Journal of Pharmacy and Pharmacology 7 (2019) 270-277
doi: 10.17265/2328-2150/2019.05.007

Histological Effect of Viola Odorata Methanolic Extract and Methotraxate Induced in Albino Male Mice

Farah T. O. Al-jumaili[1], Sura Alaa Soud[2], Mays Talip Abdalla[1], Ruqaya M. Al-Ezzy[1] and Mohammed Bdaiwy Hassan[3]

1. Departement of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

2. Microbiology Departement, College of Science, Al-Kharakh University, Baghdad, Iraq

3. AlYarmook teaching hospital, Baghdad, Iraq

Abstract: The objective of this study was to considere as an explorer for *in vivo* studies on the production of some secondary metabolites from local medical plants named *Viola odorata*. *Viola odorata* commonly known as "garden violet or sweet violet" belongs to family Violaceae, is a slow growing perennial, with stout rootstock, grows in hedgerows, rough land and margins of woodland. 200 mg/kg of methanol extract for *V. odorata* was interacted with methotrexate as a drug in albino mice to see the healing capacity for this extract. Different organs were used such as intestine, kidney, spleen, and testes for this experiment. Each organ response was recorded in this experiment. Histopathological section in the intestine of animal treated with MTX and plant showed hypertrophy and hyperplasia of goblet cells and increased cellularity of lamna properia while Histopathological section in the testes of animal treated with MTX and plant showed no sperm in the seminiferous tubules of epididymis with the round multi-nuclei cell in the lumen accompanied by homogeneous material and cellular debris while Histopathological section in the spleen of animal treated with MTX and plant proliferation of lymphocytes in the periarteriolar sheath and proliferation of mononuclear cells around sinus in red pulp and Histopathological section in the kidney of animal treated with MTX and plant mononuclear cells aggregation in the interstitial tissue mainly around blood vessels and in the adipose tissue and renal tubules. Other sections showed hydropic degeneration of renal tubules.

Key words: Medicinal plants, methotrexate, methanolic extract, violin.

1. Introduction

In a new therapeutic system, antibiotics have a big job in controlling the infectious diseases [1]. However, "the drug resistance adopted by most of the pathogens certainly needs a suitable replacement of the presently available antibiotics" [2, 3]. Besides, many antimicrobial agents are known to exhibit serious inconvenient effects on host tissues which lead to the system toxicity [4-6]. In third-world countries, it is the time to use phytochemicals for healthcare especially in remote areas [7]. "Nearly 30% of drugs across the globe are derived from plants and 252 drugs are in WHO essential medicine list" [8]. *Viola odorata* commonly known as "garden violet or sweet violet" belongs to family Violaceae, "is a slow growing perennial, with stout rootstock, grows in hedgerows, rough land and margins of woodland" [9]. *V. odorata* is considered to be a native plant of North-Western Africa and Western Asia, beside Southern Europe. The plant is rich in flavonoids, alkaloids (violin, viola-quercitin), essential oils including (ionones, alpha-ionone, beta-ionone and beta-dihydro ionone, hydroquinone dimethyl ether, linolenic) and is extensively used in diuretic, anti-inflammatory properties, abdominal pain, skin disorders, upper respiratory complications (cough, sore throat and harish) [10-16]. Antioxidants are plant origin substances, which reduce and neutralize free radicals and play a vital role in the prevention of cancer, cardiovascular diseases, Alzheimer and Parkinson diseases [17]. In *Viola odorata*, "antioxidant activity is related to the number of anthocyanins, one of the

Corresponding author: Ruqaya M. Al-Ezzy, Ph.D., assisstant professor, research field: biotechnology.

groups of flavonoids pigments. Anthocyanins occur in all tissues including stems, leaves, roots and flowers. Antioxidants like phenolic acids, polyphenols and flavonoids etc. also show their effect by scavenging free radicals, preventing the generation of reactive oxygen species (ROS) or activating detoxifying proteins" [18].

Using medicinal plants in "safe way" had been chosen in the market demand and that results in over exploitation and decline in natural habitat. All medicinal plants used across the world are getting endangered due to their severe harvesting for production of medicines as most of them grown under natural conditions. For all the above reasons we used *V. odorata* aerial parts to see their toxicity in rats.

