

## Antibacterial and Antioxidant Activities of *Balanites aegyptiaca* Kernel and Its Effects on CCl<sub>4</sub> Treated Rats

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### ABSTRACT

It is important to know the antioxidant content and their efficacy in foods, for preservation or protection against oxidative damage, to avoid deleterious changes and loss of commercial and nutritional value. Antioxidants are the compounds, which combat the free radical by intervening in any one of the free radical mediated oxidative process. The role of ethanolic extracted kernel of *Balanites aegyptiaca* (*B. aegyptiaca*) on development hepatocarcinoma and CCL<sub>4</sub> effect as an antioxidant and antimicrobial were identified in this study. The present study aimed to identify the potential hepatoprotective activity against hepatic injury produced by carbon tetrachloride CCL<sub>4</sub> in rats and to acquire information about the health aspects of *Balanites aegyptiaca*. The DPPH radical scavenging activity assay (ROS) activity of *Balanites aegyptiaca* (50 ; 100 ; 200 mg/mL) was determined against standard BHT. The Scavenging effect of kernel ethanolic extract of *Balanites aegyptiaca* (EB<sub>1</sub>) on the DPPH radical decreased in order of EB 200 > EB 100 > EB 50 at all concentrations (200 , 100 and 50 mg/mL) respectively, this actually occurred linearly with increasing concentrations and due to increase flavonoids that has apotent antioxidant activity and its strong scavengers of free radicals. The antimicrobial activity of the kernel of *Balanites aegyptiaca* (EB<sub>1</sub>) and different extracts doses well diffusion method are determined by using inhibition zone against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. The inhibition zone for *Pseudomonas aeruginosa* was 9 mm at dose EB 50 . Dose EB200 was more susceptible to inhibit bacterial activity of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis* were found to EB more sensitive than other doses of EB100 and EB50. The minimal concentration of ethanolic extract of minimum inhibitory concentration (MIC<sub>90</sub>) as more susceptible to inhibit 90% of *Staphylococcus aureus* with lower concentration 132 µl/ml while , *Streptococcus faecalis* was need for higher concentration from extract at 190 µl/ml. The biological study was carried on albino rats Liver injury induced by an oral administration of 20% Carbon tetrachloride (CCL<sub>4</sub>) which was suspended in corn oil and then injected orally (1 ml/kg body weight), twice a week, for 4 weeks. Then rats were grouped into 4 groups and dosing by 200 mg of *Balanites aegyptiaca* and compared with 50 mg per rat on Silymarin. Antioxidant and biochemical determination were recorded. *B. aegyptiaca* extracts inhibited oxidative of CCL<sub>4</sub> effect was significant decrease in treated animals for all different four groups where a significant reduction (P<0.05) in the serum at liver function (ALT and AST) levels were occurring among 200 mg fed group (3) of and also among group 4 which dosages on Silymarin treated when comparison with hepatocarcinoma group 2 of CCL<sub>4</sub> . Also there was definitely improving liver function and ALP (alkaline phosphatase), indicated that EB 200 had a significant role to recovery of some hepatocarcinoma symptoms, which occurred by CCL<sub>4</sub> treated and near to Silymarin in its activity. Also the EB extract at 200 mg it is coming in the second order after Silymarin effective when compared to antioxidative role by Glutathione reductase (GSSG-R) of Silymarin. Histopathological investigation had shown that, the kernel extract for EB 200 mg and 50 mg Silymarin were responsible to eliminate and recover treated groups by CCL<sub>4</sub> (hepatocarcinogenic agent) from inflammation. It could be concluded that, the EB 200 can provide a considerable ratio of antioxidants and anti-inflammatory were effectively upon treated CCL<sub>4</sub> and other carcinogenic agents. This result gives a new insight on beneficial effect of kernel *B. aegyptiaca* extract in antioxidant and antimicrobial as well as against hepatocarcinogenic agents such as CCL<sub>4</sub> effect.

**Keywords:** DPPH , antioxidant , *Balanites aegyptiaca* kernel, hepatocarcinoma, CCL<sub>4</sub>, MIC<sub>90</sub>, Histopathological, rats , ALP, Glutathione reductase (GSSG-R) , Silymarin

### INTRODUCTION

Traditionally used natural source of antioxidants have much more attention in the food industry, prior to their antioxidant and antibacterial properties. Nowadays, in most countries, there are some limitations in using synthetic antioxidant compounds in the food products because of their side effects, therefore natural sources have become more important to find proper and safe food antioxidants. Antioxidants also act as radical scavengers and convert radicals to less reactive species. They are helpful in reducing and preventing damage from free-radical reactions because of their ability to donate electrons (Mandal *et al.*, 2009).

