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Antioxidant and immunological evaluation of *Ceratonia siliqua* on Methotrexate induced Albino mice male

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Abstract

Carob (*Ceratonia siliqua*) plant was frequently used to cure a variety of health issues. This study aimed to evaluate the total flavonoid, phytochemical compounds, antioxidant activity *In vitro*, as well as immunological potential of *Ceratonia siliqua* methanolic extract in vivo through determination of total and absolute count of white blood cell on albino male mice. The result showed that *Ceratonia siliqua* contain (224.5±37.86) of flavonoid compound, in addition of many bioactive phytochemical compounds like (tannin, glycoside, alkaloid, polyphenol, and glycoside). Antioxidant activity indicated that the ability of methanolic extract of *Ceratonia siliqua* plant to scavenging free radical *In vitro* in all concentration uses (0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) when compared with trolox (vit E). Immunological parameters indicated the ability of plant to enhance the immunity by increase WBCs count (7166 cell/cu.mm.blood) in comparison with positive (methotrexate) and negative control, as well as increase in lymphocytes (3700 cell/cu.mm.blood) , neutrophil (2985 cell/cu.mm.blood) and monocyte (481 cell/cu.mm.blood) All of these effect attributed to active phytochemical constituents in plant extract.

Keywords: *Ceratonia siliqua*; Flavonoid; Phytochemical**;** Methotrexate

1. Introduction

History of medicine and plants dates back to remote past when herbal treatment was the only answer to all kind of ailments (1). Nowadays, greater emphasis was again being laid to phytotherapy all over the world (2). Medicinal plants, also called medicinal herbs, have been discovered and used traditional medicine practices since prehistoric times. Plants synthesis hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, and herbivorous mammals (3). The researches and utilization of herbal medicine in the treatment of diseases increases every day. In the past, medicinal plants were believed traditionally to be a therapeutic agent for the treatment of diseases such as typhoid, cholera, measles, etc (4). .

Carob (*Ceratonia siliqua*) was one of Asia and Africa's popular nutritional and medicinal crops, This unique plant has an outstanding functional properties and nutritional profile, carob has high sugar content, drought resistance and was very economical, carob fruit consists of pulp and seed that are rich sources of different bioactive components, carob has wide applications in various industries (food, pharmaceuticals and cosmetics) as an anti-oxidant, thickener, stabilizer, lactic acid production and emulsions, the trend of moving toward natural products further highlights the use of carob in different fields due to its excellent nutritional and therapeutic profile ,carob bean gum was widely used in the food industry.(5)

In view of the present study focuses on the knowledge on medicinal uses of *Ceratonia siliqua* (carob) plant and the scientific investigation to confirm their medicinal value that act as antioxidant, immunomodulator, antibacterial, antifungal, anticancer, antidiabetic, analgesic and can prevent anemia in addition of cough and flu treatment.

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2. Material and methods

2.1. *Ceratonia siliqua*

The aerial part of plant (leaves) from *Ceratonia siliqua* was supplied from the local market of Baghdad during November /2022 and recognized by Dr.Ibrahim S. Al-Jubouri, College of Pharmacy, AL-Mustansiriyah University, Iraq

2.2. Preparation of alcoholic Extract

Methanolic extract of *Ceratonia siliqua* was prepared according to (6), fifty grams of plant aerial part powdered and extracted with 80% methanol (250 ml) at 65 ºC for 3 hours using the Soxhlet apparatus. The extract solution was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried crude extract, which was frozen at -20 C until use to prepare the required doses

2.3. Dose of plant

In albino male mice, a dose of 300 mg/kg was tested depending on LD_{50} of *Ceratonia siliqua* to 50gm/kg.

2.4. *In vitro* analysis

2.4.1. Total flavonoid

The plant extract in weight (3.2 mg) was dissolved in 5 ml of 50% methanol solution 1 ml of a 5% (NaNo3) solution. After 6 min ago(1 ml) of a 10% ($AlCl_3$) solution was additional ,then the mixture was leave for a more 5 minutes before 10 ml of a 10% NaOH solution was added. The mixture had been completed to 50 ml with distilled water (DW) and mixed very well. Finally absorbance was measured at 450 nm with a spectrometer after 15 min. A similar procedure had been applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg) of rutin slandered, a standard curve was drawn . The total flavonoid content was determined sing a curve-fitting equation of the standard curve (7).

