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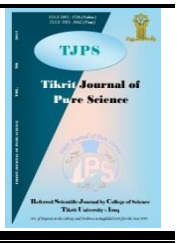

Identifying and Study of some of Phytochemical compounds and Anti-Jaundice Activity for powder of leaves and seeds of *Moringa oleifera* in male albino rats.

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Abstract

The present study aimed to revealing and identifying some of phytochemical compounds, bioactive fatty acids and antioxidants for powder of leaves and seeds of *Moringa oleifera* that cultivated in Iraq which have been many identified by using modern techniques such as gas chromatography mass spectrometry (GC-MS) technique. And then study and test the preventive and Therapy effects for powder of leaves and seeds in present study against hepatotoxicity that induced by carbon tetrachloride (CCl_4) and against jaundice. These effects were tested by measuring total bilirubin concentrations of total bilirubin and γ-glutamyl transferase (GGT) in serum of normal male rats, exposed to CCl_4 and that treated with powder of leaves and seeds of *Moringa oleifera* along the period of experiment (30) days, used (20) albino male rats divide to (4) groups, that is meaning (5) rats for each group. The rats group that exposed to CCl_4 caused a significant increase ($P<0.05$) in concentrations of total bilirubin and GGT enzyme in comparison with normal control group. Whereas the animals which exposed to CCl_4 then treated with powder of leaves and seeds of *Moringa oleifera* lead to significant decrease ($P<0.05$) in total bilirubin and GGT enzyme concentration in comparison with the group that exposed to CCl_4 only. It could be concluded of this study that using powder of leaves and seeds of *Moringa oleifera* may be have Preventive and therapeutic effects against damage and hepatotoxicity, subsequently, anti-jaundice activity through its nutritional and antioxidant influences for remove of free radicals and repairing cellular and tissues from toxic damage.

Introduction

In recent times, focus on plant research has increased all over the world because the plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities [1], as well as, throughout history, plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use. Under traditional purposes, herbal medicines and medical plants like *Moringa oleifera* Lam [2], is one of the best known and most widely distributed and naturalized species of a monogeneric family *Moringaceae* [3]. *M. oleifera*, native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia [4]. *M. oleifera* is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics'. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries [5]. In Thailand, it is locally known as 'Marum'. Seed of this plant is used as human food, medicine, in oil production, and also for efficient treatment of hard water [6]. Also, *M. oleifera* can be used to treat diabetes and intestinal worms [7].

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Figure (1): **leaves and seeds of *Moringa oleifera*** [8].

Jaundice is the most common of liver disorders. It is a condition in which yellow discoloration of the skin and mucous membranes occurs due to an increase in the bile pigments (bilirubin) in the blood [9]. Excess bilirubin in blood gives rise to jaundice. The common causes are increase destruction of red cells with rapid release of bilirubin into blood. Obstruction of bile duct cause damage to liver cells. There are many different causes of jaundice such as hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, liver cirrhosis, obstruction of bile ducts, gallstones, pancreatic cancer, alcoholic liver disease, inflammation of the liver, haemolytic anemia, typhoid, malaria, yellow fever, tuberculosis, certain medication and pregnancy.

The aim of the present study was for identified the presence of some phytochemicals and antioxidants in seeds and leaves samples of *M. oleifera* by GC-MS analysis and study of biologically active to some of phytochemicals on bilirubin level and GGT enzyme as special markers for jaundice.

Materials and methods

This study was conducted on uses the plant samples (seeds and leaves) of *Moringa oleifera* were identified and collected from Herbarium in Baghdad, Iraq. Seeds and leaves were screened to remove bad ones and dried then pulverized by using grinder transform to powder form, then sieved and put in an air-tight container and stored at 4°C until further analysis. While the animals were obtained from national center for drug control and research in Baghdad, Iraq. The animals were a wistar albino male rats (*Rattus norvegicus*) of strain Sprague dawely, the weights ranged between (275-300)g and their ages were ranged (3-4) months. The animals were housed according to the institutional guidelines for animal research in propylene cages and were provided bedding of sawdust. Animal care, handling of cages and alteration of sawdust was done continually each two days. And put under standard laboratory conditions of light a 12 h light and 12 h dark as cycle, and temperature was maintained at 22±2°C with a relative humidity of 45 ±1% and acclimated to the laboratory environment for two weeks before use. Animals had free access to sterile food (animal chow) (35% wheat, 34% corn, 20% soy-bean, 10% animalistic protein, 1% milk powder and additive 50 gm protective and antifungal substances) [10]. It's given standard food and water *ad libitum* in adequate amounts all through for the experimental period that expanded along between September/November,2015.