Secondary metabolites for most plant exhibit bioactivity of plants and health-promoting properties (George, and Pamploma-Roger, 1999). Phytonutrients are used as a flavoring agent, natural medicines by humans to cleanse and purify the body by

binding chemical carcinogens and activating detoxification enzymes, mostly in the gastrointestinal tract (Clarke, 2004). To protect the cells and organs of the body against ROS, humans have evolved a highly sophisticated and complex antioxidant protection system. This involves a variety of components, both endogenous and exogenous, that function interactively and synergistically to neutralize free radicals. The organism opposes the toxicity of oxygen through the endogenous enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). Integrated antioxidant defenses protect tissues and presumably remain equilibrium with continuously generated Reactive Oxygen Scavenging (ROS) to maintain tissues metabolically intact most of the time. Oxidative stress occurs when the formation of ROS due to the normal cellular function in the human body exceeds the availability of antioxidants. The antioxidant defense systems are important in maintaining good

health, and therefore an antioxidant-rich diet or antioxidant complements may be necessary as a health-protecting factor.

Phenolic compounds such as phenolic acids, flavonoids, stilbenes, tannins and lignans can scavenge free radicals, quench ROS, and therefore provide effective means for preventing and treating free radical-mediated diseases (Sharma, 2009). Phenolic compounds are contributing to fruit sensory and nutritive quality in terms of modifying color, taste, aroma and flavor, and also providing health-beneficial effects (Ornelas-Paz *et al.*, 2010).

Additionally, other phytonutrients are fatty acid derivatives, amino acid derivatives and minerals. Over production of free radicals are dangerous because they react with vital cellular components such as DNA or cell membranes causing damage to muscles, other tissues and have been implicated in many ailments (Halliwell and Gutteridge, 2000). Phytophenolics play a crucial role in health promotion and disease prevention by mechanisms related to cell differentiation, deactivation of pro-carcinogens, maintenance of DNA repair, and inhibition of N nitrosamine formation and change of estrogen metabolism, amongst others (Shahidi, 2004).

Consumption of flavonoid-rich foods cause a huge increase in antioxidant capacity of the blood and this is not caused directly by the flavonoids themselves, but most likely is due to increased uric acid levels that result from expelling flavonoids from the body (Ververidis *et al.*, 2007). Saponins could interfere with membrane bilayers leading to red cell lysis and the amphipathic nature of saponins makes them active surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes and they have also been used as adjuvants in vaccines (Hostettmann and Marston, 1995). Liver diseases remain as one of the serious health problems. However, we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders.

Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. A number of plants have been shown to possess hepatoprotective properties by improving the antioxidant status. However, there is still a lack of scientific proofs to authenticate the hepatoprotective properties of some plants which are used traditionally to treat liver disorders (Gupta *et al.*, 2005). A number of antioxidant enzymes provides defense against free radicals: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). SOD converts  $O_2^-$  to  $H_2O_2$ , whereas GSH-Px and CAT convert  $H_2O_2$  to  $H_2O$ . Glutathione reductase (GSSG-R) functions in regeneration of reduced glutathione, which is utilized in the degradation of  $H_2O_2$  by GSH-Px. These enzymes, coupled with other antioxidants, such as ascorbic acid and  $\alpha$ -tocopherol, serve to maintain a fine balance in reducing levels of ROS. When a shift in the balance occurs, either by increased free radical formation or decreased antioxidant defenses, oxidative stress results,

which can lead to damage to lipids, proteins, and DNA. (Olanow, 1993).

*Balanites* seed kernel is considered as an extremely useful edible product. It contains good quality oil and high protein content (Mohamed *et al.*, 2013). The seed oil of *Balanites aegyptiaca* has been used in Nigeria as ingredient and substitute to groundnut oil as food supplement and also in traditional medicine. The fleshy pulp of the fruit is eaten fresh or dried. It contains 64 – 72 % carbohydrates, plus crude protein, steroidal saponins, vitamin C, ethanol and other minerals (Abu Al-Futuh, 1983). All parts of the tree have a medicinal use including fruits, seeds, barks and roots. The most important is steroidal saponins, which yield diosgenin, a source of steroidal drugs, such as corticosteroids, contraceptives and sex hormones (Farid *et al.*, 2002). Different yields of diosgenin are obtained by acid hydrolysis of saponins isolated from fruit pulp (0.3%) and seed kernel (0.6%) (Tripathi *et al.*, 1996).

The aim of the present study was to evaluate antibacterial and antioxidant activity of ethanolic extracts of *Balanites aegyptiaca* seed kernel and hepatoprotective activity against hepatic injury produced by carbon tetrachloride  $CCL_4$  in rats and to acquire information about the health aspects of *Balanites aegyptiaca*.

## MATERIALS AND METHODS

### Materials:

#### 1. Chemical:

All chemicals were analytical grade and purchased from Sigma, Aldrich (Sigma Chemical Co., St. Louis, USA). Carbon tetrachloride was purchased from Egyptian Center Company, Cairo Egypt. Solvents and chemicals were purchased from Merck (Darmstadt, Germany). Analytic kits were used for analyzing parameters were purchased from Biosystem, Spain. Silymarin was purchased from Local Pharmacy Elezaby Pharmathetical, Giza.

