

The Effect of Aqueous Extract of *Citrullus colocynthis* Seeds on Cellular Immunity

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Abstract:

The aqueous extract of *Citrullus colocynthis* dried seeds (160 µg/ml) was *in vitro* evaluated for its effect on phagocytic index (PI) and lymphocyte transformation index (LTI) of blood cells obtained from 30 apparently healthy blood donors (15 males and 15 females). The PI was further *in vivo* evaluated in cells of peritone, spleen and liver of mice treated with the extract at a dose of 0.64 mg/kg. The results revealed that in *in vitro* study, phagocytic cells treated with the extract showed a significant increased percentage as compared with untreated cells (60.0 vs. 44.1%). Phagocytes obtained from peritone (44.1 vs. 30.0%) and spleen (45.6 vs. 39.6 %) of treated and untreated mice behaved in a similar manner, while liver phagocytes showed no significant difference between PI of immunological function of the investigated cells, and may use as therapeutic agent. treated and untreated mice. For LTI, cultures I and II shared an approximated mean (70.0 and 68.0%, respectively), but both indices were significantly higher than the recorded LTI in culture III (54.0%). These findings suggest that the plant extract is effective in enhancing the

Keywords: *Citrullus colocynthis*, Bitter apple, Aqueous extract of seeds, Lymphocyte transformation.

Introduction:

Citrullus colocynthis (Cucurbitaceae), commonly known as 'bitter apple', 'colosynth', 'vine-of-Sodom' and 'tumba' is a tropical plant that grows abundantly in the Arabian countries and widely in other parts of the world [1]. In traditional medicine, this plant has been used to treat constipation [2], diabetes [3], odema, fever, jaundice, bacterial infections and cancers, and it is also used as an abortifacient [1]. Further studies have implanted that *C. colocynthis* is rich in compounds that have antioxidant and free radical scavenging potentials [4,5]. Preliminary phytochemical screening of the plant showed the presence of large amounts of phenolic and flavonoids [6]. Flavonoids and phenolic compounds are widely

distributed in plants, and have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, and others [7]. Accordingly, the present study was planned with aims to evaluate the cellular immunity potentials of *C. colocynthis* seed aqueous extract *in vitro* and *in vivo*.

Materials and Methods:

Plant Seed Collection and Extraction: Dried seeds of *C. colocynthis* were collected from a local store of herbal medicine, and they were certified by the Herbarium of the College of Science (University of Baghdad). The seeds were powdered using coffee grinder, and 100 grams of the powder

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was subjected to successive extraction (four hours) in a Soxhlet apparatus, using 500 ml of distilled water as solvent at 50°C. The extract was then evaporated on a rotary evaporator [8]. For *in vitro* evaluation, a concentration of 160 µg/ml was used, while for *in vivo* evaluation, a dose of 0.64 mg/kg was employed.

In vitro Evaluation: Such evaluation included phagocytic index (PI) and lymphocyte transformation index (LTI) of blood cells obtained from 30 apparently healthy blood donors (15 males and 15 females) with an age range of 34.4 ±2.8 year. From each subject, 5 ml of venous blood were collected in heparinized tube under aseptic conditions. For PI, 0.5 ml blood was mixed with equal volumes of *C. colocythis* seed extract (160 µg/ml) and suspension of *Staphylococcus aureus* at a concentration of 1 x 10⁶ cell/ml (the bacterial isolate was obtained from the Department of Biology, College of Science) and incubated for 30 minutes at 37°C. After incubation, a thin blood film was made and stained with Leishman stain. The slide was examined under oil immersion lens and the percentage of phagocytic cells (PI) was recorded. For each subject, a control evaluation was also made and included similar conditions but without the seed extract [9, 10].