Design of Experiments:

In this study used (20) male albino rats. The animals were selected randomly and divided into a four (4) experimental groups, each group included 5 rats and take care the converging weights for animals, as follows: The normal control group (I): gave this group only drinking tap water and food daily for period 30 days, the group (II) were injected intraperitoneally CCl_4 (0.5ml CCl_4/kg b.w) [11] mixed in olive oil v/v twice a week. a group seeds of *M. oleifera* (III): this group had been given seeds powder (200 mg/Kg of b.w) of *M. oleifera* [12] with food daily for period 30 days, and a group leaves of *M. oleifera* (IV): this group give leaves powder (300 mg/Kg of b.w) of *M. oleifera* [12] with food daily for period 30 days.

At the end of experimental period (30 days), all animals were fasted for 24 hours, but still allowed free access to water. The animals were anesthetized by chloroform and sacrificed by severance of jugular vein. Then take approximately 1 ml of blood from each animal, put in EDTA tubes containing anticoagulant for measuring the total bilirubin and GGT enzyme concentration.

GC-MS Analysis:

The phytochemical properties and fatty acid composition of leaves and seeds of *M. oleifera* was quantified using Gas Chromatography–Mass spectrometry (GC-MS) analysis. This analysis was performed by Instrument of Gas Chromato-graphy–Mass spectrometry QP-2010Japan, in laboratories of al - Mustansiriyah university \ college of Science\ department of chemistry then were evaluated with Postrun system and searched in national institute of standard and technology center (NIST).

The biochemical tests:

Diagnostic kit were employed in the analysis of serum total bilirubin concentrations [13]. In addition to the concentration of GGT measured by modified method [14].

Statistical analysis:

Finally, the statistical analysis was carried out by using statistical program (SAS,2001) and Comparison between groups were made by using one-way analysis of variance (ANOVA), and tried out the arithmetic means for parameters by using test of duncun multiple range to delimitating significantly differents especially between groups. The level of statistical significance was taken at ($P<0.05$). All data are expressed as mean± standard error (M±S.D) and put above it duncun value (letters).

Results and Discussion:

The results of preliminary qualitative phytochemical for powder of seeds and leaves of *Moringa oleifera* showed the presence of phenols, glycosides,

flavonoids, steroids, alkaloids, terpenoids, tannins, volatile oils, vitamin C, resins, proteins and amino acids in all of samples in the iraqi *Moringa oleifera*, as well as, presence of coumarin and saponins only in leaves sample.

Table (1): Phytochemical screening of Extract from leaves and seeds of *Moringa oleifera*.

No	Active substances	*Moringa oleifera* leaves	*Moringa oleifera* seeds
1	Phenols	+	+
2	Glycosides	+	+
3	Flavonoids	+	+
4	Coumarin	+	-
5	Steroids	+	+
6	Alkaloids	+	+
7	Terpenoids	+	+
8	Tannins	+	+
9	Volatile oils	+	+
10	Saponins	+	-
11	Resins	+	+
12	Vitamin C (ascorbic acid)	+	+
13	Proteins	+	+
14	Amino acids	+	+

+, - represent presence and absence of phyto-constituents respectively.

Determination of Components by Gas Chromatography – Mass spectrometry technique: Analyzing the results of mass spectrum of GC-MS was done using the database of NIST. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and mass fragmentation of some of the components of the test materials were ascertained as follows:

