

# **Role of Aqueous Extract of *Apium graveolens* Seeds Against the Haematotoxicity Induced by Carbon Tetrachloride in Female Rats**

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## **Abstract :**

The present study investigated the role of the aqueous extract of *Apium graveolens* seeds against the hematotoxicity induced by carbon tetrachloride (CCl<sub>4</sub>) in female rats. The females were divided into three groups each group contain six animals as the following: the first group was the control group, the second group treated intraperitoneally with 1 ml / kg of B.W. of CCl<sub>4</sub> for two weeks and the third group treated intraperitoneally with 1 ml/ kg of B.W. of CCl<sub>4</sub> + oral administration of 200 mg / kg of B.W. of aqueous extract of *Apium graveolens* seeds for two weeks. The result indicated that the CCl<sub>4</sub> caused a significant reduction ( $p < 0.01$ ) in the count of the erythrocyte (R.B.C.), hemoglobin (Hb.) level, and total leukocyte (W.B.C.) count and non significant decreased in the level of mean cell volume (MCV.) and Monocyte (MON.) percentage compared with the control group, also treated with the CCl<sub>4</sub> caused non significant increased in mean corpuscular haemoglobin concentration (MCHC.) level and Lymphocyte (LYM.) percentage compared with the female rats of control group . Administered of aqueous extract of *Apium graveolens* seeds at 200 mg / kg of B.W. for two weeks showed a significant increased ( $p < 0.01$ ) in the count of erythrocyte (R.B.C.), hemoglobin (Hb.) level, and total leukocyte (W.B.C.) count, and non significant increased in the mean cell volume (MCV). level and percentage of Monocyte (MON.), also treated with aqueous extract of *Apium graveolens* seeds indicated non significant decreased in mean corpuscular haemoglobin concentration (MCHC.) level and Lymphocyte (LYM.) percentage compared with the CCl<sub>4</sub> treated group. The study indicated the important effect of aqueous extract of *Apium graveolens* seeds in

recovery of these hematological parameters above toward normal value compared with CCl<sub>4</sub> treated group and compared with control group.

**Key words:** *Apium graveolens*, hematotoxicity, CCl<sub>4</sub>.

### **Introduction :**

Carbon tetrachloride is a haloalkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, particularly in refrigerator fluids, as a propellant for aerosol cans, as a dry-cleaning agent in industry, as a household spot remover, as grain fumigant and as intermediate in the synthesis of chlorofluorocarbons. As a result of its widespread use, CCl<sub>4</sub> is a common contaminant of ground and surface waters where it persists for years. Therefore CCl<sub>4</sub> is now of greatest concern as an environmental contaminant (Guo *et al.*, 2000). CCl<sub>4</sub> is a known hepatotoxic agent, it induces hepatotoxicity by producing free radical, putting oxidative stress hence causing lipid peroxidation in liver tissues, consequently necrotic liver damage will occur (Ram and Goel, 1999), also various studies have demonstrated that CCl<sub>4</sub> causes free radical generation in many tissues such as kidney, heart, lung, testis, brain and blood in addition to liver (Dashti *et al.*, 1989; Parola *et al.*, 1992; Ko *et al.*, 1995).

*Apium graveolens* L.(A.G.) or celery is a plant from the family of Apiaceae that has been used as food and as medicine. A review of the literature indicates that celery has been cultivated for the last 3.000 years, in Indian traditional medicinal system the seeds are used to treat bronchitis, asthma, liver and spleen diseases ( Momin and Nair, 2001). Extracts of the celery seed have been shown to inhibit arthritic pain (Bjeldanes and Kim, 1977). Moreover Friedmen *et al.*, (2002) showed that the celery seeds extract have bactericidal activities against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enteric*. Other experimental studies have shown that *A. graveolens* possess nematicidal, antifungal, mosquitocidal (Momin and Nair, 2001; Rafikali *et al.*, 2000; Rafikali and Muraleedharan, 2001), and as anti-aggregation (Teng *et al.*, 1985), also its known that celery in combination with other plants can help lower blood pressure (Chevallier, 1998; Radenkovic *et al.*, 2009).