#### 2 Seeds:

Seeds of *Balanites aegyptiaca* Del, were kindly obtained from the Western desert of Egypt from (Mersa Alam) between April and August 2015 Cairo, Egypt.

#### 3. Bacterial strains:

Three bacterial strains were tested including Gram positive (+) bacteria as *Staphylococcus aureus* (12600), *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. All microbial were obtained from faculty of science, Cairo University.

### Method :

#### 2. Preparation of extracts:

Seeds were ground to a fine powder then extracted with absolute ethanol, in a Soxhlet apparatus for 10 hours, according to (AOAC, 2012) procedure. Afterward, prepare the required doses of *Balanites aegyptiaca* ( $EB_1$ ) (EB50, EB100 and EB200 mg/ml methanol), then kept in refrigerator at  $-18^\circ C$  for performing an antioxidant, antibacterial and hepatoprotective activities.

### 3. Antioxidant activity:

#### The DPPH radical scavenging activity assay (ROS) :

The DPPH assay was done according to the method of Brand-Williams *et al.* (1995). Prepared the required doses of *Balanites aegyptiaca* (EB I ) (EB 50, EB 100 and EB 200 mg/ml methanol ).The assay was performed by measurement of the decrease in the absorbance of DPPH at 517nm. The percentage of the DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1) / A_0) \times 100$$

Where:  $A_0$  is the start absorbance at time zero and  $A_1$  is the final absorbance after 60 min.

#### Disc diffusion assay:

Antimicrobial activity of the tested doses of ethanolic extract *Balanites aegyptiaca* (EB I ) (EB 50, EB 100 and EB 200 mg/ml dimethyl sulfoxide) was determined by using a modified Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966). Briefly, 100  $\mu$ l of the bacteria grown in 10 mL of fresh media until they reached a count of approximately  $10^8$  cells/ml for bacteria 100  $\mu$ l of microbial suspension was spread onto Mueller Hinton (MH) agar (National Committee for Clinical Laboratory Standards, 1993, 1997) in Petri plates corresponding to the broth in which they were maintained. An aliquot (10  $\mu$ l) of each of the ethanolic extract *Balanites aegyptiaca* was pipetted on a sterile paper disc (Whatman No. 1, 5.5 mm paper disc) on the agar surface. Standard discs of Ampicillin (Antibacterial agent), served as positive controls for antimicrobial activity but filter discs impregnated with 10  $\mu$ l of solvent (distilled water, chloroform, and DMSO) were used as a negative control.

The plates were inverted and incubated at 35-37°C for 24-48 hours. Microbial inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc and recorded as diameter of inhibition zone in millimeter. The zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1993).

#### Determination of minimum inhibitory concentration (MIC<sub>90</sub>):

The minimum inhibitory concentration (MIC<sub>90</sub>) of which inhibits 90% of growth of tested microorganism (*Staphylococcus aureus* and *Streptococcus faecalis*) that treated with ethanolic extract of *B.aegyptiaca* seeds and compared to the control reported by (National Committee for Clinical Laboratory Standards, 1997).

### 5. Hepatoprotective studies:

#### Animal:

Adult male Albino rats weighing (185  $\pm$  5g) were obtained from Faculty of Veterinary Medicine, Cairo University. The experimental animals were carried out according to the ethical committee and standard regulations by the animal's house of Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt.

#### Carbon tetrachloride induction of hepatic injury:

Liver injury was induced by an oral administration of 20% Carbon tetrachloride (CCL<sub>4</sub>)

which was suspended in corn oil and then injected orally (1 ml/kg body weight), twice a week, for 4 weeks.

#### Experimental animals and study design:

The animals were housed in separated cage and maintained under control laboratory conditions at 25°C  $\pm$  2°C on a 12 h light: 12 h dark cycle for one week with free access to standard food and water *ad libitum*. The animals were fed with standard diet consisted of casein 20%, sucrose 10%, cellulose 5%, corn oil 10%, salts mixture 4%, vitamin mixture 1% and completed to 100% with corn starch AOAC (2012). Then, after one week of acclimation, rats were classified into eight groups each containing five animals and received treatment for 28 days as follows

Group 1: control group.

Group 2: hepatotoxin control group and oral administered with CCl<sub>4</sub> (1ml/kg body weight) twice a week.

Group 3: CCl<sub>4</sub> (1ml/kg body weight) twice a week and seed extract (200mg/kg body weight) treated rats.

Group 4: CCl<sub>4</sub> (1ml/kg body weight) twice a week and Silymarin (50 mg/kg body weight).