In vivo Evaluation: In such evaluation, the PI was recorded for phagocytes obtained from the peritone, spleen and liver of albino male mice (8-9 weeks old), which were distributed into two groups. The first group (5 mice) was administrated orally with 0.25 ml of the extract (25 mg/kg) daily for two weeks, while the second group (5 mice) was treated with distilled water in a similar manner. In both cases and at the end of treatment period, each mouse was injected intraperitoneally with 0.1 ml of formalin-killed

Staphylococcus aureus at a concentration of 1 × 10⁶ cell/ml, and after 24 hours, the animals were sacrificed, dissected and the phagocytes were obtained from the peritone, spleen and liver. These cells were processed as in *in vitro* evaluation to record the PI [9, 10]. All studies were conducted accordance with National Institute of Health's Guide for the Care and Use of Laboratory Animals [11]

Lymphocyte transformation test: In the assessment of LTI, three cultures were set-up for each subject. Culture I included mixing 2.5 ml RPMI-1640 medium, 0.5 ml extract (160 µg/ml), 0.3 ml PHA and 0.2 ml blood. In culture II, similar components were mixed but without the extract (Control), while in culture III, the PHA was replaced by the extract. The three cultures were incubated at 37°C for 72 hours, and after that, they were processed with a hypotonic solution (0.075M KCl) and the obtained cells were fixed (3 parts of absolute methanol with 1 part of glacial acetic acid), and then, 4-5 drops of the cell suspension were dropped on a clean slide from a height of about two feet. The slide was air-dried at room temperature, and by then it was stained with Giemsa stain for 15 minutes and rinsed with distilled water. The slide was examined under oil emersion lens (100X), and at least 500 cells were examined, and percentage of blast and dividing cells (LTI) was recorded [12].

Statistical Analysis: Data are expressed as mean ± standard error (S.E.), t-test was used to determine the differences between the groups by using the computer program SPSS version 13.0. Statistical significance was considered at $P \leq 0.05$.

Results and Discussion:

Phagocytic index (PI): In *in vitro* study, phagocytic cells were treated

with the extract showed a significant increased percentage as compared with untreated cells (60.0 vs. 44.1%) (Table 1). Phagocytes obtained from peritone (44.1 vs. 30.0%) and spleen (45.6 vs. 39.6 %) of treated and untreated mice behaved in a similar manner, while liver phagocytes showed no significant difference between PI of treated and untreated mice (Table 2).

Lymphocyte Transformation Index (LTI): Cultures I and II shared an approximated mean of LTI (70.0 and 68.0%, respectively), but both indices were significantly higher than the recorded LTI in culture III (54.0%) (Table 1).

Table 1: Phagocytic index and lymphocyte transformation index of blood cells treated with 160 µg/ml of *Citrullus colocynthis* aqueous seed extract.

Parameter	Mean ± S.E. (%)			ANOVA Probability ≤ 0.05
	Treated	Untreated		
Phagocytic Index	60.0 ± 4.0	44.1 ± 2.5		0.05
Lymphocyte Transformation Index	Culture I	Culture II	Culture III	I vs. III P ≤ 0.05 II vs. III p ≤ 0.05
	70.0 ± 5.6	68.0 ± 3.2	54.0 ± 3.0	

Table 2: Phagocytic index of phagocytes obtained from peritone, spleen and liver of mice treated with 25 mg/kg of *Citrullus colocynthis* aqueous seed extract.

Phagocyte Sources	Phagocytic Index (Mean ± S.E.; %)		ANOVA Probability ≤ 0.05
	Treated	Untreated	
Peritone	44.1 ± 2.1	30.0 ± 1.7	0.05
Spleen	45.6 ± 1.3	39.6 ± 0.7	0.05
Liver	35.3 ± 3.2	33.2 ± 2.4	Not Significant