2. Materials and Methods

2.1 Plant Collection and Identification

The aerial parts of the plant were collected from the local markets during September (2017), which had been identified previously by National Herbarium of Iraq.

2.2 Plant extract Preparation

Methanolic extract of *V. odorata* was prepared according to Ref. [19]. Fifty grams of the plant aerial parts powdered and extracted with 80% methanol (250 ml) at 65 ℃ 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 ℃ until use to prepare the required doses.

2.3 Animal's Preparation

Six–eight weeks aged Albino male mice weighted 23-25 g, and were purchased from Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq. Four animals were housed in each cage with *ad libitum* access to water and food pellets. They were divided into three groups as in Table 1 below.

2.4 The Experiment

This experiment was designed to get an interaction between plant extract (200 mg/kg) and the drug methotrexate through post-treatment with the plant extract, in which the animals were injected with methotrexate in day 1, while in days 2-7, they were injected with the plant extract (single dose/day). The animals were sacrificed on day 8 for laboratory assessments. Details of this experiment are summarized in Table 1.

3. Results

According to previous results plants extract is found the best dose to be used at 200 mg/kg plus MTX (40 mg/kg [20]. Therefore, in this paper the results represent the best concentration compared with the drug and control.

3.1 In Intestine

As shown in Fig. 1 normal appearance of intestine but when treated with methotrexate slight necrosis and degeneration of nephrocytes are observed (Fig. 2) and histopathological section in the intestine of animal treated with MTX and plant showed hypertrophy and

Table 1 Groups of animals investigated in the laboratory tests.

	Type of interaction	Dose mg/kg	Laboratory tests & number of animals (Histological examination for intestine, spleen, kidney and testes)
1	Distilled water	----------	4
2	Methotrexate		4
3	Methotrexate + viola extract	40	4
	Total number of animals	40 + 200	12

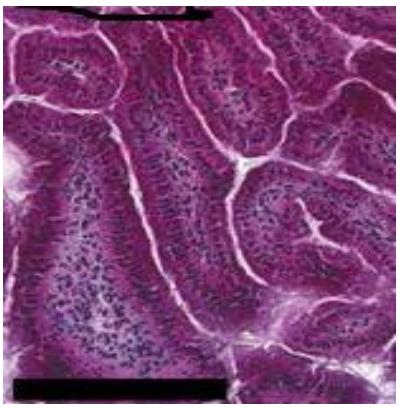

Fig. 1 Section showing normal appearance of intestine.

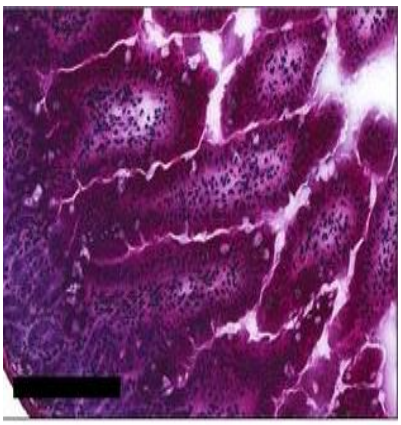

Fig. 2 Section of a intestine tissue in mouse treated with methotrexate. Slight necrosis and degeneration of nephrocytes are observed (200×; H and E).

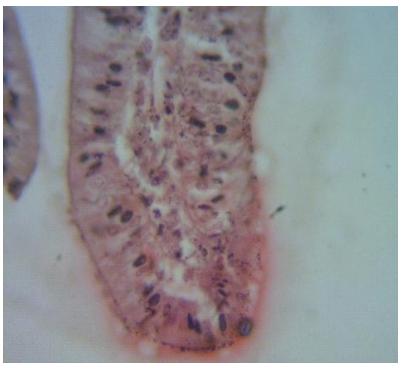

Fig. 3 Histopathological section in the intestine of animal treated with MTX and plant showed hypertrophy and hyperplasia of goblet cells and increased cellularity of lamna properia (H&E stain 40×).

hyperplasia of goblet cells and increased cellularity of lamna properia as in Fig. 3.

