International Journal for Research in Applied Sciences and Biotechnology

ISSN: 2349-8889
Volume-8, Issue-6 (November 2021)

www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.6.13

In Vivo Histological Study the Potential of *Borago officinalis* on Spleen and Testis Histo-Architecture on CCL4 Induced Albino Male Mice

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ABSTRACT

History of medicine and plants dates backside to seclude past when herbal treatment was used and be the only answer to all kind of pain and disease. Nowadays, greater prominence is again to use phytotherapy all over the world. Herbal medicine is a traditional or folk medicine that based on the use of plants' seeds, berries, roots, leaves, barks, flowers and plant extracts for medicinal purposes. This research study focused the line on the potentail of aqueous and methanolic extract of Borago officinalis (Borago; BO) on spleen and testis of albino male mice alone or after interaction between both plant extracts with CCL4 (toxic compound) in comparison to controls group (negative control; without any treatment and positive control; mice treated with CCL4 only). The results indicated the ability of plant extracts to modulate toxic effect resulted from CCL4 treatment.

Keywords- Borago, medicinal plant, cancer, inflammation and *gamma-linolenic acid* (GLA).

I. INTRODUCTION

Herbal medicine consider as the oldest form of health care known to mankind, and it had been used since ancient time. These plants represent an integral part of modern civilization progress (Fuemmeler *et al*., 2009). Herbal medicine is a major component of all indigenous people's traditional medicine and a common element in Ayurvedic, Homoeopathic, Naturopathic, Traditional Arabic, Oriental and Native American Indian medicine, and the WHO predictable further that out of 119 plants derived pharmaceutical medicines, approximately 74% are used in modern medicine (Toni *et al*., 2010). Borago officinalis or borago or starflower characterized as an annual herb which possessed medicinal and culinary uses with the most important active compound obtained from borago seed oil. Gamma-linolenic acid (GLA) represented the richest borago plant source from seeds oil which is used as dietary or food supplement *(Zemmouri et al.,2019).* One of the main features of borago is its ability to resist to microbes when grow well in wet soils with good drainage, weedy places and in complete exposure to sunlight (Colombo *et al.*, 2010). Fatty acids from borago are used in healing and treatment of thrombosis, inflammation and cancer (Zahn *et al*., 2019) Also, borago raw leaf obtained is used as supplements. In addition to all that, the plant used for the treatment of various diseases such as multiple sclerosis, diabetes, heart diseases, all these plant activities attributed to its active components represented by fatty acids such as linoleic acid (Lim *et al*.,2019).

II. METHODS

Plant collection, identification and extractions (aqueous and methanolic)

- Plant collected from local Baghdad market during the period sep,2020 which previously identified by Iraqi center of herbarium
- Plant extraction: two types of extraction used in this study which were aqueous or methanol extraction, in both methods fifty grams of the dried aerial parts of plant were extracted for 3 hours in 250 ml of the solvent (either distilled water or methanol) using the soxhlet apparatus. After that the leaf extract solution evaporated at 45°C using a rotary evaporator, and then stored by frozen at -20°C until use (Nadir *et al*., 1986).

- ***Experimental design of animals:***

Six mice groups used in this stage which classified to

Group 1: negative control without any treatment

Group 2: positive control (mice treated with CCL4)

Group 3: mice treated with aqueous extract of borago plant at dose 200 mg\kg

Group 4: an interaction between CCL4 and borago aqueous extract

Group 5: mice treated with borago methanolic extract only at dose 200mg\kg

group 6: an interaction between CCL4 and borago methanolic extract

All mice (these were supplied from Biotechnology Research Center\Al-Nahrain University; their age 8 weeks and weight 23-25grams and their feed *add labitum*) injected intraperitonially with tested compounds for seven days then at day 8 the animals scarified and spleen and testis of each mice group take and preserved in formalin (10%) to prepare histological section of spleen and testis. total numbers of animals (24 mice)

- ***Histological section of spleen and testis:***

After fixation with 10% formalin for 24h, all samples dehydrated with gradual series of alcohol (30-

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www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.6.13

100 %) for (5) min following by xylene treated before embedded in paraffin wax for sectioning, both sections (spleen and testis) stained with hematoxylin (Harison) and eosin according to standard method and examined under light microscope.

