Iraqi Journal of Science, 2022, Vol. 63, No. 9, pp: 3703-3710
DOI: 10.24996/ijs.2022.63.9.4

ISSN: 0067-2904

Cytogenetic and Cytotoxic Potentials of *Borago officinalis* on Albino Male Mice

Ruqaya Mohammed Al-ezzy[1]*, Rafal Shakeeb alanee [2], Hadeel Mohamed khalaf[1]
[1]*Molecular and Medical Biotechnology Department, College of Biotechnology , Al- Nahrain University Baghdad, Iraq*
[2] *College of Pharmacy , Al- Nahrain University, Baghdad, Iraq*

Received: 23/11/2021 Accepted: 17/2/2022 Published: 30/9/2022

Abstract

The current study is an attempt to assess the cytogenotoxic potential of the ethanolic extract from the leaves of *Borago officinalis* on Swiss albino male mice. Young Swiss albino mice were orally administered with leaf ethanolic extract of *Borago officinalis*. Three mice groups were used using different doses of plant extract (T1: 100, T2: 200, and T3:400 mg\kg) in addition to the control negative group (untreated mice) for 7 days to assess mitotic index or 28 days to assess meiotic index. The results revealed that the extract significantly induced the division of disruptive chromosomal changes in the bone marrow and the mean of abnormality was (3.5±0.47, 4.43±0.83, and 5.83±0.96%) for T1, T2, and T3 respectively, as well as in primary spermatocytes. It is planned that the extract might have interfered with the spindle protein or protein packing.

Keywords: - *Borago officinalis*, Cytotoxic, chromosome, bone marrow, spindle protien.

التاثيرات السمية والوراثية الخلوية للورد الماوي على ذكور الفئران البيض

رقيه محمد العزي*[1]، رفل شكيب العاني[2]، هديل محمد خلف[1]
[1] قسم التقنيات الحيوية الجزيئية والطبية، كلية التقنيات الاحيائية، جامعه النهرين، بغداد، العراق
[2]كلية الصيدلة، جامعه النهرين، بغداد، العراق

الخلاصه

نبات الورد الماوي هو واحد من النباتات الطبية. هدفت الدراسة الحالية الى تقييم التاثيرات السمية الخلوية و الوراثية للمستخلص الايثانولي لنبات الورد الماوي على ذكور الفئران البيض باستخدام ثلاث جرع للنبات (مجموعه الاولى:100ملغم\كغم , مجموعه ثانية 200 ملغم\كغم, ومجموعه ثالثة 400 ملغم\كغم) بالاضافه الى المجموعه السالبة (مجموعه السيطرة الغير معامله) حيث تمت المعامله عن طريق التجريع بالفم لمدة اسبوع لقياس الانقسام الخلوي داخل نخاع العظم او 28 يوم للانقسام الحيونات المنوية.اظهرت النتائج أن النبات ادى الى تغيرات على الكروموسومات داخل نخاع العظام بمعدل تشوهات (3.5±0.47 , 4.43 ±0.83 و 5.83±0.96 %) للمجاميع الاولى والثانية والثالثة على التوالي وكذلك في الحيوانات المنوية الأولية, ويعود السبب في ذلك الى ان المستخلص النباتي ممكن ان يتداخل مع البروتين المغزل أو البروتين التعبئة.