3.2 Testes

Testis tissue in control negative mice showed normal seminiferous tubules, spermatids and spermatogenic cells at different stages of developemant as shown in Fig. 4, but widespread apoptotic germinal cells in seminiferous tubule germinal epithelium and in the lumen when mice treated with methotrexate drug as shown in Fig. 5 and Histopathological section in the testes of animal treated with MTX and plant showed no sperm in the seminiferous tubules of epididymis with the round multi-nuclei cell in the lumen accompanied by homogeneous material and cellular debris (Fig. 6).

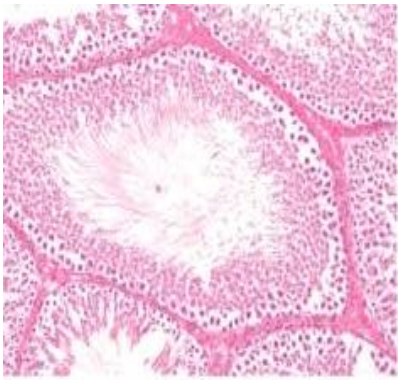

Fig. 4 Section of testis tissue in control negative mice showing normal seminiferous tubules, spermatids and spermatogenic cells at different stages of developemant (400×; H and E).

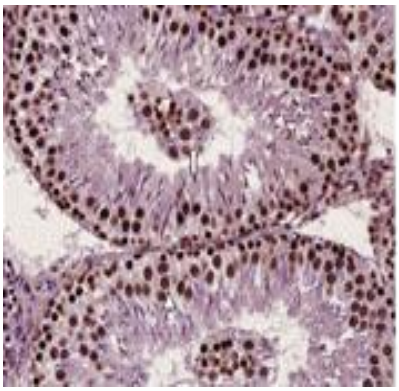

Fig. 5 Section of testis tissue in mice treated with methotrexate drug, widespread of apoptotic germinal cells in seminiferous tubule germinal epithelium and in the lumen (200×; H and E).

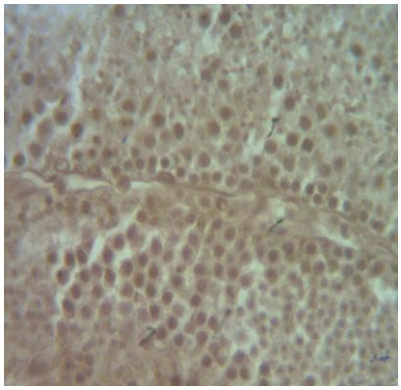

Fig. 6 Histopathological section in the testes of animal treated with MTX and plant showed no sperm in the seminiferous tubules of epididymis with the round multi-nuclei cell in the lumen accompanied by homogeneous material and cellular debris.

3.3 Spleen

Fig. 7 shows spleen tissue in control negative mice. A cross section in spleen showing white, W, red R palps and scattered but Transverse section of the red pulp area in the spleen treated with methotrexate showing the infiltration of polymorphonucleur cells, degenerating lymphocytes D and necrosis of the tissue Megakaryocytes as in Fig. 8 while Histopathological section in the spleen of animal treated with MTX and plant proliferation of lymphocytes in the periarteriolar sheath and proliferation of mononuclear cells around sinus in red pulp as shown in Fig. 9.

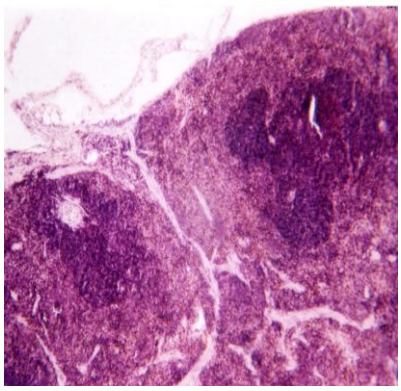

Fig. 7 Section of spleen tissue in control negative mice. A cross section in spleen showing white, W, red R palps and scattered (200×; H and E).