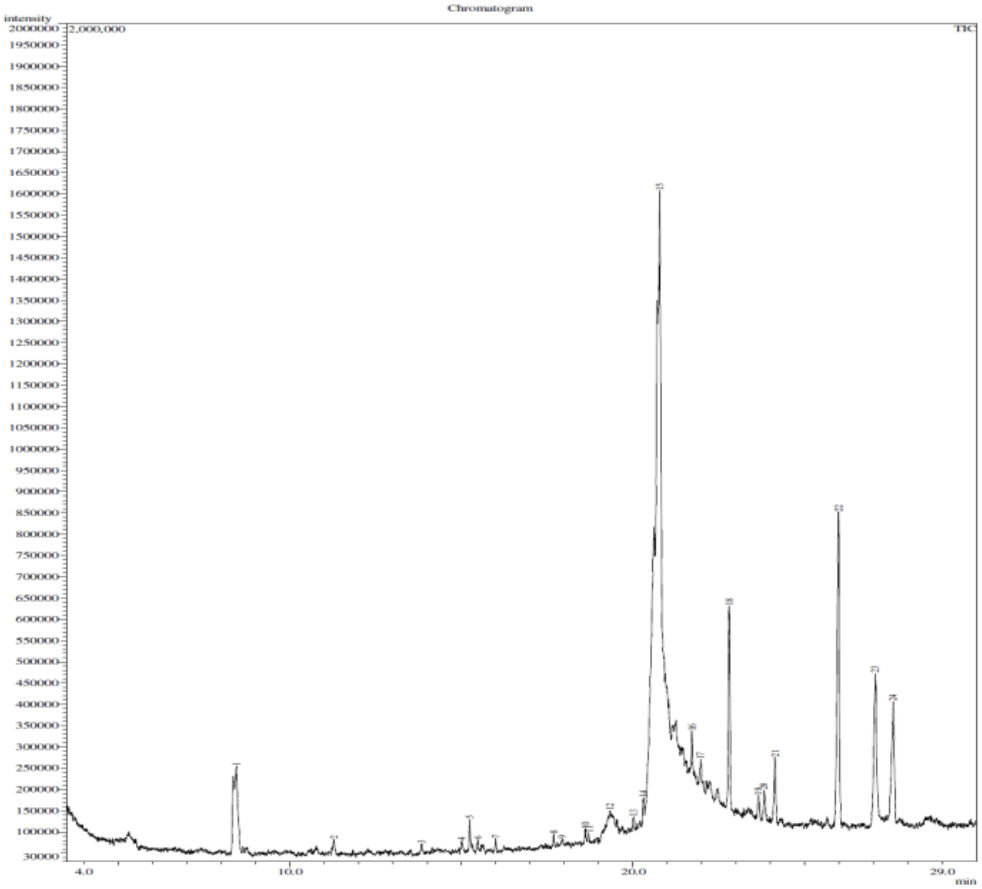

Figure (2): GC-MS Chromatogram for leaves powder of *M. oleifera*.

The results concerning to GC-MS technique led to the determination of number of compounds in leaves and seeds powder of *M. oleifera*. GC-MS chromatogram revealed many climaxes that indicating to numerous of compounds (figure 2), (figure 3), (Table. 2).

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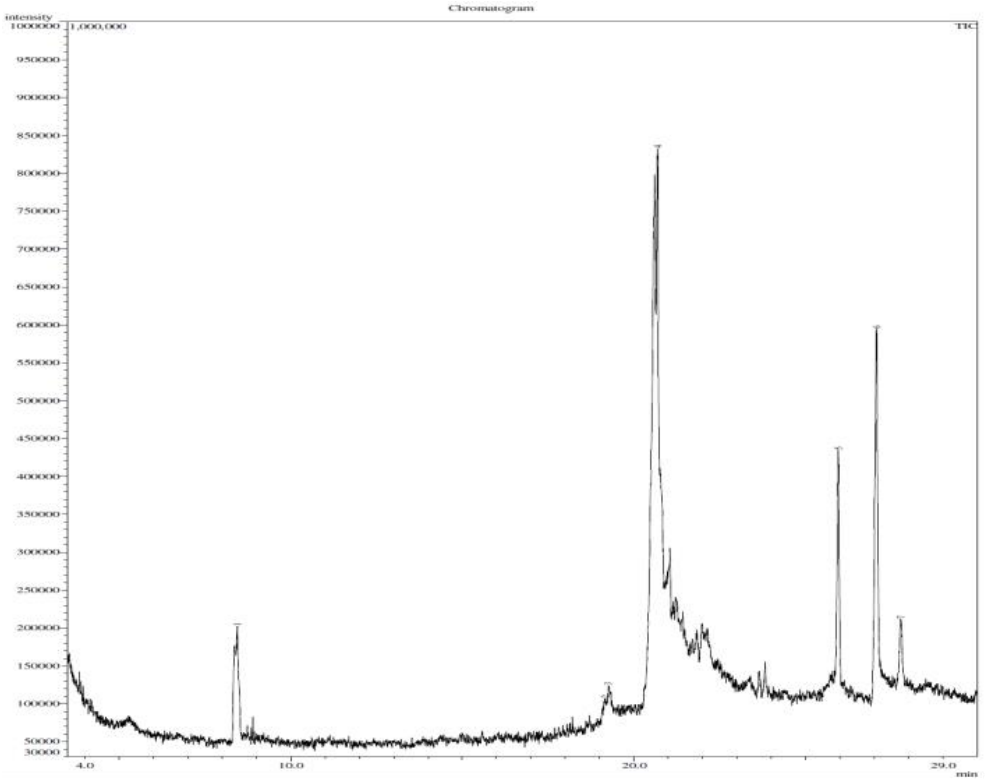