*Email: ruqaia.alezzy83@yahoo.com

1. Introduction

One such medicinal plant is ***Borago officinails***. **Starflower or *Borago officinalis*** is a member of the primrose family (Boraginaceae) [1]. The genus name is from the Latin meaning "one-third of a foot" which corresponds to the plant's average height [2]. The species name refers to being from the north, Borage is a medicinal plant which has in pharmaceutical, industrial and forage fields and is used in the production of drinks and salads. Starflower is one of the most studied plants and is widely used in all ages in medicine [3]. The species is presently cultivated worldwide because of its ability to adapt to different climatic conditions. Biological and pharmacological activities attributed to type of solvent used, extracts and products like oil differ in its composition according to parts of the plant that's led to diverse types[4]. At first, malaria was treated by starflower, and then after used to treat different diseases. Also, a decoction of the leaves and stem-bark is used in the treatment of fever by water therapy or inhalation [5]. Therefore, starflower is used in integrated pest management and its bioactivity can control more than 300 insects [6]. The products are also recommended for the treatment of large number of diseases caused by infection. Though apparently believed non-toxic, a clean chit offered to these plant products require strict scientific test, because these natural products may contain a few harmful ingredients as secondary metabolites [7], toxic effects likely to be produced at one or the other level [8]. The present work is an attempt to assess the cytogenotoxic potential of the extract from the leaves of *Borago officinalis* on Swiss albino mice (*Mus musculus*).

2. Methodology

2.1. Plant collection and extraction

Mature leaves from ***Borago officinalis*** were collected from the field which were previously identified by the National Herbarium of Iraq then the plant was washed with running tap water. Air dried (in shade) Leaves were kept in an oven for about 36 hours at 60°C, coarsely powdered in glass mortar with the aid of a pestle. The powdered leaves were then put in Soxhlat with 80% ethanol (v/v) at 60°C using a Soxhlat apparatus (Borocil, Bombay, India) for about 72 hours for full extraction. Free ethanol extract is made by using a rotatory evaporator and finally dried on water bath (at 60°C) to convert it to a powder [9]. Experiments were performed in healthy laboratory Swiss albino mice (6-8 weeks old) of body weight 22-28gram supplied from Biotechnology Research Center affiliated to Al-Nahrain University. Food and water were available to mice all the time (*ad libitium).*

2.2. Experimental Design

Two groups were used in this study as indicated below, each group contained eight mice (total number of mice 32)

1. Control group (untreated group): The mice of untreated groups were given 0.1ml distilled water per day using a curved needle.
2. Treated group: Each time freshly prepared ethanol soluble extract was administered for 7 or 28 consecutive days to the animals (Treated group) at the rate of 100 mg\kg ($_{T1}$), 2oomg\kg ($_{T2}$), and 400mg\kg ($_{T3}$) according to the required protocol. Each mouse was administrated with 0.1 ml as a single dose\day and sacrificed on day 8 or 29 for (mitotic test or meiotic test) respectively.

2.3. Experimental protocol:

- **Mitotic chromosome test:** Animals were sacrificed after 7 days of treatment. The experimental animals were injected with colchicine intra-peritoneally (0.5 mg in 1 ml), 24 hours after the last treatment, and then were sacrificed after 90 minutes by cervical dislocation. Bone marrow from both femora was flushed out in warm hypotonic Kcl solution (0.075M) for 45 minutes and slides were prepared by the standard aceto-alcohol - flame drying -Giemsa staining technique [10]. About 600 well-spread metaphase plates at 100

plates/animal were screened from 6 animals in each group. If any abnormalities were detected, they were put into two categories namely structural damage (Chromatid break, gap, acentric fragment, and minute fragment) and division disruptions (aneuploidy, polyploidy centric fusion, C-mitosis, precocious separation, clumping, and stickiness).

- **Meiotic chromosome test:** Animals were sacrificed after 28 days of treatment for harvesting the treated cells as primary spermatocytes. Animals have injected colchicines intraperitoneally (0.5 mg in 1ml) for 90 minutes prior to sacrifice. Slides were prepared from the testicular cell suspension by the standard aceto-alcohol-flame drying - Giemsa staining schedule [11].

In both experiments, the slide was examined under an oil emersion lens (100X) of a light microscope. A total of 500 metaphase- I plates for each experimental group of animals about 100 metaphase- I were screened per animal. The detected chromosomal abnormalities were classified to, namely

Structural damage: Chromatid breaks, gap, fragments and heterozygous reciprocal translocation (HRT)

Division disruptive:

(a)***Numerical variation:-*** Polyploidy, Hypoploidy, C-mitosis, and precocious separation

(b)***Gross changes:-*** Stickiness, clumping and Heteropycnosis

Synaptical changes:- Univalent, Quadrivalent, and X-Y separation.