On the other hand Kamal *et al.*, (2009) reported hypolipidemic effect of *Apium graveolens* extract. Nitrogenous compounds from essential oil of celery seed have been reported

to have effect on the central nervous system (Al-Hindawi *et al.*, 1989). Seeds of the *A. graveolens* are employed as a traditional medicine in India and other countries used as a tranquilizer, antispasmodic, nerve tonic, diuretic, and antirheumatic (Kapoor, 1990), inhibitory effect of celery seeds extract on chemically induced hepatocarcinogenesis (Sultana *et al.*, 2005). *A. graveolens* was reported to exhibit anti-inflammatory activity in experimental animals (Atta and Alkofahi, 1998; Butters *et al.*, 1999; Whitehouse *et al.*, 2000, 2001; Kitajima *et al.*, 2003; Michio, 2005), antiulcer activity in rodent models (Whitehouse *et al.*, 2000, 2001; Kitajima *et al.*, 2003; Butters *et al.*, 1999; Michio, 2005). Also *Apium graveolens* seeds have gastro protective effect probably mediated through non-prostaglandin E2 production (Whitehouse *et al.*, 2001). Hepatoprotective effect of methanolic extract of *A. graveolens* seeds has been reported in rats against various hepatotoxicants (Singh and Handa, 1995; Ahmed *et al.*, 2002), also the methanol extract of celery showed a significant hepatoprotective activity compared to the paracetamol and thioacetamide treated rats (Singh and Handa, 1995; Bahar *et al.*, 2002). Besides, the extract of leaves has also shown its effectiveness in lowering oxidative stress and hepatoprotective effects of celery extracts induced by CCl<sub>4</sub> in mice (Min *et al.*, 1996; Jakovljevi *et al.*, 2002; Popovi *et al.*, 2006). Moreover, it has also been reported to modulate reproductive toxicity induced by sodium valproate (Hamza and Amin, 2007).

The present study aimed to evaluate the protective efficiency of aqueous extract of *Apium graveolens* (A.G.) seeds against the hematotoxicity of ccl<sub>4</sub> in female rats.

### **Materials and Methods:**

#### **Plant materials and Preparation of Extracts:**

Fresh plant materials of *Apium graveolens* seeds were purchased from a local market in Al-Nassiriyai city / Thi-Qar / Iraq. 25 g/ 250 ml of plant materials were boiled with distilled water for 5 minutes, after cooling and filtering by using funnel and filter paper, clear supernatants were taken with concentrate about 200 mg /kg of B.W.

#### **Animals**

Eighteen white female rats weighing ( 95 - 100 ) gm and aged between (5 - 6) weeks were used in this study, all animals were obtained from animal house of biology department /college of sciences / university of Thi-Qar / Iraq. The rats were divided randomly into three groups of six rats.

**Group I:** as the control group, received only food and water for two weeks.

**Group II:** treated intraperitoneally with 1 ml /kg of B.W. from CCl<sub>4</sub> with two doses (Ray *et al.*, 2006), at the first day from each week for two weeks. (each week one dose).

**Group III:** treated intraperitoneally with 1 ml /kg of B.W. from CCl<sub>4</sub> with two doses, at the first day from each week for two weeks (each week one dose) + 0.2 ml of 200 mg/ kg of aqueous extract of *Apium graveolens* seeds daily oral intake for two weeks.

After two weeks of treatment, the animals were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture, 5 ml of blood samples were collected from heart and put into EDTA tubes for measurement of the blood parameters. The blood parameters included erythrocyte (R.B.C.) Hemoglobin (Hb.), mean cell volume (MCV.), mean corpuscular haemoglobin concentration (MCHC.), leukocytes (W.B.C), Monocyte (MON.), and Lymphocyte (LYM.)

**Result :**

Table 1 showed the effect of aqueous extract of *Apium graveolens* seeds on the R.B.C. and Hb. of the female rats treated with CCl<sub>4</sub>. There are a significant decreased ( $p < 0.01$ ) in the count of the R.B.C. and Hb. level after treatment with CCl<sub>4</sub> compared with the control group, oral administration of aqueous extract of *Apium graveolens* seeds resulted in increasing of these parameters significantly ( $p < 0.01$ ) and were likely to be approaching normal values when compared with that of toxic group ( also see Figure 1).