2.5. Determination of the active phytochemicals in of *Ceratonia siliqua* plant

According to (8), the qualitative phytochemicals investigations of *Ceratonia siliqua* were carried out as follows as**.**

2.5.1. Detection of Tannins tests

A few drops of the 1% Lead acetate solution have been added to the alcoholic plant extract. A gelatinous or white precipitate have been made that the presence of tannin.

2.5.2. Detection of glycosides

A liquate of 1 ml of the alcoholic plant extract was mixed with 2 ml of the Benedict reagent, then place the mixture in a boiling water bath for 5 minutes and left to cool. The red deposit indicated that a presence of polysaccharides.

2.5.3. Detection of alkaloids (Dragangroff test)

A solution of 60 mg of Bismuth sub-nitrate have been dissolved in 0.2 ml HCl (solution A) and Solution B contains 600 mg potassium iodide in 1 ml D.W The solution A + B were mixed and added to the plant alcoholic extract, an orange to brown color will detected expression the presence of alkaloids.

2.5.4. Detection of the Saponins

These detection process will be happening by shaking the solution of the plant alcoholic extract well. Formation of foam at the top of the extract will be show presence of saponins.

2.5.5. Detection of Flavonoids

The test of Alkaline reagent: was prepared by using Sodium hydroxide solution had been mixed with restricted amount of plant extract solutions and left, a bright yellow color was showed that presence of flavonoids.

2.5.6. Detection of Polyphenolic Compound

A little few drops of 3% ferric chloride (FeCl3) solution have been added to the plant alcoholic extract solution a brown deposition will be showen.

2.6. *In vivo* analysis

2.6.1. Laboratory Animals

Albino male mice (*Mus musculus)* were the laboratory animals.They were supplied by the Biotechnology Research Center (Al-Nahrain University). Their ages at the start of experiments were 8-10 weeks , and their weight was 23-27 grams. They were distributed into groups, and each group was kept in a separate plastic cage (details of these groups are given in the section of experimental design). The animals were maintained at room temperature , and had free excess to food (standard pellets) and water *(ad libitum).*

2.7. Experimental Design

The experimental designs were divided in four groups each group contain six animles as the following:

- Group I: Mice were administrated with D.W (negative controls).
- Group II: Mice were administrated with *Ceratonia siliqua* methanolic extract at dose of (300mg/kg).
- Group III: Mice were administrated with the MTX (1st and 2ed days) + *Ceratonia siliqua* methanolic extract (from 3rd to 7th day).
- Group IV: Mice were administrated with the MTX (7th days) at dose of (200mg/kg).

2.8. Total and absolute count of leucocytes

Blood samples were collected by heart puncture using a disposable insulin syringe (1ml). The method of (9) was followed.

A drop of blood was smeared on a clean slide and air-dried. The smear was stained with Leishman stain for 5 minutes and buffered for 10 minutes, and then washed with tap water. The slide was air-dried, and then examined under oil immersion lens (100X). At least 100 leucocytes were examined, and percentage of each cell type was recorded, while absolute count of each type of leucocytes was obtained by using (9).

3. Results and discussion

3.1. *In vitro* Analyses

3.1.1. Total flavonoid

The total flavonoid of *Ceratonia silique* methanolic extract was (224.5±37.86) table (1).

Table 1 Concentration of total flavonoid of *Ceratonia silique*

Mean ± Std	Total Flavonoid
224.5±37.86	*Ceratonia siliqua*

3.1.2. Phytochemical contents

Ceratonia silique methanolic extract had many chemical composition (phytochemical) as showed in table (2)

Table 2 Investigation of main bioactive phytochemical compounds of *Ceratonia silique*

Test name	Alcoholic extract
Tannins	++ve
Glycoside	-ve
Alkaloids	+ve
Saponins	-ve
Flavonoids	+ve

Polyphenols	+ve

3.1.3. Reductive Ability (FRAP assay)

In all tested concentrations (0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), the absorbance of *Ceratonia siliqua* methanolic extract was significantly higher than trolox (vitamin E), and such findings suggest that the plant extract was more effective than trolox in the reductive ability, which was concentration-dependent, as showed in table (3).