2.4. Statistical Analysis

The values of the investigated parameters were assessed by analysis of variance (ANOVA) followed by the least significant difference (LSD) or Duncan test, using the computer programme SPSS version 13.0.

3. Results and discussion

3.1. Effect of *Borago officinalis* extract on mitotic chromosome division:

Amidst 600 metaphase plates screened, 3.5%, 4.43%, and 5.83% abnormal metaphase cells were found in leaf extract in mice treated with T_1, T_2, and T_3 in comparison to untreated control in which the percentage was 1.43%. Total chromosome abnormalities were 1.66%, 2.16%, and 3% in Treated groups (T_1, T_2, and T_3) in comparison to control 1.33%. These were dose-dependent effects Figure 1. There were no significant changes in the individual type of abnormalities except T3, these included chromatid breaks, chromatid gap, acentric fragment, minute fragments (due to the deletion of a telomeric or interstitial part while ring chromosome (due to double breaks) were the most common. However, a significant increase was found in the gross type, where polyploidy, hypoploidy C-mitosis, precocious separation, stickiness, and clumping were the most frequent. In gross type, only T3 and T4 possessed significant changes as shown in Figures 2,3, Table 1.

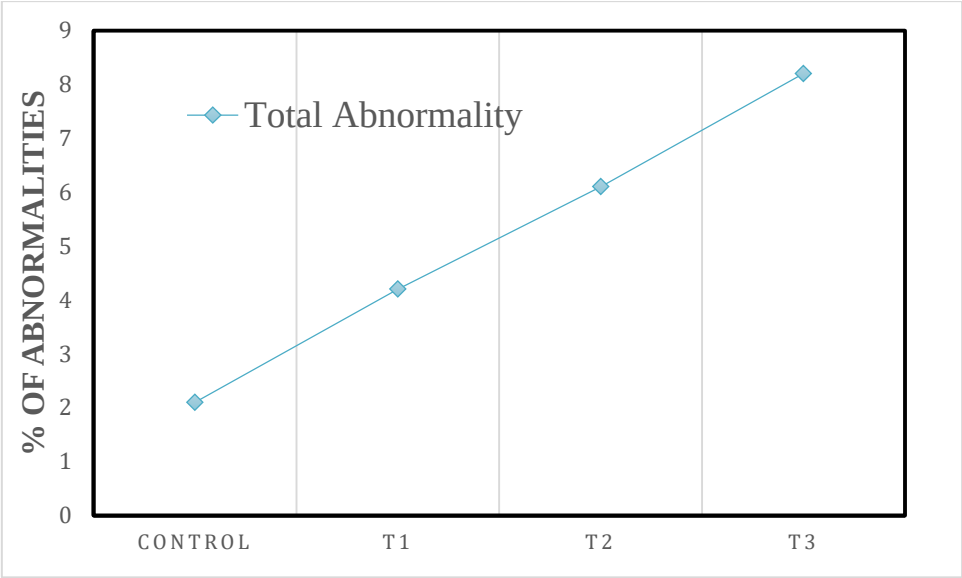

Figure 1- Graph showing the dose-depending effect of leaf extract on albino male mice

Table 1- Frequency of Chromosomal abnormalities (mean % ± S.E) in bone marrow cells of mice fed with ***Borago officinalis*** leaf extract after 7 days of exposure, 600 spots were analyzed.