III. RESULTS AND DISCUSSION

The results of all treated group with plant extracts (aqueous or methanol) either alone or after interaction with CCL4 represented the ability of borago to counteract the toxic effect of CCL4 as shown in Figurers below.

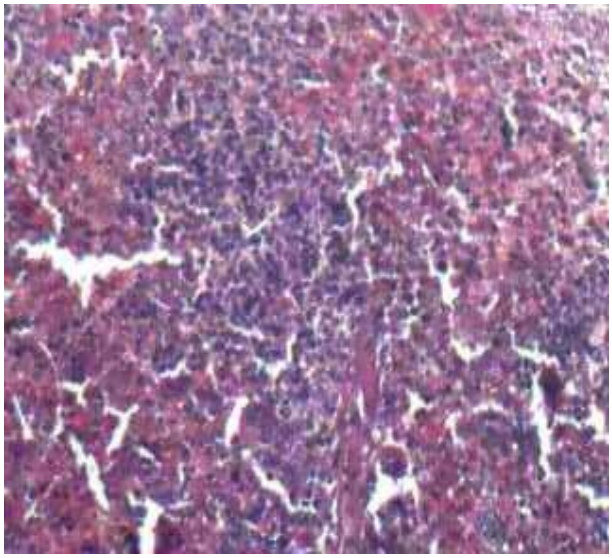

Figure (1) (spleen): normal spleenic section (negative control) (400X; H and E)

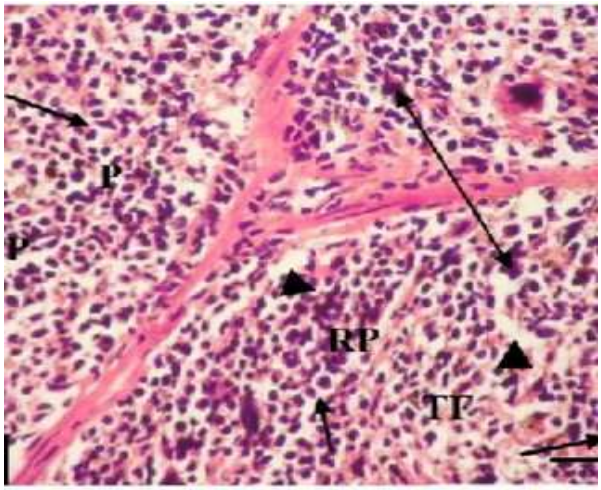

Figure (2) (spleen): section showing necrosis of splenic cells after CCL4 treatment. (400X; H and E)

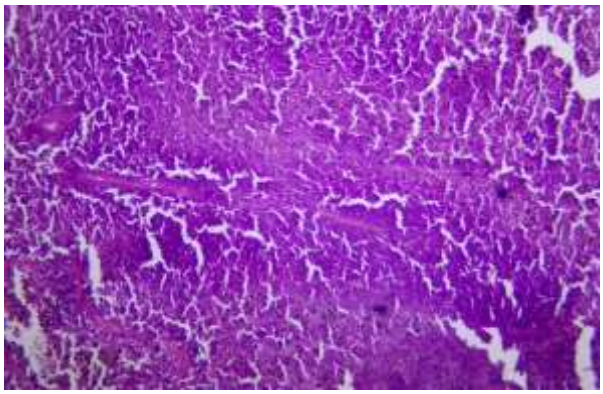

Figure (3): Section f the spleen showing diffuse hyperplasia of lymphoid cels in spleen of mice treated with borago aqueous extract. (H&E) (X10).