Figure (3): GC-MS Chromatogram for seeds powder of *M. oleifera*.

Table (2): Phyto-components identified in the leaves and seeds of *M. oleifera*.

Leaves of *M. oleifera*.				
Peak	**Name of compound**	**R. Time**	**Area**	**Height**
1	Acetic acid	8.44	1783088	199226
2	Butanoic acid, 2-methyl-	11.28	149499	34578
3	Hexanoic acid	13.84	66282	22869
4	2-Butenedioic acid (Z)-, dibutyl ester	15.01	107504	27986
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.25	161307	69837
6	2-Hexenoic acid	15.48	77016	27140
7	6-Octen-1-ol, 3,7-dimethyl-, acetate	15.99	74073	31952
8	2-Pentadecanone, 6,10,14-trimethyl-	17.69	49922	27521
9	Nonanoic acid	17.94	24021	10124
10	1,2,3-Propanetriol, 1-acetate	18.60	82741	32226
11	Hexadecanoic acid, methyl ester	18.70	34292	19035
12	Octadecanoic acid	19.32	652266	50030
13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	20.02	93529	31397
14	1-Hexadecanol	20.30	122054	51053
15	Octadec-9-enoic acid	20.78	17262437	1336010
16	Triacontane	21.71	222834	91605
17	Phthalic acid, butyl tetradecyl ester	21.97	128023	48636
18	Phytol	22.80	1425307	457853
19	Tetradecanoic acid	23.65	124798	45198
20	Dibutyl phthalate	23.81	245107	68550
21	Hexatriacontane	24.13	497730	145944
22	Hexanedioic acid, bis(2-ethylhexyl) ester	25.98	3231000	726892
23	Pentadecanoic acid	27.05	1699455	326391
24	Triacontane	27.58	1491057	276364
Seeds of *M. oleifera*.				
1	Acetic acid	8.44	1298796	147139
2	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	19.17	64268	13843
3	Octadecanoic acid	19.26	141134	26318
4	Octadec-9-enoic acid	20.69	7605563	598888
5	Hexanedioic acid, bis(2-ethylhexyl) ester	25.95	1219171	293831
6	n-Hexadecanoic acid	27.06	2488024	452188
7	18-Nonadecenoic acid	27.77	365643	74425

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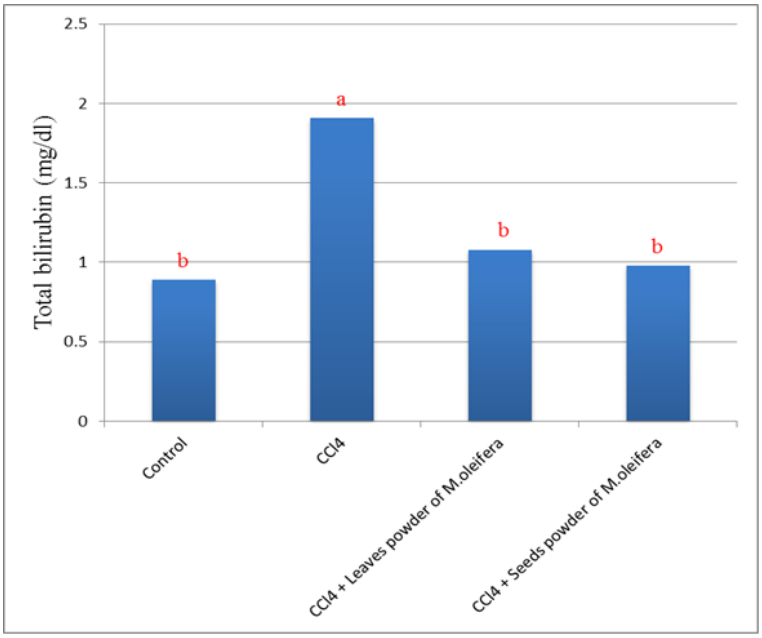
Total bilirubin concentration in serum of male albino rats:

Results showed in figure (4) that there was a high significant increase ($p<0.05$) of total bilirubin concentration in serum of male albino rat group which administrated carbon tetrachloride CCl_4 in comparison with normal control group.