Table 3 Reductive Ability (FRAP assay) of *Ceratonia siliqua* methanolic extract

	Reductive Ability Absorbance (Mean ± SD)	
Con. (mg/ml)	***Ceratonia siliqua***	**Trolox (Vitamin E)**
0.64	0.375±0.11	0.211 ± 0.015
0.32	0.281±0.010	0.132 ± 0.007
0.16	0.252±0.009	0.114 ± 0.004
0.08	0.248±0.008	0.108 ± 0.001
0.04	0.245±0.008	0.101 ± 0.001

3.2. Evaluation of methanolic extract of *Ceratonia siliqua* on immunological parameters

3.2.1. Total white blood cell (WBC) counts

The typical WBC count in mice the range is 2000 to 10,000 per microliter. This value had been shown in the control negative (group no.1), in which the total count was 4500 cell/cu.mm.blood ,as showed in table (4)An increase in the WBC count was observed in mice administered *Ceratonia siliqua* (group no.2), the measurement of WBC was 7166 cell/cu.mm.blood .When interact *Ceratonia siliqua* with MTX (group no.3), the count increased to 5971 cell/cu.mm.blood, but MTX decrease the WBC count, which this appearance at mice group no.4), in which the total count was 3000 cell/cu.mm.blood , as showed in table (4).

Table 4 The mean of total WBC count in albino male mice

No	**Groups**	**Dose**	**Mean ± SD (cells/cu.mm.blood)**
I	Control negative	**---------**	6001±0.152
II	*Ceratonia siliqua*	300mg/kg	7166±0.264
III	*Ceratonia siliqua* + Methotrexate	300+200mg/kg	5971±0.300
IV	Methotrexate	200 mg/kg	3000±0.404

3.3. Differential count of WBCs

3.3.1. Total lymphocyte count.

- In the control negative group (group no.1), the total lymphocyte count was 3161 cell/cu.mm.blood ,as showed in table (5).
- In mice (group no.2) treated with *Ceratonia siliqua* lymphocytes increased to 3700 cell/cu.mm.blood ,when it compared with control negative.
- In mice treated with *Ceratonia siliqua* and MTX (group no.3), the count of lymphocyte also increased to 3151 cell/cu.mm.blood, but when treated with MTX (group no.4), the lymphocyte count decreased to 1800 cell/cu.mm.blood.

3.3.2. Total neutrophils count

Neutrophils are a kind of white platelet (WBC or granulocyte) that shield us from diseases. In mice, Neutrophils are the first of all cells reach on the scene when we experience microorganisms.

- In the control negative (group no.1), the total neutrophils was 1430 cell/cu.mm.blood, as showed in table (5).
- In mice treated with *Ceratonia siliqua* (group no.2), neutrophils count increased to 2985 cell/cu.mm.blood, when it compared with control negative.
- In mice treaded with *Ceratonia siliqua* and MTX (group no.3), the result showed also increased in neutrophils count (2400), but when treated with MTX (group no.4), the neutrophils decreased to 1080 cell/cu.mm.blood .

3.3.3. Total monocytes count

Monocytes are a type of WBC. They help fight bacteria, viruses, and other infections in the body. Along with other types of WBC, monocytes are a key element of your immune response. Monocytes in mice equal or less than 3%.

- In the control negative (group no.1), the total count of monocytes was 170cell/cu.mm.blood, as showed in table (5).
- In mice treated with *Ceratonia siliqua* (group no.2), monocytes count increased to 481 cell/cu.mm.blood.
- In mice treaded with *Ceratonia siliqua* and MTX (group no.3), monocytes count also increased to 420 cell/cu.mm.blood. But when treated with MTX (group no.4), the count of monocytes was decreased to 120 cell/cu.mm.blood.