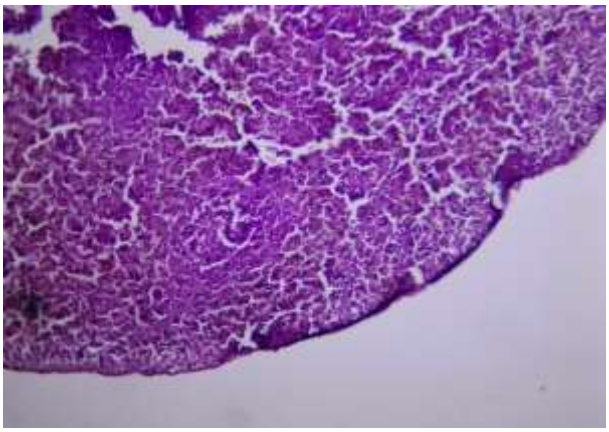

Figure (4): showing spleenic hyperplasia of lymphoid cells after interaction between borago aqueous extract and CCL4. (H & E) (X10).

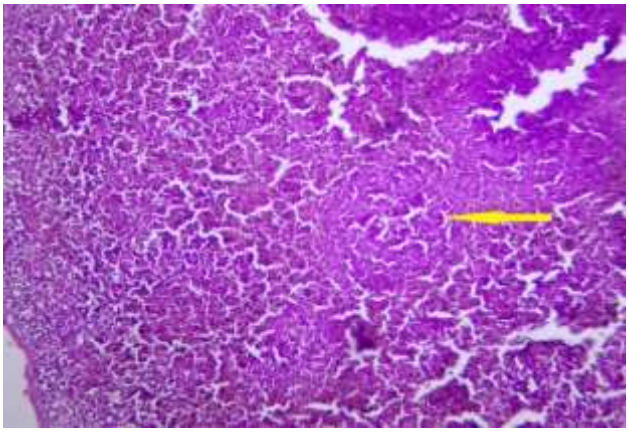

Figure (5): Showing lymphoid follicular hyperplasia on albino mice treated with borago methanolic extract. (H & E) (x10).

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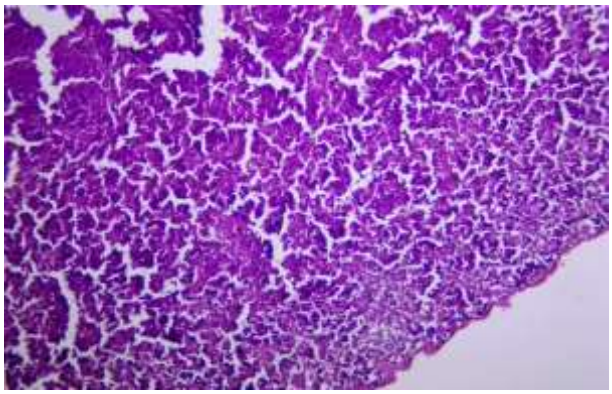

Figure (6): Showing lymphoid cells hyperplasia after interaction between borago methanolic extract and CCL4. (H & E) (X10).