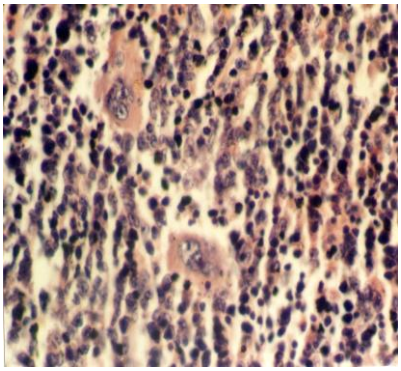

Fig. 8 Transverse section of the red pulp area in the spleen treated with methotrexate showing the infiltration of Polymorphonucleur cells, degeneration of lymphocytes D and necrosis of the tissue Megakaryocytes(200×; H and E).

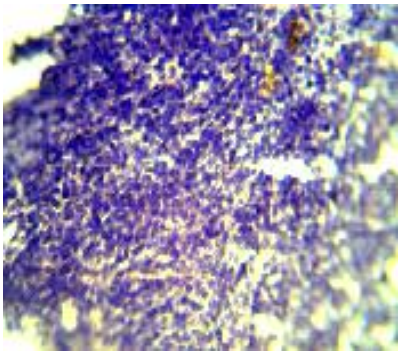

Fig. 9 Histopathological section in the spleen of animal treated with MTX and plant proliferation of lymphocytes in the periarteriolar sheath and proliferation of mononuclear cells around sinus in red pulp.

3.4 Kidney

In Fig. 10 normal appearance of kidney shown and when mice treated with methotrexate drug necrosis is present together with mild inflammatory cell infiltrate (mononuclear cells) and fatty changes in kidney tissue as shown in Fig. 11 and Histopathological section in the kidney of animal treated with MTX and plant mononuclear cells aggregation in the interstitial tissue mainly around blood vessels and in the adipose tissue and renal tubules. Other sections showed hydropic degeneration of renal tubules as shown in Fig. 12.

4. Discussion

Herbs or medicinal plants are used for ages to maintain and enhance health against body infections. This is due to "their availability and arguably efficacious state, therefore offering an alternative remedy

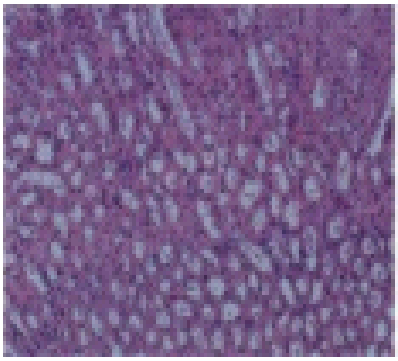

Fig. 10 Section showing normal appearance of kidney (200 ×; H and E).

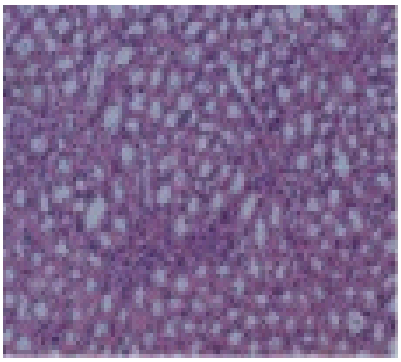

Fig. 11 Necrosis is present together with mild inflammatory cell infiltrate (mononuclear cells) and fatty changes in kidney tissue of mice treated with methotrexate drug (200 ×; H and E).

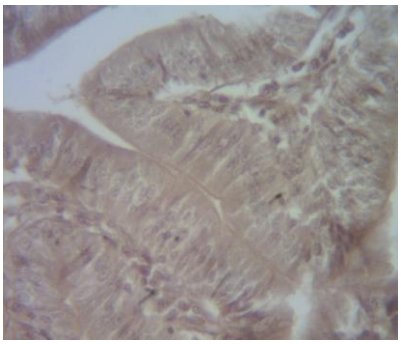

Fig. 12 Histopathological section in the kidney of animal treated with MTX and plant mononuclear cells aggregation in the interstitial tissue mainly around blood vessels and in the adipose tissue and renal tubules. In other section showed hydropic degeneration of renal tubules (H&E stain 40 ×).

in enhancing hematological parameters" [21].