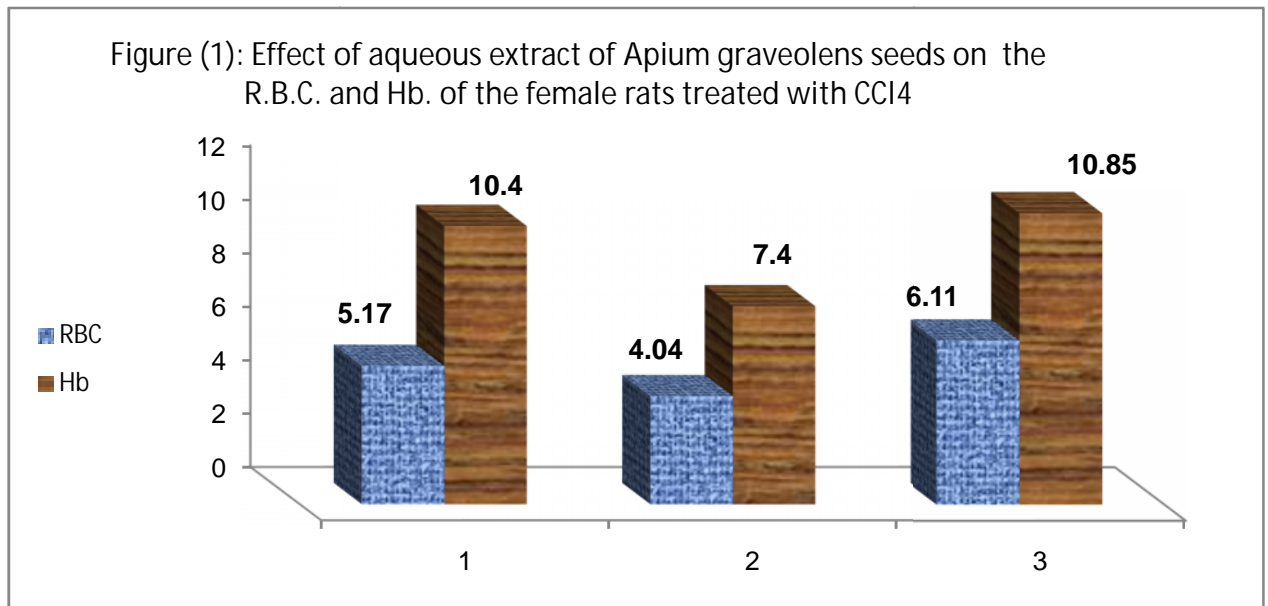
**Table (1): Effect of aqueous extract of *Apium graveolens* seeds on R.B.C. and Hb. of female rats treated with CCl<sub>4</sub>**

Group No.	R.B.C. (10 <sup>6</sup> /mm <sup>3</sup> )	Hb. (g/dL)
Group I	5.17 ± 0.37 a	10.4 ± 1.30 a
Group II	4.04 ± 0.44 b	7.4 ± 0.89 b
Group III	6.11 ± 0.17 a	10.85 ± 0.17 a

Values given as mean of 6 rats ± S.E.

Means with the same letter are not significantly different at  $p < 0.01$

The different letters refers to a significant differences at  $p < 0.01$



The results of the effect of aqueous extract of *Apium graveolens* seeds on the MCV. , MCVC. levels after administration of CCl<sub>4</sub> to the female rats were illustrated in Table 2. The results indicated that there is non significant decreased in the MCV. level, and non significant increased in the MCHC. level of females treated with CCl<sub>4</sub> compared with the control group. Administration of the A. G. extract result in non significant increased in the MCV. level, and non significant decreased in the MCHC. level (also see Figure 2).

**Table (2): Effect of aqueous extract of *Apium graveolens* seeds on the MCV., MCHC. of female rats treated with CCl<sub>4</sub>**