Blood samples were collected from the retro-orbital vein of each animal at the end of experiment using a glass capillary tube. Then the animals were sacrificed and the liver from each group were fixed in 10% buffered formalin solution for further examination.

Serum and plasma preparation: The serum and plasma were separated by centrifuging (Hettich, Universal 16, German) at 3000 rpm for 20 min at 4°C, then collected into sterilized tubes and stored at 20°C for biochemical parameters and antioxidant studies.

#### Liver functions:

Serum enzymes activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were assayed according to the methods of Gella *et al.*, (1985), while Alkaline Phosphatase (ALP) was determined according to the method of Rosalki *et al.*, (1993). The level of lipid peroxidation was determined as malondialdehyde (MDA) according to the method reported by Onkawa *et al.*, (1979).

#### Antioxidant enzyme determination:

It was determined by a number of antioxidant enzymes in Plasma enzymes activities of Glutathione reductase (GSSG-R) functions, and catalase (CAT) were measured according to the method reported by Goldberger and Spooner (1983) and Aebi (1983), respectively.

#### Histopathological studies:

Liver samples from each group were fixed in 10% buffered formalin and embedded in paraffin wax. Microtome sections of 3-4  $\mu$ m thickness were prepared according to the standard procedure and stained with haematoxylin and eosin. Sections were then examined for pathological findings of such as centrilobular necrosis, fatty and lymphocytes infiltration by the light microscope (Bancroft *et al.*, 1996).

#### Statistical analysis:

All data were subjected to statistical analysis, including the calculation of the mean and standard error

(mean±SE). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA), and Duncan's multiple range test was used to detect the least significance difference among the means in between groups. Differences were considered significant at P<0.05 level (Snedecor and Cochran 1982) using SPSS (version 10) computer program.

## RESULTS AND DISCUSSION

### The DPPH radical scavenging activity assay (ROS):

Figure 1 demonstrated the Reactive Oxygen Scavenging activity (ROS) of the natural antioxidant of the ethanolic extract of the kernel of *Balanites aegyptiaca* (EB<sub>i</sub>) and compared with standard synthetic antioxidant of BHT. The ROS activity increased as the dose of extracts dependent. In this system the extract exhibited highest antioxidant activity surpassing all of

the tested doses from (50 up to 200 mg/ml) of extracts. Among the extracts dose 200 mg/ml exhibited highest activity being close effectiveness to the standard BHT. While other doses extracts from 50-100 mg/ml showed the lowest antioxidant activity (ROS).

Furthermore, the scavenging effect of *Balanites aegyptiaca* (EB<sub>i</sub>) on the DPPH radical decreased in order of EB 200 > EB 100 > EB 50 at all concentrations (200, 100 and 50 mg/mL) respectively, this actually occurred linearity with increasing concentrations. The natural compounds like flavonoids that possess potent antioxidant activity are strong scavengers of free radicals. In order to evaluate the effectiveness of flavonoid naringenin as an antioxidant, we used the DPPH radical scavenging capacity assay. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, et al., 1997).

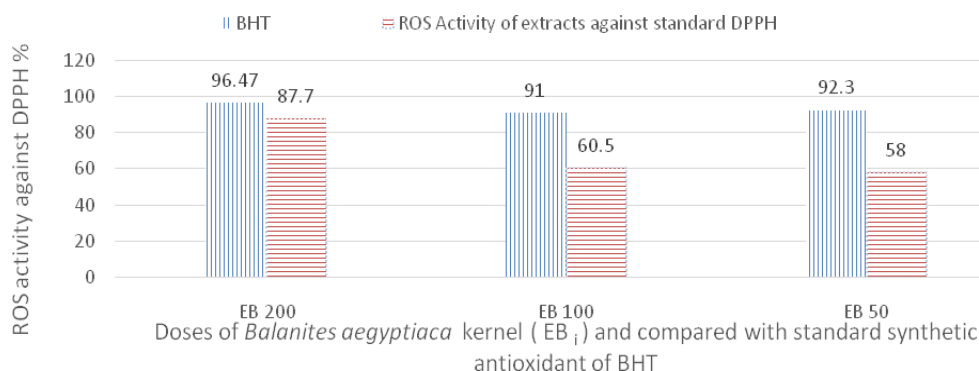


Figure 1. Antioxidant activity by Reactive Oxygen Scavenging (ROS) by using DPPH radical scavenging activity assay.