V. odorata in many reports had indicated anti-inflammatory, antipyretic, antioxidant and antibacterial activities [22-24]. Fresh leaves of this plant have been used externally and internally in the treatment of cancer. Decoction, poultice, infusion of leaves, syrup made from petals or a liquid extract of *V. odorata* fresh leaves are used for throat and tongue cancers [23, 25]. However, some Violaceae compounds (cyclotide and flavonoids) demonstrated significant anti-carcinogenic and antioxidant activity [26, 27]. Compounds isolated from Viola include flavonoids, phenylpropanoids, terpenoids, amides, sterols, essential oils, saccharides, aromatic acids and cyclotides. Viola has exhibited a number of activities such as antioxidant, antibacterial, anti-inflammatory, immunomodulatory, antimalarial, insecticidal, anticancer, anti-HIV, anxiolytic, anticonvulsant, cytotoxic, hepatoprotective and lung protective activity [28]. *Viola odorata* exhibited hepatoprotective activity

against paracetamol induced hepatotoxicity due to the presence of isorhamnetin and luteolin (flavonoids) [29]. Similarly, during the phytochemical investigation of the *Viola canescens*, polyphenols and flavonoids, beside saponins, triterpenes and alkaloids were extracted [30]. Methotrexate (MTX) was discovered 50 years ago and was primarily used to treat malignancies such as breast cancer [31]. "It can be used in the treatment of autoimmune diseases such as rheumatoid arthritis (RA), graft vs. host disease (GVD), psoriasis and Chron's disease. This anti-inflammatory use started about 20 years ago in the treatment of RA" [32].

MTX is similar in structure to dihydrofolate (FH2) and is a competitive inhibitor of DHFR with the result that the tetrahydrofolate (FH4), essential for DNA synthesis, is then not produced. This interferes with the mitosis of cancerous cells by inhibiting the de novo synthetic pathways for purines, pyrimidines, formation of polyamines and transmethylation of DNA, RNA, phospholipids and proteins [33]. After entering the cell, MTX undergoes polyglutamation as in natural folates [34]. When this happens, up to five additional of glutamates can be added to the molecule [31]. The products of intracellular metabolism are MTX-polyglutamates, 7-hydroxymethotrexate and diamino-2,4 N10 methylptero ̈c acid (DAMPA) [35]. MTX-polyglutamates also inhibit the enzymatic conversion of 5-aminoimidazole-4-carboxamide-ribonucleotide (AICAR) to formyl-AICAR by the enzyme AICAR-transformylase.

References

[1] Khan, M. F. 1978. "The New Development in Antibiotics." *Ann. Intern. Med*. 89: 849-53.

[2] Threlfall, E., et al. 1996. "Increasing Spectrum of Resistance in Multiresistant Salmonella Typhimurium." *The Lancet* 347 (9007): 1053-4.

[3] Conly, J., et al. 1992. "Disseminated Candidiasis due to Amphotericin B-Resistant Candida Albicans." *Journal of Infectious Diseases* 165 (4): 761-4.

[4] Calderwood, S. B., and Moellering Jr, R. C. 1980. "Common Adverse Effects of Antibacterial Agents on Major Organ Systems." *Surgical Clinics of North America* 60 (1): 65-81.

[5] Di Matteo, V., and Esposito, E. 2003. "Biochemical and Therapeutic Effects of Antioxidants in the Treatment of Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis." *Current Drug Targets-Cns And Neurological Disorders* 2 (2): 95-108.

[6] Bolard, J. 1986. "How Do the Polyene Macrolide Antibiotics Affect the Cellular Membrane Properties?" *Biochimica et Biophysica Acta (BBA)* 864 (3-4): 257-304.

[7] Gul, F., Shinwari, Z. K., and Afzal, I. 2012. "Screening of Indigenous Knowledge of Herbal Remedies for Skin Diseases among Local Communities of North West Punjab, Pakistan." *Pakistan J Bot*. 5: 1609-16.

[8] Sahoo, N., Manchikanti, P., and Dey, S. 2010. "Herbal Drugs: Standards and Regulation." *Fitoterapia* 81 (6): 462-71.

[9] Tobyn, G., Denham, A., and Whitelegg, M. 2011. "Viola Odorata, Sweet Violet." *Viola Tricolor*, 2011: 337-48.

[10] Kathi, K. 1991. *The Illustrated Herb Encyclopedia (a Complete Culinary, Cosmetic, Medicinal and Ornamental Guide to Herbs)*. New York: Mallard Press.