Experimental group	Abnormal Metaphase Cell			Chromosomal abnormalities					
				Individual type			Gross type		
	Mean %	±	S.E	Mean %	±	S.E	Mean %	±	S.E
C.G	1.43	±	0.47	1.33	±	0.47	1.00	±	0.40
T_1	3.5	±	0.63	1.66	±	0.52	2.5	±	0.63
T_2	4.43	±	0.83*	2.16	±	0.59	3.83	±	0.78*
T_3	5.83	±	0.96*	3.00	±	0.79*	5.00	±	0.88*

* Indicate a significant difference in the corresponding value between the treatment and control group (SPSS test was used)

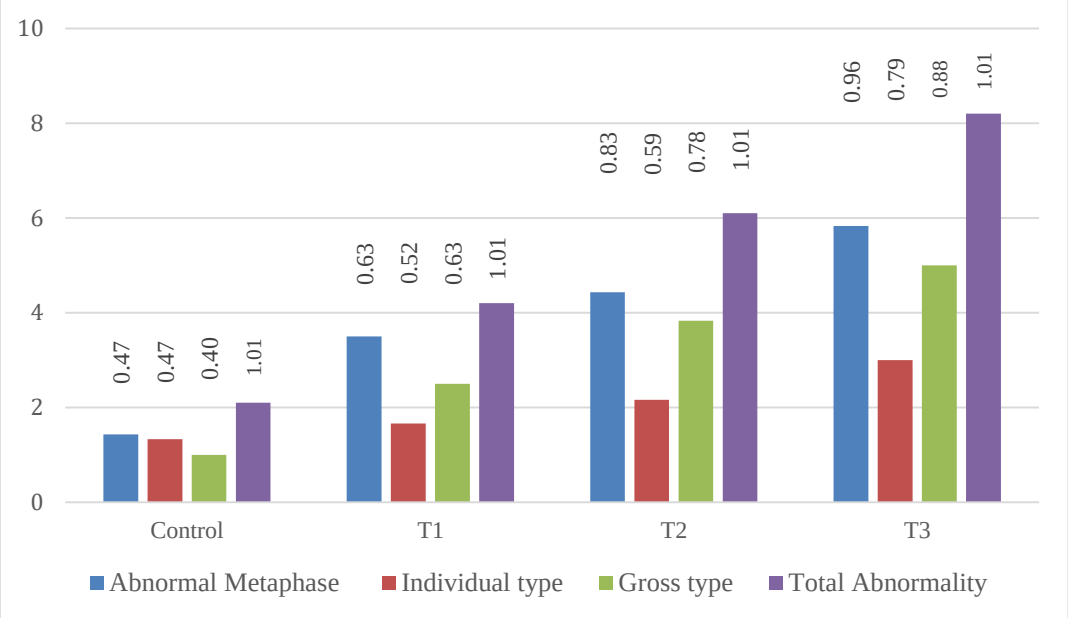

Figure 2- represented abnormal metaphase, individual type, Gross type, and total abnormality in different mice groups