### Antibacterial activity of ethanolic extracted doses from the kernel of *Balanites aegyptiaca* (EB<sub>i</sub>):

As shown in Table (1), the antibacterial activity of *Balanites aegyptiaca* kernel (EB<sub>i</sub>) and different extracts doses was assessed using the Mueller Hinton (MH) agar well diffusion method by measuring the diameter of growth inhibition zones at different concentrations. The results of antimicrobial activity of *Balanites aegyptiaca* kernel (EB<sub>i</sub>) and different extracts doses well diffusion method are presented in Table 1. All bacterial species (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*) tested were sensitive to *Balanites aegyptiaca* (EB<sub>i</sub>) and different extracts doses with the inhibition zone 12, 12 and 11 mm, respectively by using concentrated dose 200 mg/ml of ethanolic kernel extract of *Balanites aegyptiaca* (EB 200). Generally, the inhibition zone for *Pseudomonas aeruginosa* bacteria 9 mm. at dose EB 50. Dose EB 200 was more susceptible to inhibit bacterial activity of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis* were found to EB 200 the more sensitive than dose EB 100 and EB 50.

The minimum inhibitory concentration 90 (MIC 90) was assessed into *Staphylococcus aureus* at 132

µl/ml and 190 µl/ml against *Streptococcus faecalis* (Table 2). The minimal concentration of ethnic extract was more susceptible to inhibit 90% of *Staphylococcus aureus* with lower concentration 132 µl/ml while, *Streptococcus faecalis* required for higher concentration from extract at 190 µl/ml.

Table 1. Antibacterial activity of ethanolic extracted dose from the kernel of *Balanites aegyptiaca* (EB<sub>i</sub>) (mg/ml).

| Bacterial                     | Sample concentrate (mg/ml)           |       |       |
|-------------------------------|--------------------------------------|-------|-------|
|                               | EB50                                 | EB100 | EB200 |
|                               | Inhibition zone diameter (mm/sample) |       |       |
| <i>Pseudomonas aeruginosa</i> | 9                                    | 10    | 12    |
| <i>Staphylococcus aureus</i>  | 10                                   | 10    | 12    |
| <i>Streptococcus faecalis</i> | 11                                   | 11    | 11    |

Table 2. Minimum inhibitory concentration (MIC<sub>90</sub>) of Ethanolic extract of kernel *B. aegyptiaca* seeds (EB<sub>i</sub>).

| Ethanolic extract of kernel <i>B. aegyptiaca</i> seeds | MIC <sub>90</sub> (µl/ml)    |                               |
|--|------------------------------|-------------------------------|
|  | <i>Staphylococcus aureus</i> | <i>Streptococcus faecalis</i> |
|  | 132                          | 190                           |

**Biochemical parameters:**

The mean values of serum biochemical parameters of treated rats with CCL<sub>4</sub> at different extracted dosages of EB 200 concentrates and Silymarin are depicted in Table (3) . By the end of treated animals for all different four groups a significant reduction (P<0.05) in the serum at liver function (ALT and AST) levels were occurring among 200 mg fed group (3) of and also among group 4 which dosages on Silymarin treated when comparison with hepatocarcinoma group 2 of CCL<sub>4</sub> . On the other hand, these affected groups 3 and 4 were similar to control group (1) in their liver function. In respect to group 4 which treated with Silymarin was similar to group 3 in

such AST and ALT of liver function. These results recorded for ALT and AST among groups 3 and 4 as (53.67 and 42.00U/L)and (40.00 and 34.40 U/L) , respectively. Generally, group 2 which treated by CCL<sub>4</sub> had a highest level of liver function and also shown as the worst group when compared to other treated groups.

B. aegyptiaca extract at dose 200 mg shown a significant decrease in ALP (U/L) in compared with group 2. ALP concentration was decreased from 155.00 U/L among group 2 into 116.17 among group (3). Otherwise, group 4 of Silymarin showing a similar concentration with normal control group about ≈ 92.00 U/L.

**Tables 3. Effect of *B. aegyptiaca* kernel extract and Silymarin on some biochemical parameters in treating rats with hepatodocarcinoma inducer of CCL<sub>4</sub>. (mean±SD).**

| Treatment groups                          | Liver Function           |                          | Biochemical parameters              |  |                                     |                             |
|---|--------------------------|--------------------------|-------------------------------------|--|-------------------------------------|-----------------------------|
|   | ALT (U/L)                | AST (U/L)                | ALP (U/L)<br>(Alkaline Phosphatase) | GSSG-R (U/ml)<br>Glutathione reductase | MDA<br>(nmol/ml)<br>(Malonaldehyde) | CAT<br>(U/ml)<br>(Catalase) |
| Group A (Normal control)                  | 43.00 <sup>c</sup> ±0.67 | 34.00 <sup>b</sup> ±0.73 | 92.67 <sup>c</sup> ±0.67            | 19.07 <sup>a</sup> ±0.32               | 11.65 <sup>c</sup> ±0.21            | 44.75 <sup>a</sup> ±0.20    |
| Group 2 (CCl <sub>4</sub> )               | 71.00 <sup>a</sup> ±2.82 | 56.33 <sup>a</sup> ±4.08 | 155.00 <sup>a</sup> ±1.59           | 11.83 <sup>c</sup> ±0.60               | 21.48 <sup>a</sup> ±0.55            | 31.80 <sup>c</sup> ±0.80    |
| Group 3(200 mg ethanol extract/kg B.W)    | 53.67 <sup>b</sup> ±1.58 | 40.00 <sup>c</sup> ±0.93 | 116.17 <sup>b</sup> ±1.85           | 15.67 <sup>b</sup> ±0.42               | 15.50 <sup>b</sup> ±0.43            | 38.50 <sup>b</sup> ±0.80    |
| Group 4 Silymarin (50 mg/kg body weight ) | 42.00 <sup>c</sup> ±0.86 | 34.42 <sup>b</sup> ±0.42 | 92.17 <sup>c</sup> ±0.84            | 18.92 <sup>a</sup> ±0.58               | 11.85 <sup>c</sup> ±0.28            | 44.52 <sup>a</sup> ±0.50    |
| LSD P < 0.05                              | 5.03                     | 6.30                     | 3.80                                | 1.46                                   | 1.15                                | 1.88                        |