Table 5 The mean of differential Lymphocyte, Neutrophil and Monocyte in albino male mice

NO.	Groups	Dose	Mean ± SD (cells/cu.mm.blood)		
			Lympho	Neutro	Mono
I	Control negative	---------	3161	2415	425
II	*Ceratonia siliqua*	300 mg/kg	3700	2985	481
III	*Ceratonia siliqua* + Methotrexate	300+200mg/kg	3151	2400	420
IV	Methotrexate	200 mg/kg	1800	1080	120

Plants are of great interest in drug discovery and are the main source of our modern medicine. About 25% of modern medicines were derived from a plant origin and only 5–15% of plants are being investigated for their medicinal use (10) Today, medicinal herbs and functional foods were being widely studied resulting inlucrative therapeutic potentials (11) Where the secondary metabolites, which possessed antioxidant properties can fight several pathological disorders such as cancer, heart disease, hypertension, dementia, and stroke (12). .

As it is known, oxidative stress occurs when there is an imbalance as it is known, oxidative stress occurs when there is an imbalance between antioxidation and oxidation, there was a mechanism in metabolism consisting of endogenous and exogenous antioxidants to counteract the oxidative stress that occurs in normal physiological conditions (13). However, oxidative stress that occurs when the antioxidant system of metabolism is not sufficient to respond to different xenobiotics can cause various chronic and degenerative disorders such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases ,carob contains a wide range of biologically active compounds, including polyphenols, sugars, cyclitols, amino acids, fibers, and minerals, whereas carob seeds contain gum, polyphenols, and proteins (14). Due to its chemical composition, carob and its ethanolic extract have a strong antioxidant activity and possess several valuable therapeutic functions, such as lipid-lowering , anti-proliferative, anti-cardiovascular, and nephroprotective properties (15) . In addition, carob has exhibited significant pharmacological activities in the digestive tract including antidiarrheal, antibacterial, anti-ulcer, and anti-inflammatory actions, and possesses a laxative effect on gastrointestinal propulsion (16). Despite carob's importance in functional food development, most Arab countries use carob to make a popular drink consumed mainly in the month of Ramadan. Carob is also used in the preparation of special traditional types of Arabic confectionery. Due to the nutritional value of carob powder water extract which is known to contain dietary fiber, tannins, flavonoids, and gallic acid, which account for its antioxidant properties, Phenolic compounds as depicted in carob pods are good electron donors and could terminate the radical chain reaction by converting free radicals into more stable products (17), The antioxidant activity of phenolics depends on the number and substitution of the hydroxyl group. As such, carob's antioxidant activity can thus

be attributed to the presence of gallic acid, protocatechuic, catechin, p-hydroxybenzoic, and vanillic acid, according to the study of ,the methanolic extracts from a variety of Moroccan carob barks showed good antioxidant power *In vitro*. It was previously reported C. siliqua leaves from Morocco exhibited high antioxidant activity *In vitro*, the DPPH discoloration degree in a solution reveals the ability of extracts to release H+ protons and it is likely due to the presence of products having the capacity to interact with free radicals as electron donors and, therefore, inhibiting the ROS such the hydroxyl radical, superoxide anion (18).

Compound identification using High Performance Liquid Chromatography (HPLC) showed that carob contain flavonoids of quercetin glycosides, catechin and epicatechin gallate. Flavonoids are considered as plant secondary metabolites that numerous pharmacological functions are attributed to them including antioxidant, anti-mutagenic, antibacterial, anti-angiogenic, anti-inflammatory, anti-allergic, enzyme modulation, and anti-cancer (19) .they are defined as phytochemicals which exist either as free aglycones or glycosidic conjugates. Flavonoids are polyphenolic with a wide range of structures Based on this diversity, they are categorized mainly into flavones, flavanols, isoflavones, flavonols, flavanones, flavanonols, and chalcones. The diverse structures of flavonoids have resulted in many properties including anti-cancer and anti-inflammatory effects (20). Recently, it has been shown that flavonoids can affect immune system response and might have immune-modulator effects.

4. Conclusion

Ceratonia species have long been used as traditional herbal remedies for many diseases related to antioxidant , immunity and respiratory tract, or even in; digestive tract, malignancy, etc.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

References

[1] Welz, A. N., Emberger-Klein, A., and Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. Biomedcentral complementary and alternative medicine. 18(1): 92.