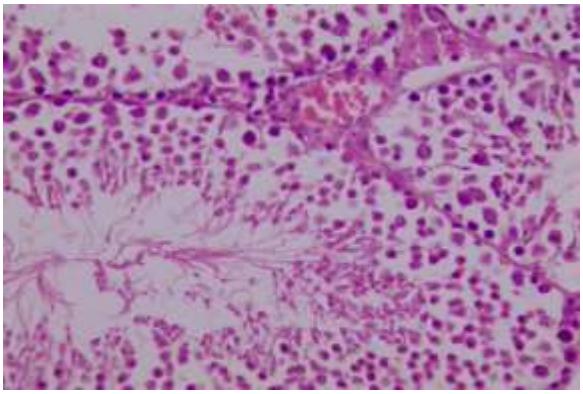

Figure (7): Normal section of testis in control negative group

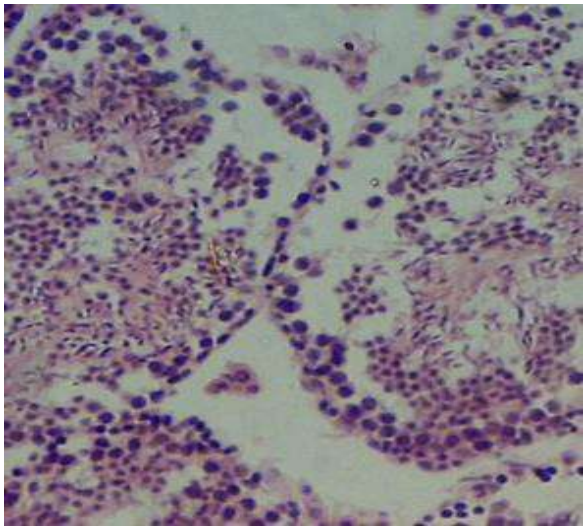

Figure (8): testis after mice treated with CCL4 showed nonappearance of spermatogenesis through tinny of the wall OF SEMINIFEROUS TUBE OF THE TESTES. (400X; H AND E)

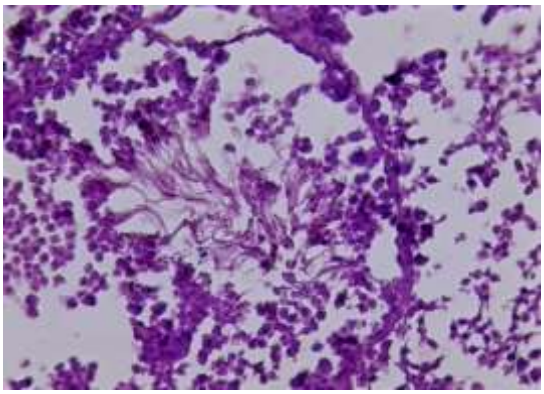

Figure (9): Section of the testis showing normal maturation of seminephrous tubules with presence of sperms inside the lumen of the tubules on aqueous borago extract treated mice. (H & E) (X40).

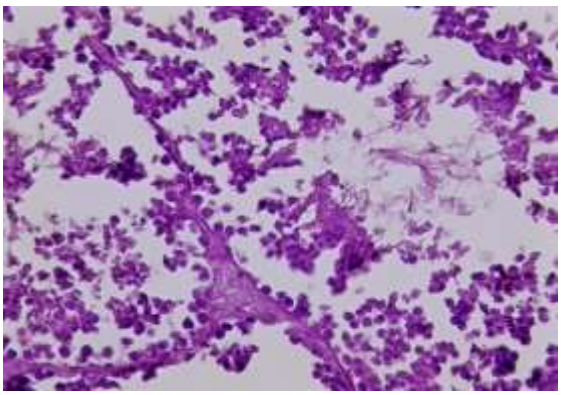

Figure (10): Showing normal maturation of seminephrous tubules but with immaturity and abnormality of the sperms inside the lumen in an interaction group between aqueous extract and CCL4. (H & E) (X40).

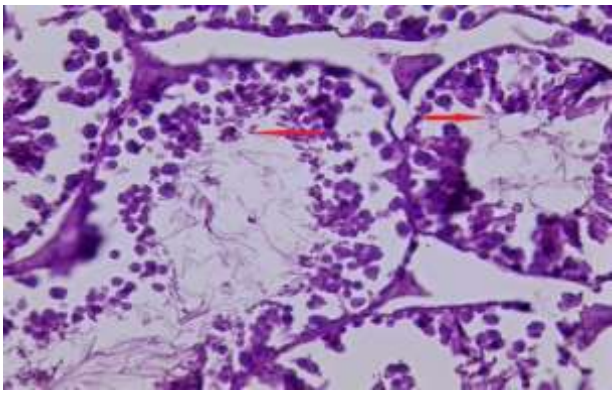

Figure (11): showing normal development but with immaturity of sperms on albino mice treated with borago methanolic extract. (H & E) (X40).