the present results demonstrated the plant extract was significantly effective in enhancing phagocytosis in both *in vitro* and *in vivo*, and accordingly it is possible to suggest that the extract may contain chemical compounds that enhanced the non-specific cellular immunity of phagocytes reviewing the literature revealed that *C. colocynthis* is rich in three flavonoids glycosides (isosaponarin, isoviterxin and isoorientin 3'-O-methyl ether), protein (rich in isoleusine, leusine and tryptophan amino acids), lipids, saponins, flavonoids (such as apigenin and quercetin and leuteolin), and a considerable amount of ions (potassium, phosphorus and iron) such constituents have been demonstrated to have immunomodulatory effects [6, 13, 14, 15, 16, 17]. Another active material that founded in the *C. colocynthis* seed extract that have

antimicrobial properties is Alkaloids, tannins, steroids, pigments and iridoids [18]. In agreement with such augmentation [6, 13, 14, 15, 16, 17] were able to demonstrate that these constituents have a wide range of biological effects, *C. colocynthis* is an Iranian medicinal plant that has traditionally been used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes [6], the plant extract had decrease the formation of granuloma tissue in chronic inflammation model in male albino rats this investigation support that *C. colocynthis* is used as anti-inflammatory agent [15]. including treatment of diabetic people in Mediterranean countries, and used for treatment many inflammatory disease, constipation, odema, fever, jaundice, leukemia, bacterial infections, cancer, used as abortifacient

and it had antibacterial and antifungal properties, which have confirmed by others [1, 2, 3, 18, 19]. Furthermore, the Folkloric application of the plant (for instance anti-oxidant, antibacterial, antifungal and anti-leukemic activity) can also be interpreted on such ground, especially it use consider the anti-oxidant potential of the plant or these active ingredient [4, 5, 6, 7, 20, 21, 21, 23]. With respect to LTI, the extract was less effective than PHA in cross-linking the lymphocyte receptors, and therefore the LTI value came lower in culture III as compared with culture II. However a value of 54 % for LTI in culture III cannot be ignored and some potential effects in enhancing the cellular response of T lymphocyte can be suggested and it might also be related to the chemical constituents of the plant.

From the findings of both indices, it is possible to suggest that the plant may be characterized with immunomodulation potentials; but it is too early to reach a final conclusion, and further investigations are required to cover such subject.

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تأثير المستخلص المائي لبذور نبات الحنظل *Citrullus colocynthis* على المناعة الخلوية

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الخلاصة:

تم تقييم تأثير المستخلص المائي لبذور نبات الحنظل *Citrullus colocynthis* الجافة بتركيز 160 ميكروغرام / مليلتر خارج الجسم الحي من خلال معامل البلعمة (PI) ومعامل التحول للمفاوي (LTI) لـ 30 عينة دم لمتبرعين اصحاء ظاهرياً (15 ذكر و15 أنثى). وكذلك تم تقييم تأثير المستخلص داخل الجسم الحي من خلال معامل البلعمة لخلايا الغشاء البريتوني ، الطحال والكبد لفئران معاملة بتركيز مستخلص 25 ملغم / كغم. وأظهرت النتائج التي أجريت خارج الجسم الحي بوجود زيادة معنوية بين الخلايا البلعمية المعاملة بالمستخلص بالمقارنة مع الخلايا غير المعاملة (60.0 vs. 44.1%). كما كانت نسبة معامل البلعمة للخلايا البلعمية التي تم الحصول عليها من الغشاء البريتوني (44.1 vs. 30.0%) والطحال (45.6 vs. 39.6 %) للفئران المعاملة بالمستخلص وغير المعاملة متماثلة، في حين لم تظهر فروق معنوية لمعامل البلعمة للخلايا البلعمية التي تم الحصول عليها من الكبد من الفئران المعاملة وغير المعاملة بالمستخلص ، أما نتائج التحول و الأقسام للمفاوي فكان متوسطي الزرع الأول والثاني متقاربين (70.0 and 68.0%) والذان أظهرتا زيادة معنوية بالمقارنة مع الزرع الثالث. وتشير هذه النتائج إلى أن للمستخلص النباتي تأثير في تحفيز الجهاز المناعي وإمكانية استخدامه كعامل علاجي.