Each value represents the mean of three replicates ± SE. The various superscript letters indicate statistically significant differences in the Duncan test, with (p< 0.05).

From these obtained results of improving liver function and ALP (alkaline phosphatase), indicated that EB 200 had a significant role to recovery of some hepatocarcinoma symptoms, which occurred by CCL<sub>4</sub> treated and near to Silymarin in its activity. These results also advocated by Sarker *et al.*, (2000) in his report, there is a considerable interest in developing .

new antioxidant plant material to increasing evidence of resistance against hepatocarcinoma. Phytochemical investigations on *Balanites aegyptiaca* yielded in the isolation of several classes of secondary metabolites, many of which expressed biological activities such as coumarins, flavonoids and steroidal saponins.

Respect to group 2 which was worst group , the plasma Glutathione reductase (GSSG-R) level is has significantly increased among all groups under this current study. There were non –significant difference (P>0.05) occurred between group 4 and group 1 in their Glutathione reductase (GSSG-R) . This means that Silymarin had affecting action role similar to the control group. The EB extract at 200 mg was in the second order after Silymarin effective when compared to antioxidative role by Glutathione reductase (GSSG-R) of Silymarin.

Revealed to the enzymatic activity of CAT catalase concentration, significantly decreased in plasma of worst group 2. While all groups in this current study such as group 1 (44.75 U/ml) and 4 (44.52 U/ml) shown a higher concentration of CAT and is followed by group 3 (38.50 u/ml) in their CAT activity. This is in

company with occurred a significant reduction of plasma levels was recorded by Silymarin and control group and followed by group 3 (Table 3) . This increase in MDA level was higher in CCL<sub>4</sub> treated (group 2) than other groups of group 3,4, and control.

This result indicated by Speroni *et al.*, (2005) that, the determination of oxidant and antioxidant markers in rats' sera and muscles of all tested groups. GSH (non-enzymatic antioxidant) depletion and MDA accumulation in rats' sera and muscles of infected-untreated group suggested an oxidative stress and progress of lipid peroxidation. A balance between oxidants and antioxidants was known to exist under physiological conditions. However, even small changes in oxidant and/or antioxidant levels might disturb that balance. *Balanites aegyptiaca* extract treated group showed a marked reduction of the inflammation reaction compared to al Bndazole-treated and infected-untreated groups. This might EB attributed to the anti-inflammatory effect of methanolic extract of *B. aegyptiaca* fruits .

**Histological studies:**

Specimens from a histological liver section of treated grouped of rats depending on different dosage of EB 200 , Silymarin after treated by CCL<sub>4</sub> , showed a significant changes in there liver sections structure as observed in Figures 2 A, 2B , 3A , 3B , 4A, 4B, 5A ,and 5B. There was no histological effect on structure of liver observed in the hepatic lobule of the liver among group 1 (Fig. 2A and Fig. 2B). Fibroblasts and focal hepatic and inflammatory could observed around bile duct of liver section for group 2. This group 2 had

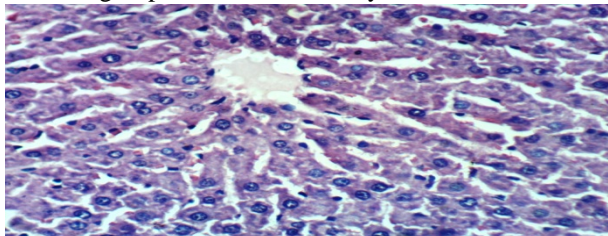


significant inflammatory structure than later groups of 3, 4 and 1.