[2] Raman, R. P. (2017). Applicability, feasibility and efficacy of phytotherapy in aquatic animal health management. American Journal of Plant Sciences.8(02): 257

[3] Gershenzon J., and Ullah C (January 2022). "Plants protect themselves from herbivores by optimizing the distribution of chemical defenses". Proc Natl Acad Sci USA.119 (4).

[4] Mintah, S. O., Asafo-Agyei, T., Archer, M. A., Junior, P. A. A., Boamah, D.,Kumadoh, D., and Agyare C. (2019). Medicinal plants for treatment of prevalent diseases. In Pharmacognosy-Medicinal Plants. Intech Open.

[5] Zunaira Basharat, Muhammad Afzaal, Farhan Saeed, Fakhar Islam, Muzzamal Hussain, Ali Ikram, Muhammad Usama Pervaiz and Chinaza Godswill Awuchi. (2023). Nutritional and functional profile of carob bean (*Ceratonia siliqua*): a comprehensive review. International Journal of Food Properties. 26:1, 389-413.

[6] Ibraheem, R. M., Mhawesh, A. A., and Abood, K. W. (2018). Estimation of the whole flavonoid, antioxidant, antibacterial challenge concerning Viola odorata (banafsha) methanolic extract. Iraqi Journal of Agricultural Sciences. 49(4) 655–662.

[7] Sakanaka, S., Tachibana, Y., and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chemistry.89(4)569–575.

[8] Sticher, O. (2008). Natural product isolation. Natural Product Reports, 25(3).

[9] Sood, R. (1986). Hematology for Students and Practitioners. Jaypee Brothers, New Delhi, India

[10] Khan F., Sarker M.M.R., Ming L.C., Mohamed I.N., Zhao C., Sheikh B.Y., Tsong H.F., and Rashid M.A.(2019).Comprehensive review on phytochemicals,pharmacological and clinical potentials of gymnema sylvestre. Front. Pharmacol.10:1–19.

[11] Atanasov A.G., Zotchev S.B., Dirsch V.M., Orhan I.E., Banach M., Rollinger J.M., Barreca D., Weckwerth W., and Bauer R., Bayer E.A.,.(2021). Natural products in drug discovery: Advances and opportunities. Nat. Rev. Drug Discov. 20:200–216.

[12] Fanzo J., Hunter D., Borelli T., and Mattei F.(2013). Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health. Routledge;London, UK

[13] Pizzino G., Bitto A. (2014).Interdonato M., et al. Oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to heavy metals living in the Milazzo-Valle del Mela area (Sicily, Italy) Redox Biology.2:686–693.

[14] Zhu B.-J., Zayed M.Z., Zhu H.-X., Zhao J., and Li S.-P.(2019). Functional polysaccharides of carob fruit: Areview. Chin. Med.14:1–10.3.

[15] Rtibi K., Selmi S., Jabri M.-A., El-Benna J., Amri M., Marzouki L., and Sebai H. (2016).Protective effect of *Ceratonia siliqua* L. against a dextran sulfate sodiuminduced alterations in liver and kidney in rat. J. Med. Food. 19:882–889.

[16] Rtibi K., Selmi S., Grami D., Amri M., Eto B., El-Benna J., Sebai H., and Marzouki L. (2017).Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua* L.) on the gastrointestinal tract: A review. Biomed.Pharmacother.93:522–528.

[17] Ayache S.B., Saafi E.B., Emhemmed F., Flamini G., Achour L., and Muller C.D.(2020).Biological activities of aqueous extracts from Carob. Molecules. 25:1–16.

[18] Tagne, R.S., Telefo, P., Nyemb, J.N., Yemele, D.M., Njina, S.N., Goka, S.M.C.,Lienou, L.L., Kamdje, A.H.N., Moundipa, P.F., and Farooq, A.D.(2014). Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pac. J. Trop. Med. 7, S442–S447.

[19] Cardenas C, Quesada AR., and Medina MA.(2011). Anti-angiogenic and anti-inflammatory properties of kahweol, a coffee diterpene. PLoS ONE 6:e23407.

[20] Ravishankar D., Rajora AK., Greco F., and Osborn HM.(2013). Flavonoids as prospective compounds for anti-cancer therapy. Int J Biochem Cell Biol.45:2821–31.