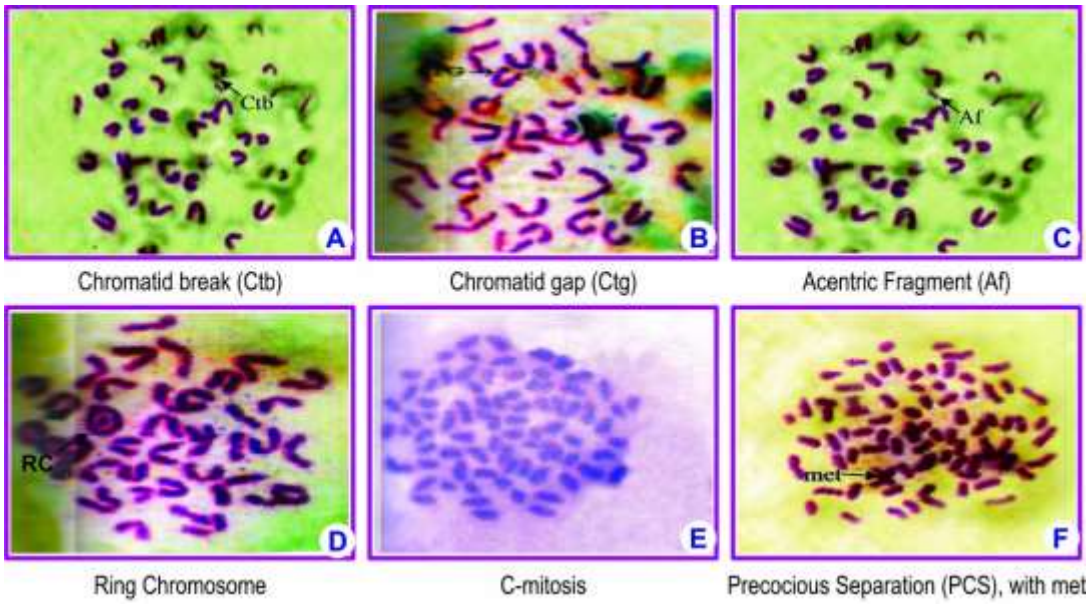

Figure 3- explained mitotic chromosomal abnormalities (chromatid breaks A, chromatid gap B, acentric fragment C, ring chromosome D, C-mitosis E, and precocious separation F) (Giemsa stain, 100X)

3.2. Effect on meiotic chromosome :

Amidst 500 metaphase- I plates screened. The frequency of Total abnormalities in treated groups T_1 (2.2%), T_2 (3.8%), and T_3 (6%) were significantly higher than the control (1.2%). However, structural abnormalities did not show significant changes while in univalent type only the T3 group and gross type exhibited significant activity Figures 4, 5, Table 2.

Table 2- Frequency of Chromosomal abnormalities (% ± S.E) in Primary spermatocytes of mice given ***Borago officinalis*** leaf extract. After 28 days of exposure, 500 metaphase cells were screened.

Experimental group	Abnormal Metaphase-I Cell			Structural changes			Univalent type (Synaptic disturbance)			Gross type (Meiosis disturbance or division disruptive)		
	Mean %	±	S.E	Mean %	±	S.E	Mean %	±	S.E	Mean %	±	S.E
C.G	1.2	±	6	1.00	±	0.44	0.3	±	0.28	0.3	±	0.18
T_1	2.2	±	0.63	1.2	±	0.48	1.2	±	0.52	1.9	±	0.49*
T_2	3.8	±	0.82*	1.6	±	0.56	4	±	0.62	2.5	±	0.78*
T_3	6.00	±	1.08*	2.2	±	0.65	3.8	±	0.73*	2.4	±	0.91*

* Indicate a significant difference in the corresponding value between the treatment and control group (SPSS test was used)