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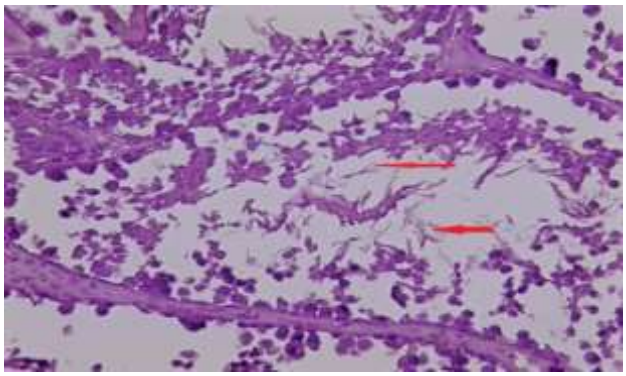

Figure (12): Showing normal development of seminephrous tubular cells but with immaturity of sperms inside the lumen after interaction between borago methanolic extract and CCL4. (H & E) (X40).

Different medicinal plants like salvia, hypericum, cyperus, borago and many others which possessed secondary active metabolite like flavonoids have shown to have multiple medicinal activity such as diuretic, laxative, antispasmodic, anti-hypertensive, and anti-inflammatory actions and immunomodulatory potentials (Lu *et al*., 2013). CCl_4 possessed its organ toxicity due to its metabolite CCl_3^-, which is a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids. In the presence of oxygen, lipid peroxides are produced, leading to organ damage through organ cirrhosis and necrosis (Zeashan *et al*., 2008). These oxidative stress causes organ injury and carcinogenesis (Cheng *et al*., 2013). One clinical strategy for prevention and reduction the toxic effects of CCl_4-induced toxicity was reducing the creation of reactive metabolites (Wong *et al*., 2012). The histopathological examinations of spleen and testis sections in CCl_4-treated mice revealed a regeneration of organ cells after treatments with the plant extract at a dose of 200 mg/kg; therefore, it is possible to suggest that borago plant extract being able to state the spleen and testis cells to a status of accelerated renewal (Aydin *et al*., 2014). Flavonoids and terpenoids among the most important plant constituents gave the protective effect of borago against the organ toxicity induced by CCl_4 (*Yuce and Bagci, 2012*) with most important properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action leading to different organ protection (*Tanemossu et al*., 2014). These effect simply be due to an anti-oxidative property, and other mechanism. Borage oil contains high amount of GLA which possess anticonvulsant, bronchodilator, vasodilator. It also has cardio-depression property. Previous study revealed that Borage plant had the ability to increase in urine excretion and regulate blood pressure and kidney function. Borago had the ability to increase immunity due 70% GLA containing that possess potentials effect on gene transcription responsible for cytokine reproduction IL-2 and IFN-γ, indicating a direct effect of the GLA and fatty acids or their metabolites on gene transcription. This is also supported that the borago active constituents is probably affected on monocytes activated by T cells, or it could be either T cells or monocytes or both (Harbige and Fisher, 1997).

Our results of present study agreed with who found that in mice fed BO was effective as an anti-obesity by reducing adipocyte hypertrophy and modulate the expression of adipogenesis-associated genes (yong et al., 2.19). Also, **another scientist found that borago exhibited antitumor activity through** immunomodulatory function by improving the reproductive activity of spleen lymphocyte induced by Con A and the activity of NK cells and the phagocytosis of peritoneal macrophage and all these may be related to regulating the cellular immunity (Ramezani *et al*.,2020). Finally, GLA which is main constituents of borago can act as a substrate for a series of enzyme reactions giving rise to series 1 eicosanoids (biologically active substances, including prostaglandins), which have a wide range of actions in the body (Hafid *et al*., 2002).

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ISSN: 2349-8889
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