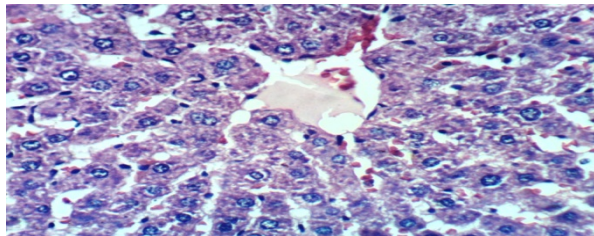
Consider to group 3 which was treated by EB 200 had shown no histopathological alteration in the liver section, except for some dilation and congestion in the central vein of conducted livers of group 3. A similar histopathological structure could investigate, consider group 4 which treated by submarine and also

except some fibrosis proliferation around bile duct. Silymarin and EB 200 had a recovering role in reducing inflammatory effect after treating by CCL<sub>4</sub>.

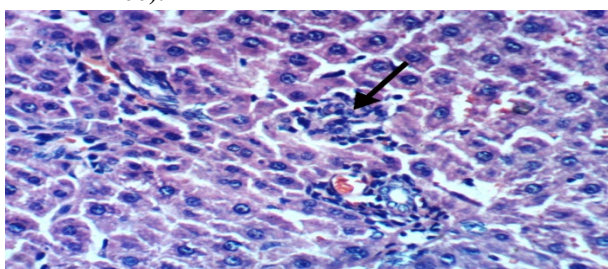
Concerning histopathological indicated that EB 200 and Silymarin were responsible to eliminate and recover treated groups by CCL<sub>4</sub> (hepatocarcinogenic agent) from inflammation.



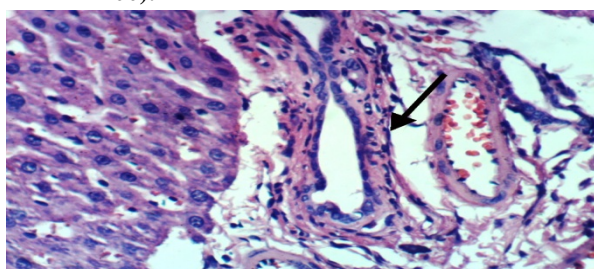
**Fig 2A** Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).



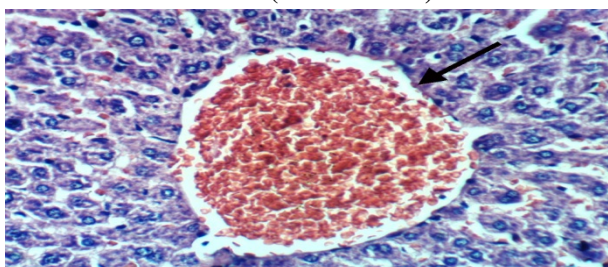
**Fig 2B** Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).



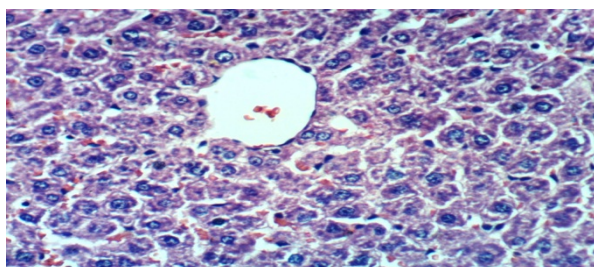
**Fig 3A** Liver of rat from group 2 showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).



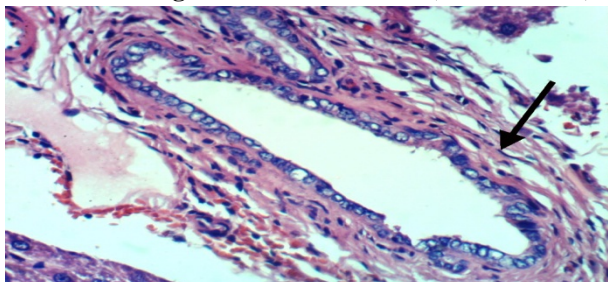
**Fig 3B** Liver of rat from group 2 showing fibroblasts proliferation around bile duct (H & E X 400).



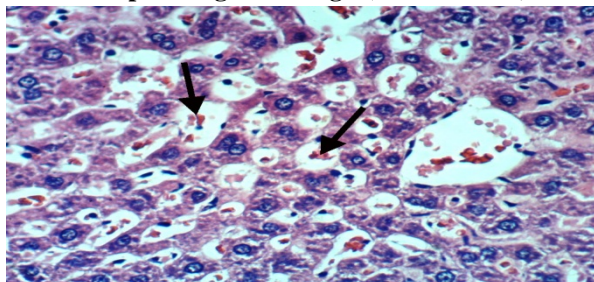
**Fig 4A** Liver of rat from group 3 showing dilation and congestion of central vein (H & E X 400).



**Fig 4B** Liver of rat from group 3 showing no histopathological changes (H & E X 400).



**Fig 5A** Liver of rat from group 4 showing fibroblasts proliferation around bile duct (H & E X 400).



**Fig 5B** Liver of rat from group 4 showing slight dilatation with congestion of hepatic sinusoids (H & E X 400).

## CONCLUSION

From this study, can conclude that, the EB 200 can provide a considerable ratio of antioxidants and antiinflammatory were effectively upon treated CCL<sub>4</sub> and other carcinogenic agents.