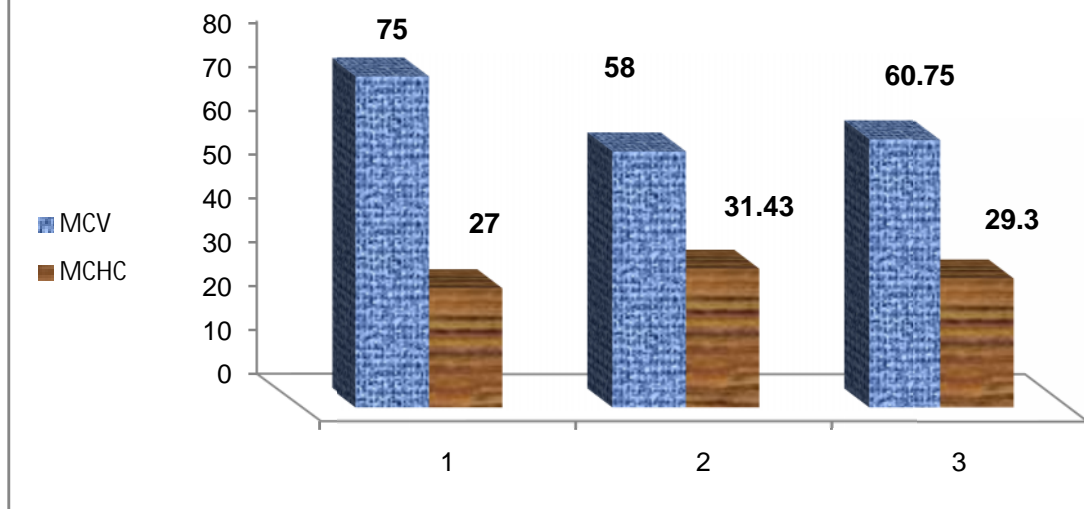
Group No.	MCV. m <sup>3</sup> (μ	MCHC. g/dL)(
Group I	75 ± 13.00 a	27.85 ± 6.25 a
Group II	58 ± 1.00 bc	31.43 ± 0.29 a
Group III	60.75 ± 1.03 ab	29.3 ± 0.41 a

Values given as mean of 6 rats ± S.E.

Means with the same letter are not significantly different at  $p < 0.01$

The different letters refers to a significant differences at  $p < 0.01$

Figure (2): Effect of aqueous extract of *Apium graveolens* seeds on the MCV, MCHC. of female rats treated with CCl<sub>4</sub>



The hepatoprotective effect of the aqueous extract of *Apium graveolens* seeds on the W.B.C., MON., and LYM. of female rats treated with CCl<sub>4</sub> are summarized in Table 3. The table showed the comparison of effects among untreated or control group and carbon tetrachloride treated group with the plant treated group of rats. There is a significant reduction ( $p < 0.01$ ) in the total count of W.B.C and non significant reduction in the MON. percentage, and non significant elevation in the percentages of LYM. were found in toxic group, when compared with the control group. While treatment of female rats with aqueous extract of *A. graveolens* seeds caused a significant increased ( $p < 0.01$ ) in the total W.B.C. count and non significant increased in the MON. percentage also treated with the plant extract resulted in non significant decreased in the LYM. percentage compared with females treated with CCl<sub>4</sub> (see Figure 3).

Table (3): Effect of aqueous extract of *Apium graveolens* seeds on the W.B.C., MON. and LYM. of female rats treated with CCl<sub>4</sub> treated

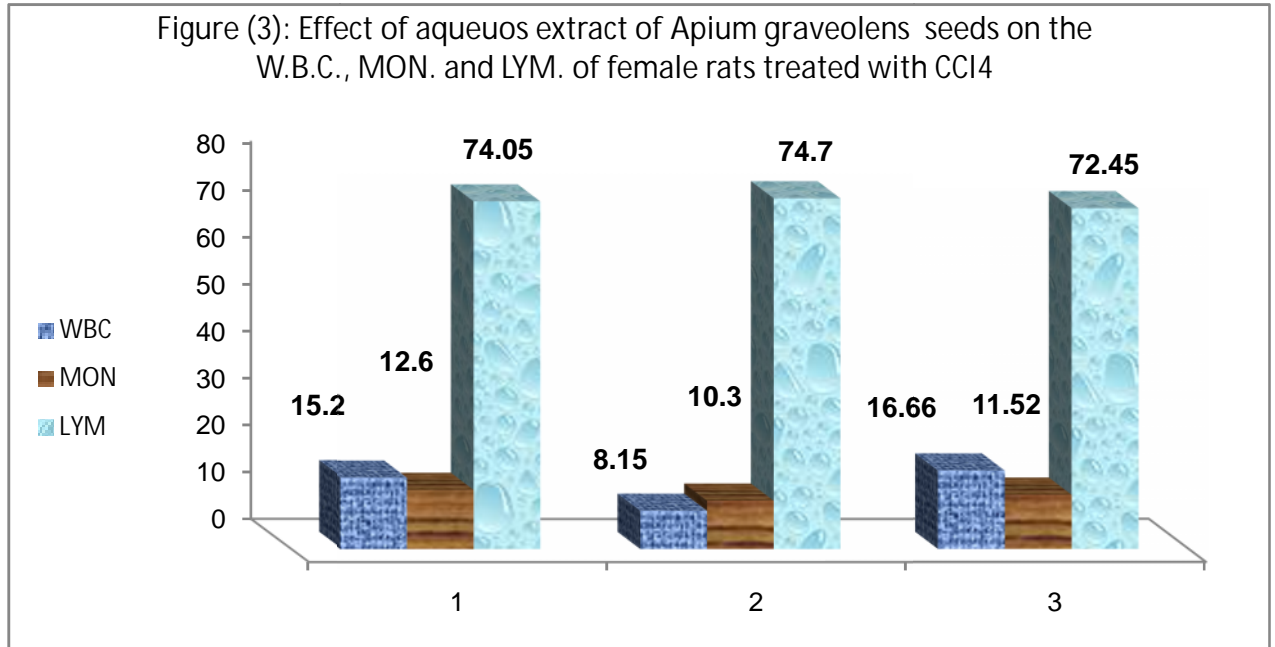
Group No.	W.B.C. ( $10^3/\text{mm}^3$ )	MON. (%)	LYM. (%)
Group I	15.2 ± 0.01 a	12.6 ± 2.40 a	74.05 ± 9.55 a
Group II	8.15 ± 0.02 b	10.3 ± 1.89 a	74.7 ± 2.96 a
Group III	16.66 ± 0.63 a	11.52 ± 1.04 a	72.45 ± 3.16 a