Figure(4): Effect of treatment by leaves powder (300 mg/kg of b.w) and seeds powder (200 mg/Kg of b.w) of *Moringa oleifera* on total bilirubin concentra-tion in male albino rats that exposed to liver toxicity Induced by CCl_4 .

CCl4 is one of the most commonly used hepatotoxins in induced the liver injury by associated with oxidative stress and free radicals. The hepatotoxicity of CCl4 is due to reductive dehalogenation products, such as trichloromethyl ($CCl3^{\cdot}$) and trichloro-methyl peroxyl ($CCl_3O_2^{\cdot}$) radicals, these radicals can bind to organic compounds such as lipids, proteins and nucleic acids, thus occurrence lipid peroxidation and damage in hepatocytes [15], and subsequently, elevate concentration of serum bilirubin.

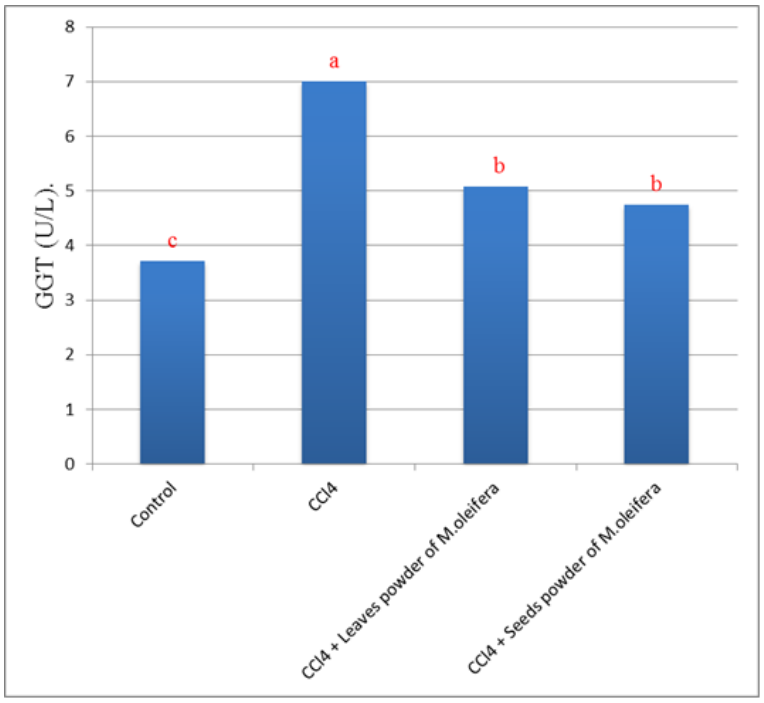
While the groups that administrated CCl_4 then treated by leaves and seeds powder of *Moringa oleifera* observed significant decrease ($p>0.05$) in total bilirubin concentration in comparison with group which administrated CCl_4 only. This may be due to that treatment with parts of *M. oleifera* which contain a rich source of proteins, β-carotene, vitamins A, B, C, E, riboflavin, nicotinic acid, folic acid and pyridoxine, amino acids, unsaturated fatty acids, minerals and various phenolic compounds [16], this effect may lead to repairing damage in tissue and cells of liver, and respectively, decrease of serum bilirubin concentration.

Whereas the results showed no significant variations in TB concentration in all groups that administrated CCl_4 then treated by leaves and seeds powder of *Moringa oleifera* in comparison with normal control group.

γ-glutamyl transferase (GGT) concentration in serum of male albino rats:

The results in figure (5) indicates that there was a significant increase ($p<0.05$) in concentration of GGT in serum of male albino rats group which administrated CCl4 only and the groups which administrated CCl_4 then treated by leaves and seeds powder of *Moringa oleifera* successively, in comparison with normal control group. The causes in increasing of GGT may be due to; when cell membrane of hepatocyte is damaged, a variety of enzymes normally that located in the cytosol are released into the blood stream [17]. The activity of serum GGT is generally elevated as a result of liver disease [18]. Also, elevated of GGT in serum may be result from biliary epithelial necrosis, intra-hepatic cholestasis or hepatic infiltration [19], that harm induced by CCl_4. Moreover, if the liver is damaged or the normal flow of blood or bile is obstructed, the cellular content of GGT enzyme leak or secreted into the blood and thus may result in increase of GGT concentration.