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## التأثيرات البكتريولوجية والمضاد للاكسدة لمستخلصات نوي الاهيليج (بلح الصحراء) (*Balanitesaegyptiaca*) للفئران المعاملة برابع كلوريد الكربون

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تعتبر مضادات الاكسدة ذات دور مهم في منع حدوث التحطيم الأوكسيدي في الأغذية وذلك بالحفاظ علي القيمة الغذائية والأقتصادية للاغذية ومنع تكوين الشقوق الحرة بصفة عامة وبالتالي تمنع الاكسدة وقد تطرق البحث الي إستخدام مستخلص أنوية بلح الاهيليج (بلح الصحراء) في دورها بمنع تحطم خلايا الكبد بعد معاملتها باستخدام رابع كلوريد الكربون لحيوانات التجارب وذلك بالأعتماد علي التراكيب الكيميائية لذلك المستخلص في كونها مضادة للاكسدة . أستهدفت هذه الدراسة الي تحديد دور المستخلص الايثانولي لنوي بلح الاهيليج الي ايقاف الأثر التدميري لخلايا الكبد بعد معاملة فئران التجارب برابع كلوريد الكربون وذلك بمعاملة الفئران بجرعات وتركيزات مختلفة من المستخلص الايثانولي لنوي بلح الصحراء من 50 ، 100 ، 200 مجم /مل ومقارنتها مع بعضها في إيجاد التأثير كمضاد للاكسدة باستخدام داي فنيل بكريل هيدرازيل DPPH المعروف بقدرته العالية علي خلب الشقوق الحرة ووجد ان التركيز 200 مجم /مل من مستخلص النوي كان له تأثير معنوي في زيادة القدرة علي خلب وإيقاف الشقوق الحرة Redox Oxygen Scavenger وقد يعزي هذا الي محتوى الفلوفونات الموجودة في المستخلص مما يكون لها الاثر الواضح لخلب الشقوق الحرة ومنع دخولها في سلاسل الاكسدة المتتالية . تم إجراء تقديرات ميكروبيولوجية لتحديد التركيز الامثل في مستخلص الاهيليج كمثبط لنمو الميكروبات محل الدراسة *Psedomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. وكانت *Psedomonas aeruginosa* حدث لها تثبيط عند مستوي 50 مجم/مل بقطر 9 مم أما بالنسبة لل 200 مجم /مل كان لها التأثير الاكبر علي *Psedomonas aeruginosa*, *Staphylococcus aureus*. التي أظهرت أعلى حساسية وتثبيط عند ذلك التركيز بينما ميكروب *Streptococcus faecalis*. أظهر تثبيط بداية من تركيز 50-100 جم / مل من مستخلص نوي الاهيليج . أيضا تم تقدير اقل حد من التركيز يكون قادر علي قتل 90% من الميكروبات محل الدراسة حيث أن تثبيط ال *Staphylococcus aureus* كانت عند تركيز أقل من 132 ميكروليتر/مل بينما التركيز الاعلي من 190 ميكروليتر /مل المستخلص نوي الاهيليج الذي له القدرة علي تثبيط *Streptococcus faecalis*. وبإجراء التجربة البيولوجية علي فئران التجارب التي تم معاملة الفئران جميعها برابع كلوريد الكربون وقسمت بعد المعاملة الي أربعة مجموعات ووجد أن تركيز 200 مجم /مل من مستخلص نوي الاهيليج كانت أكثر تأثيرا في المجموعات وكانت متقاربة في نتائجها من التحاليل البيولوجية للمجموعة المعاملة بتركيز 50 مجم/مل من السيليمارين وتبين هذا في دور مجموعة المستخلص من نوي الاهيليج والسيليمارين من خفض مستوي أنزيمات الكبد للفئران بعد المعاملة برابع كلوريد الكربون المتسبب في تحطيم بعض خلايا الكبد وحدث أيضا تحسن معنوي في مستوي انزيم جلوتاتيون المختزلبيسرم الدم لتلك المجموعات وذلك عند مقارنتها مع الكنترول المعامل فقط برابع كلوريد الكربون.تبين من الدراسات الهستولوجية لقطاعات الكبد من تحسن نسبي في خلايا كبد المجموعات المعاملة ب 200 مجم /مل من مستخلص نوي الاهيليج و 50 مجم /مل سيليمارين من تحسن الصور الهستولوجية للكبد وبالتالي يعتبر رابع كلوريد الكربون من محفزات السرطان وبالتالي يعتبر المستخلص نوي الاهيليج عند 200 مجم/مل ذو كفاءة عالية كمضاد للاكسدة ومنع لحدوث الأورام والالتهابات وكذلك مانع للنمو المرصضات البكتيرية.