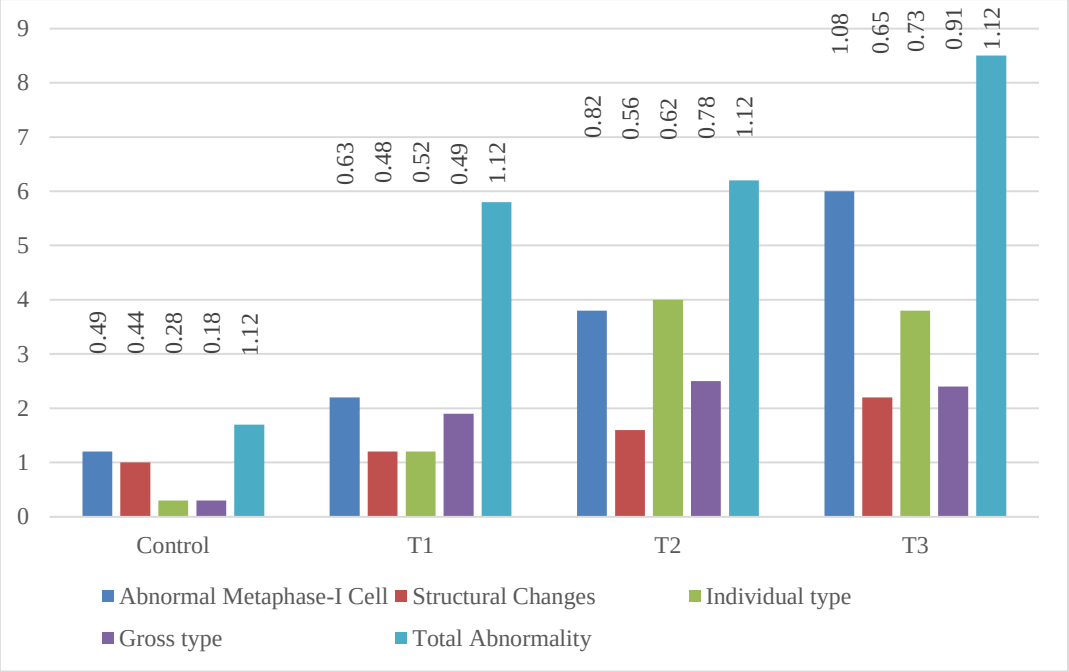

Figure 4- Graph showing chromosomal abnormalities in primary spermatocytes

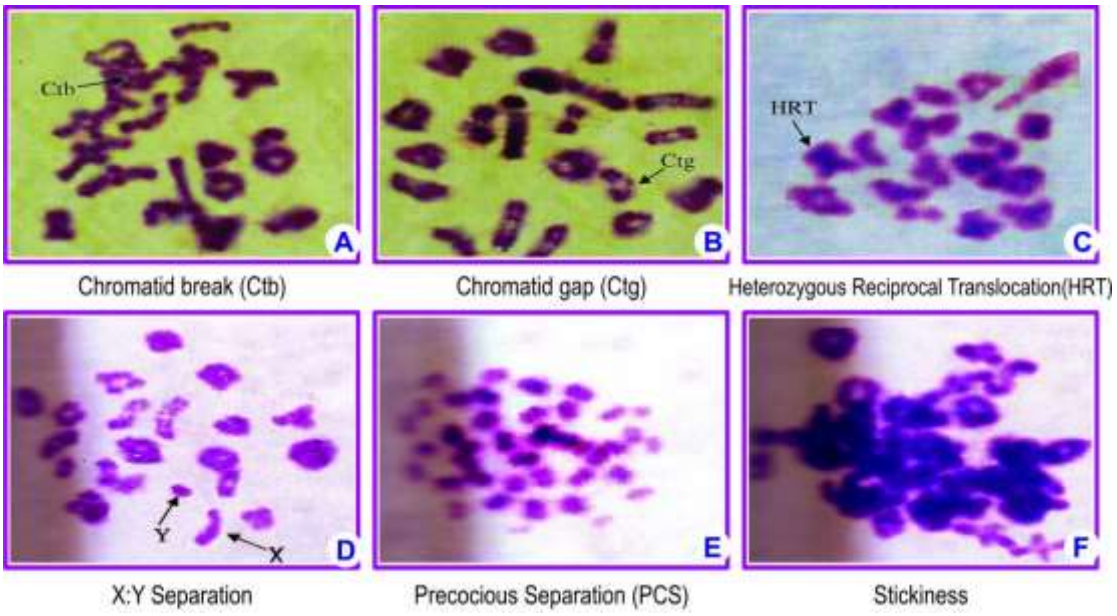

Figure 5-figure indicated meiotic chromosomal abnormalities (chromatid break A, chromatid gap B, heterozygous reciprocal translocation C, X: Y separation D, precocious separation E, and stickiness F) (Giemsa stain 100X)

The significant increase in the total abnormalities was due to a marked increase in division disruptive were polyploidy, C-mitosis, Hypoploidy, were more common and synaptic disturbance, in which univalent, X-Y separation precocious separation were more frequent. In gross type stickiness, clumping and hetero pycnosis were more common. Since no significant increase was exhibited in the structural changes. The overall effects of *Borago officinalis* showed that the abnormalities were increased with the increase in doses. Thus the effects were dose-dependent.

A quantitative estimation of results revealed that the abnormalities were dose-dependent (increased as the dose increased). Also, the results showed that gross types of damages were

more prominent than the individual type. However, previous studies concluded that biomutagens could induce more gross abnormalities than the individual types [12,13].