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Values given as mean of 6 rats  $\pm$  S.E.

Means with the same letter are not significantly different at  $p < 0.01$

The different letters refers to a significant differences at  $p < 0.01$



### Discussion:

The present study was conducted to evaluate the beneficial effects of the aqueous extract of *A. graveolens* seeds on the blood toxicity induced by CCl<sub>4</sub> in female rats. Intraperitoneal administration of CCl<sub>4</sub> greatly affected hematological parameters. It cause hematotoxicity occurred as a decreases in erythrocytes count, hemoglobin, MCV. values and also obvious thrombocytopenia. Depletion in the count of R.B.C. along with the Hb. level was detected in mice treated with CCl<sub>4</sub> at a dose of 0.05 ml per mouse for five weeks (Mandal *et al.*, 1998) this is similarly consistent with previous reported of anemia in CCl<sub>4</sub> exposed experimental animals ( Mortiz and Pankow, 1989; Adaramoye and Akinloye, 2000). According to Ballinger (2007), depletion in erythrocytes count and Hb. level leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture, also hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation this could be another reason for decreasing hematological values (Tung *et al.*, 1975). The depression in R.B.C. count and Hb. level recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their

formation and / or their enhanced removal from circulation due to CCl<sub>4</sub> toxicity. Also treatment with CCl<sub>4</sub> induced marked leucopenia, this result supports the finding of Jirova *et al.* (1996) and Mandal *et al.*, (1998) who stated that exposure to CCl<sub>4</sub> induced non significant decrease in leukocytes count in peripheral blood of mice compared with normal rats . In this present the decreasing in leukocytes count may occurs due to lysis of white blood cells.

The present study showed that the aqueous extract of *A. graveolens* seeds caused an improvement of these parameters and return it to the normal value in females treated with CCl<sub>4</sub>, this protective effect may be mediated through several mechanisms since the extract itself is a complex mixture of many chemicals. Phytochemical analysis of the plant showed that *A. graveolens* consist of calcium (Teng *et al.*, 1985). Also, Mitra *et al.*, (2001) and Belal, (2011) were found that the *Apium graveolens* is excellent source of Vitamin C, and it's a good source of dietary fibers, potassium, magnesium, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B6, calcium, phosphorus, and iron. Other phytochemical analysis of the *A. graveolens* demonstrated the presence of flavonoids, apigenin, luteolin and quercetin (Anthony and Dweck, 2009; Tang *et al.*, 1990). Besides these *A. graveolens* contains -tocopherol and glucosides (Ching and Mohammed, 2001; Kitajima *et al.*, 2003). Vitamin C is a strong antioxidant (Rao, 1997; Sato *et al.*, 1997). The detoxification effect of Vitamin C is manifested by the removal or minimization of free radicals produced by mercury (Gebhart, 1984; Herbaczynska *et al.*, 1995). Also Vitamin C protects DNA from oxidative damage (Eylar *et al.*, 1996; Antunes and Takahashi, 1999), reduces DNA damage exerted by irradiation (Green *et al.*, 1994) and reduces micronucleus (MN) frequencies in polychromatic erythrocytes of bone marrow in rodents exposed to heavy metals and radiation (Chorvatovicova' *et al.*, 1991; Konopacka *et al.*, 1998). On the other hand the antioxidant activity of the plant may be due to present of flavonoids in its constituents which have shown to posses various biological properties related to antioxidant mechanism(Latha *et al.*, 2003). These phytoconstituents have antioxidant effect (Momin and Nair, 2001; Jakovljevi *et al.*, 2002; Sultana *et al.*, 2005; Popovi *et al.*, 2006). It probably does so by free radical scavenging which has been established in the *in vitro* studies indicating its antioxidant activity (Popovic *et al.*, 2006; Wei and Shibamoto, 2007), this antioxidant activity may also play an important role in cell protection from enhancement of per-



