protected group (0.3 ml *Aloe vera* gel) showing inflammatory cells infiltration (f) and multiple newly formed bile ductless in the portal area on using 0.3 ml.

**Fig. 4-B** Protected group (0.6 ml *Aloe vera* gel) showing inflammatory cells infiltration in portal area.

**Fig. 4-A** Protected group (0.9 ml *Aloe vera* gel) showing mild dilatation of central vein (CV).

**Fig. 4-D** Injected group (0.3 ml *Aloe vera* gel) showing congestion in central vein (CV).

**Fig. 4-E** Injected group (0.6 ml *Aloe vera* gel) showing Dilatation portal vein (PV) with oedema (O) in the portal area.

**Fig. 4-F** Injected group (0.9 ml *Aloe vera* gel) showing mild congestion in portal vein (PV) with edema (O) in portal area.
Fig (5). Histological changes in the kidney stained with (H&E X40) using different doses of *Aloe vera* in protected and injected groups

**Control (-)**

1. Control (-) showing normal histological structure of the glomeruli (g) and tubules (t) or the cortea
2. Control (+) showing sewer congestion in the blood vessels (v) and glomeruli (g).
3. Control (+) showing necrosis in the lining epithelium of some tubules (t) at the cortex.

**Protected Groups**

A. Protected group (0.3ml Aloe vera gel) showing congestion in cortical blood vessels (V) and glameruli (g).
B. Protected group (0.6ml Aloe vera gel) showing congestion in cortical blood vessels (V).
C. Protected group (0.9ml Aloe vera gel) showing congestion in cortical blood vessels.

**Injected Groups**

D. Injected group (0.3ml Aloe vera gel) showing congestion in cortical blood vessels.
E. Injected group (0.6ml Aloe vera gel) showing normal histological structure.
F. Injected group (0.9ml Aloe vera gel) showing normal histological structure.
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REFERENCES


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التأثير الوقائي للجل المستخلص من نبات الصبار على أمراض الكبد في فئران التجربة

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المستخلص

تهدف هذه الدراسة إلى تحديد الدور الوقائي للجل المستخلص من نبات الصبار على التغيرات في انزيمات كبد فئران الأليبيون المعدلة بواسطة رابع كوريد الكرون، حيث يشير نبات الصبار من النباتات الطبية، والتي تعد علامة قوية، والذين يدور فيها滥用 في التطبيقات الطبية.

استخدم في هذا الدراسة مجموعات من 40 فئران أوزانها 130 ± 5 جرام، وتتم تقسيمها إلى 8 مجموعات بكامل من 8 فئران. المجموعة الأولى (المجموعة الضارة السالبة) قادرة على إعطاء الإقبال لعدها سهلاً. المجموعة الثانية (المجموعة الضارة الموجبة) تحتوي تحت الجلد براع كوريد الكرون المخلوط بزيت البارفين بنسبة 50% حجم/حجم 2 ملي/كلو من وزن الفأر مرتين أسبوعياً لمدة أسبوعين، وذلك لتحديد الأثرات على الالتهاب مزمن في نسيج الكبد. الاستمرار في التغذية على الفئران المضيفة. المجموعة الثلاثة والرابعة والخامسة تحت تحت الجلد براع كوريد الكرون المخلوط بزيت البارفين بنسبة 50% حجم/حجم مرتين أسبوعياً لمدة أسبوعين ثم تختفيها بنسبة 0.3 وم 0.6 وم 0.9 ملي من مستخلص جل نبات الصبار يتركز 50 ملجرام/مل على التوالي لمدة أربعة أسابيع (المجموعات المصابة). أما المجموعة السابعة والسابعة والمئات ثم تختفيها على الانتظار فالمعدلة أربعة أسابيع ثم ثم تختفيها براع كوريد الكرون المخلوط بزيت البارفين بنسبة 50% حجم/حجم تحت الجلد مرتين أسبوعياً لمدة أسبوعين، فيأخد التغير في التغذية على مستخلص جل نبات الصبار بنفس النسب (المجموعات الوقائية).

وقد أظهرت النتائج أن محصور جيل نبات الصبار أدى إلى ارتفاع في القيمتها الطبية في كل المكونات (بروتين، دهون، كربوهيدرات والألياف الخام) وكذلك أظهرت ارتفاع نسبة فيتامين E (6,5 ميكروجرام/مل) والفيتامون (0,39 ملجرام/100مل).

كما أظهرت النتائج ارتفاع في نسبة المنوي للزيادة في وزن الجسم في المجموعات الوقائية 58.90% في نسبة 0.3 مل مستخلص جل نبات الصبار بالمخلط مع المناطق الضارة السالبة (92.40%)، كما أظهرت نتائج معيّنة ملجرام/مل على التوالي بمعدلة أربعة أسابيع (المجموعات المصابة) بتراج ما بين 0.9 من مستخلص جل الصبار بعد ستة أسابيع وهي مدة التجربة. كما أظهرت النتائج أن تغذية الفئران على جانب مختلفة من مستخلص جل الصبار أحدث انخفاض في انزيمات الكبد ALT، AST. هو مقارنة بالمجموعة الضارة السالبة، وكذلك انخفاض في مستوى قيم حمض البورك والألياف في كل النوعية 0.3 مل مستخلص جل نبات الصبار بالمخلط مع المناطق الضارة السالبة (92.40%). المعايير تحت الاختبار مقارنة بالمجموعة الضارة السالبة (92.40%) تم تأكيد نتائج في مجموعات الدوافع كان ذلك 12.41±89.6 في مجموعات الدوافع الثلاثية ذات التركيز المركزي 0.9، وبورك نسبة (0.6) 81±10.8) مقارنة بالمجموعة الوقائية.

كما أظهرت النتائج العينة التشريحي حدوث تحسين في تغيرات نسب الكبد والكلي للفئران التي تم أصابتهم براي من كوريد الكرون المخلوط بزيت البارفين، حيث تظهر على نسبة مختلفة من مستخلص جل نبات الصبار في المجموعات الوقائية.