Other studies showed that the genotoxic effect of synthetic pesticides, fertilizer, distillery, synthetic silk dyeing waste had the ability to enhance individual type of damage and were more frequent than the gross type [14,15] due to the formation of electrophilic ions and reactive radical during the metabolization of mutagens [16,17]. Such electrophilic reactive radicals or ions might attack the nucleophilic site of DNA leading to structural changes in chromosomes [18].

The significant increase of gross type abnormality in chromosomes may be, due to the interference with the spindle apparatus of dividing cells. Hence, the mechanism of action proposed is spindle poison [19,20]. However, It is proposed that biomutagens produce micro tubule specific metabolites and active radicals inside the cells [21].

These metabolites and radicals may directly bind to tubulin dimers at the assembly end or bind to a free subunit thereby blocking further addition of monomers. Meanwhile, they may initiate depolymerization at the opposite end of the microtubules leading to their dissociation [22,23].

4. CONCLUSION:

The current study assessed the cytogenotoxic potential of the extract from the leaves of *Borago officinalis* on some cytogenetics parameters in swiss albino mice. Results revealed that the extract induced the division disruptive chromosomal changes in bone marrow as well as in primary spermatocytes and such effects may be attributed to that leaf extract might be producing the damage of two different levels. First by affecting the cells chromosome morphology leading to structural type of changes and second by affecting gross type chromosomal abnormalities.

ETHICAL CLEARANCE The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

CONFLICT OF INTEREST The authors declare that they have no conflict of interest.

FUNDING: Self-funding

REFERENCES

[1] M. Zając, I. Duda, Ł. Skoczylas and M. Tabaszewska "Potential Use of Hyssopus officinalis and Borago officinalis as Curing Ingredients in Pork Meat Formulations", *Animals (Basel)*, vol. 10 , no. 12, pp. 2327, 2020.

[2] R. M. Al-Ezzy, A. Mhawesh and K. W. Abbood "Estimation of the whole flavonoid, antioxidant, anti-bacterial challenge concerning viola odorata (banafsha) methanolic extract", *Iraqi Journal of Agricultural Sciences*, vol. 49, no. 4, pp.566, 2018.

[3] D. Charlotte, Q. Muriel, B. Aurélie and J. Anne-Laure "Temperature and water stress affect plant–pollinator interactions in Borago officinalis (Boraginaceae)", *Ecology and evolution*, vol. 8, no. 6, pp. 3443-3456, 2018.

[4] A. Catharine, B. Ian, H. Jason, S. Ses and E. Sue "A systematic review and quality assessment of case reports of adverse events for borage (*Borago officinalis*) *coltsfoot (Tussilago farfara) and comfrey (Symphytum officinale)", Fitoterapia*, PP. 104519, 2020.

[5] C.Q. Descamps, B. Muriel, J. Aurélie and L. Anne "Temperature and water stress affect plant–pollinator interactions in Borago officinalis (Boraginaceae) ", *Ecology and evolution*, vol. 8, no.6, pp. 3443-3456, 2018.

[6] S. Hammami, H. Debbabi, I. R. K. JlassiJoshi and R. El Mokni "Chemical composition and antimicrobial activity of essential oil from the aerial parts of Plantago afra L.(Plantaginaceae) growing wild in Tunisia", *South African Journal of Botany*, vol.132, pp. 410-414, 2020.

[7] M. L. Colombo, F. Assisi, T. Della Puppa, , F.M. Moro Sesana, and M. Bissoli "Most commonly plant exposures and intoxications from outdoor toxic plants", *J Pharm Sci Res*., vol. 2,pp. 417-425, 2010.

[8] A. Hamedi, A. Pasdaran, A. Zebarjad and A. A. Moein "Survey on Chemical Constituents and Indications of Aromatic Waters Soft Drinks (Hydrosols) Used in Persian Nutrition Culture and Folk Medicine for Neurological Disorders and Mental Health" *J Evid Based Complementary Altern Med*., vol. 22, no. 4, pp. 744-752, 2017.