While the groups that administrated CCl_4 then treated by leaves and seeds powder of *M. oleifera* showed significant decrease ($p>0.05$) in GGT concentration in comparison with group which administrated CCl_4 only. This effect may be refer that treatment with *M.* leaves can be cause increase of antioxidant activity and remove of free radicals, subsequently, reduced oxidative stress and damage for major biomolecules and prevent tissue damage by free radicals (oxidative stress) [20], therefore, indicating on promising hepatoprotective activity of Moringa leaves that may conserve the structural integrity of hepatocytes membranes and consequently preventing GGT enzyme leakage into blood stream.



Figure(5): Effect of treatment by leaves powder (300 mg/kg of b.w) and seeds powder (200 mg/Kg of b.w) of *Moringa oleifera* on GGT concentration in male albino rats that exposed to liver toxicity Induced by CCl_4

Conclusion

The results of the present study indicated that under the present experimental conditions, administration of leaves and seeds powder of *Moringa oleifera* showed hepatoprotective abilities by improved values of total bilirubin and GGT enzyme against carbon tetrachloride which induced liver damage in male albino rats.

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تشخيص ودراسة بعض المركبات الكيميائية النباتية والنشاط المضاد لليرقان لمسحوق أوراق وبذور نبات مورنجا اوليفيرا *Moringa oleifera* في ذكور الجرذان البيض

وجدان ابراهيم عباس عبدالوهاب

قسم علوم الحياة ، كلية التربية ، جامعة سامراء ، سامراء ، العراق

الملخص

استهدفت هذه الدراسة الكشف وتشخيص بعض المواد والمركبات الكيميائية النباتية والاحماض الدهنية الفعالة بيولوجياً ومضادات الاكسدة في مسحوق اوراق وبذور نبات مورنجا اوليفيرا *Moringa oleifera* المزروع في العراق والتي تم تحديد العديد منها وباستخدام التقنيات الحديثة مثل تقنية كروماتوكرافيا الغاز GC-MS. ومن ثم دراسة واختبار التأثيرات الوقائية والعلاجية لمسحوق الاوراق والبذور للنبات قيد الدراسة ضد التسمم الكبدي المستحدث بوساطة رابع كلوريد الكاربون CCl_4 وضد اليرقان. وتم اختبار هذه التأثيرات من خلال قياس تراكيز البيليروبين الكلي وانزيم كاما كلوتامايل ترانسفيريز GGT في مصل دم ذكور الجرذان السليمة والمعرضة لـ CCl_4 والمعاملة بمسحوق اوراق وبذور مورنجا اوليفيرا وطيلة فترة التجربة البالغة 30 يوماً. إذ استخدم 20 من ذكور الجرذان البيض قسمت الى 4 مجاميع، أي 5 حيوانات في كل مجموعة. إذ أدى تعريض الحيوانات لـ CCl_4 الى ارتفاع معنوي ($P<0.05$) في تراكيز البيليروبين الكلي وانزيم GGT بالمقارنة مع مجموعة السيطرة السليمة. في حين ان معاملة مجاميع الحيوانات المعرضة لـ CCl_4 بمسحوق اوراق وبذور مورنجا اوليفيرا أدت الى انخفاض معنوي ($P<0.05$) في تراكيز البيليروبين الكلي وانزيم GGT بالمقارنة مع المجموعة المعرضة لـ CCl_4 فقط. وقد استنتج من الدراسة الحالية ان استخدام مسحوق اوراق وبذور المورنجا اوليفيرا قد يكون له تأثيرات وقائية وعلاجية ضد التلف والتسمم الكبدي وبالتالي ضد اليرقان من خلال فعاليتها التغذوية والمضادة للأكسدة لازالة الجذور الحرة واصلاح التلف الخلوي والنسجي.

الكلمات المفتاحية: مورنجا اوليفيرا، CCl_4، GC-MS، البيليروبين الكلي، انزيم GGT.