oxidative injuries, also the ability of *A. graveolens* for healing comes from these important Vitamins and Minerals in there components.

### **Conclusion:**

The present results show that the aqueous extract of *A. graveolens* seeds for two weeks is safe for consumption at the dose tested, succeeded in the improvement of hematological parameters and protect the haematopoietic cells from the damaging effects from exposure to CCl<sub>4</sub>.

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دور المستخلص المائي لبذور نبات الكرفس ضد سمية الدم ألمحدثه بواسطة  
رباعي كلوريد الكربون في إناث الجرذان  
أوراس سعد خيون  
قسم العلوم الاساسيه / كلية التمريض /

أخلاصه:

وجد في الدراسة أالحاليه دور المستخلص المائي لبذور نبات الكرفس ضد سمية الدم ألمحدثه بواسطة رباعي كلوريد الكربون في إناث الجرذان. تم تقسيم الجرذان إلى ثلاث مجاميع كل مجموعته تحتوي على ستة حيوانات و كالتالي: ألمجموعه الأولى : مجموعته السيطرة , ألمجموعه ألتانيه تم حقنها برباعي كلوريد 1 / كغم داخل البريتون و ألمجموعه ألتالثه تم حقنها برباعي كلوريد الكربون 1 / داخل البريتون + التجريب الفموي للمستخلص المائي لبذور نبات الكرفس 200 / . ألمعامله برباعي كلوريد الكربون سببت انخفاضا ملحوظا ( $p < 0.01$ ) في عدد كريات الدم الحمر R.B.C , قيمة الهيموكلوبين Hb, والعدد الكلي لكريات الدم البيضاء W.B.C, وانخفاضا غير ملحوظ في قيمة معدل حجم الخلايا MCV, و النسبة المنوية لخلايا Monocyte مقارنة مع مجموعته السيطرة , أيضا

سببت معاملة الجردان برباعي كلوريد الكربون زيادة غير ملحوظة في قيمة معدل تركيز الهيموكلوبين في ريات الحمراء. **MCHC** والنسبة المئوية للخلايا اللمفاوية **Lymphocyte** في مجموعة السيطرة. إضافة المستخلص المائي لبذور نبات الكرفس بتركيز (200 / ) لمدة أسبوعين أظهرت زيادة ملحوظة ( $p < 0.01$ ) في عدد كريات الدم الحمراء **R.B.C**, قيمة الهيموكلوبين **Hb** وعدد كريات الدم البيضاء **W.B.C** وزيادة غير ملحوظة في قيمة معدل حجم الخلايا **MCV** و النسبة المئوية لخلايا **Monocyte** , أيضا أدت المعاملة بالمستخلص المائي لبذور نبات الكرفس إلى نقصان غير ملحوظ في قيمة معدل تركيز الهيموكلوبين في الكريات الحمر **MCHC** المئوية للخلايا اللمفاوية **Lymphocyte** مقارنة مع المجموعه المعامله برباعي كلوريد الكربون. أظهرت الدراسة أالحاليه التأثير المهم للمستخلص المائي لبذور نبات الكرفس في تحسين معدلات القياسات أنفة الذكر ورجوعها إلى القياسات أطيبيعه مقارنة مع أالمجموعه المعامله برباعي كلوريد الكربون مقارنة مع مجموعة السيطرة .

الكلمات أالمفتاحيه: , سمية الدم, رباعي كلوريد الكربون.