[9] W. Fua, J. Chena, Y. Caia, Y. Leia, L. Chenb, L. Peic, , D. Zhoua, X. Lianga and J. Ruana, "Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.)" *Ching. J Ethnopharmacol.,* vol. 130, pp. 521-528, 2010.

[10] J.W. Allen, C.V. Shuler, R.W. Mendes and S.A. Latt "A simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromo-deoxy uridine tablets", *Cytogenet Cell Genet*., vol.18, pp. 231-237, 1977.

[11] M. El Marraki "Counting the number of spanning trees in the star flower planar map", *Applied Mathematical Sciences*, vol. 6, no.49, pp: 2418-2411, 2010.

[12] P. Friedl and B. Weigelin " Interstitial leukocyte migration and immune function" *Nat Immunol*.,vol. 9, pp. 960-969, 2008.

[13] C. L. Gorlenko, H. Y. Kiselev, Z. Budanova, A. Andrey and L. N. Ikryannikova "Plant Secondary Metabolites in the Battle of Drugs and Drug-Resistant Bacteria New Heroes or Worse Clones of Antibiotics", *Antibiotics*,vol.9, no. 4, pp.170, 2020

[14] M. Gupta and S. Singh "Borago officinalis Linn an important medicinal plant of Mediterranean region: a review", *Int J Pharm Sci Rev Res.*, vol.5, pp.27-34, 2010.

[15] H. Aslam, A.U. Khan, N. U. Rehman, F. Ali, H. Nadeem and S.M. Shah "Anti-hyperglycemic activity of Heliotropium strigosum (Boraginaecae) whole plant extract in alloxan-induced diabetic mice" *Tropical Journal of Pharmaceutical Research*, vol.16, no. 10, pp. 2425–2430 , 2017.

[16] M. Igarashi "Development of new antituberculosis drugs from natural products", *Bioscience, biotechnology, and biochemistry*, vol.81, no. 1, pp. 32-37, 2017.

[17] K. Jaffel, S. Sai, N.K. Bouraoui, R.B. Ammar, L. Legendre and M. Lachâal "Influence of salt stress on growth, lipid peroxidation andantioxidative enzyme activity in borage (*Borago officinalis L*", *Plant Biosys* , vol.145,pp. 362-369, 2010.

[18] S. Khalaf, I.J. Abed "Evaluating the in vitro Cytotoxicity of Thymus vulgaris Essential Oil on MCF-7 and HeLa Cancer Cell Lines", *Iraqi Journal of Science*, vol. 62, no. 9, pp: 2862-287, 2021.

[19] S. S. Kamble and R. N. Gacche "Evaluation ofanti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants ", *European Journal of Integrative Medicine*, vol.25, pp. 13-19, 2019.

[20] M.E. Abdalah, R.M. Al-Ezzy and Z.A. Okhti, "Protective role of Viola odorata against hepatotoxicity induced by methotrexate in albino male mice ", *journal. Pharm. Sci. & Res*., vol.10, no. 11, pp. 2775-2785, 2018.

[21] N. M. Al-abide, "Taxonomical and Anatomical Study of the Species Nonea echioides (L.) Roem. & Sehult (Boraginceae) in Northern Iraq", *Iraqi Journal of Science*, vol. 62, no. 11, pp: 3842-3850, 2021.

[22] Z. Hanene, A. Sonda, B. Amel, M. Mahfoud, E. Abdelfattah and B. Mohamed "Chemical composition and antioxidant activity of *Borago officinalis* L. leaf extract growing in Algeria", *Arabian journal of chemistry*, vol.12, no. 8, pp.1954-1963, 2019.

[23] C. Avila, B. Ian, H. Jason, S. Ses, E. Sue "A systematic review and quality assessment of case reports of adverse events for borage (Borago officinalis) .coltsfoot (Tussilago farfara) and comfrey (Symphytum officinale)", *Fitoterapia*, pp. 104519, 2020.