[11] Lamaison, J., Petitjean, F., and Carnat, A. 1991. "The Violet Flower: A Comparison between *Viola lutea*, *Viola calerata* and *Viola odorata*." *Plants Medicinal-et-Phytotherapie* 25: 79-88.

[12] Ibrahim, R. M. 2018. "Immunological, Cytogenetic and Hepatoprotective Effect of Viola odorata Methanolic Extract on Methotrexate Induced Albino Male Mice." *Journal of Biotechnology Research Center* 11: 2 .

[13] Jackson, D. 2005. *Alternative Nature Online Herbal.* Edited by K. Bergeron.

[14] Svangård, E., et al. 2004. "Cytotoxic Cyclotides from Viola t Ricolor." *Journal of Natural Products* 67 (2): 144-7.

[15] Walter, C., et al. 2011. "Antibacterial Activity in Herbal Products Used in Pakistan." *Pak J Bot* 43: 155-62.

[16] Ibraheem, R. M., Mhawesh, A. A., and Abood, K. W. 2018. "Estimation of the Whole Flavonoid, Antioxidant, Anti-bacterial Challenge Concerning *Viola odorata* (banafsha) Methanolic Extract." *Iraqi Journal of Agricultural Sciences* 49 (4): 566-51.

[17] Gerber, M., et al. 2002. "Food and Cancer: State of the Art about the Protective Effect of Fruits and Vegetables." *Bulletin Du Cancer* 89 (3): 293-312.

[18] Halliwell, B., Gutteridge, J. M., and Cross, C. E. 1992. "Free Radicals, Antioxidants, and Human Disease: Where Are We Now?" *The Journal of Laboratory and Clinical Medicine* 119 (6): 598-620.

[19] Fu, W., et al. 2010. "Antioxidant, Free Radical Scavenging, Anti-inflammatory and Hepatoprotective Potential of the Extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching." *Journal of Ethnopharmacology*

130 (3): 521-8.

[20] Ibrahim, R. M. 2017. "Immunological, Cytogenetic and Hepatoprotective Effect of *Viola odorata* Methanolic Extract on Methotrexate Treated Albino Male Mice."

[21] Salve, T., et al. 2014. "A Review Article on Banafsha (*Viola odarata* Linn.)." *PunarnaV: Int. Peer Rev. Ayurved J*. 2: 1-8.

[22] Elhassaneen, Y., et al. 2013. "Effect of Sweet Violet (*Viola odorata* L.) Blossoms Powder on Liver and Kidney Functions as Well as Serum Lipid Peroxidation of Rats Treated with Carbon Tetrachloride." *J Am Sci*. 9 (5): 88-95.

[23] Ebrahimzadeh, M. A., et al. 2010. "Antioxidant and Free Radical Scavenging Activity of *H. officinalis* L. var. Angustifolius, *V. odorata*, *B. hyrcana* and *C. speciosum*." *Pak J Pharm Sci*. 23 (1): 29-34.

[24] Stojković, D., et al. 2011. "Free Radical Scavenging Activity of *Viola odorata* Water Extracts." *Journal of Herbs, Spices & Medicinal Plants* 17 (3): 285-90.

[25] Barekat, T., et al. 2013. "A Novel Approach for Breaking Seed Dormancy and Germination in *Viola odorata* (A Medicinal Plant)." *J Nov. Appl Sci*. 2 (10): 513-6.

[26] Burman, R., et al. 2010. "Evaluation of Toxicity and Antitumor Activity of Cycloviolacin O_2 in Mice." *Peptide Science* 94 (5): 626-34.

[27] Vukics, V., et al. 2008. "Major Flavonoid Components of Heartsease (*Viola tricolor* L.) and Their Antioxidant Activities." *Analytical and Bioanalytical Chemistry* 390 (7): 1917-25.

[28] Khan, M. A., et al. 2017. "Hepatoprotective Effect of the Solvent Extracts of Viola Canescens Wall. ex. Roxb. against CCl 4 Induced Toxicity through Antioxidant and Membrane Stabilizing Activity." *BMC Complementary and Alternative Medicine* 17 (1): 10.

[29] Qadir, M. I., et al. 2014. "Hepatoprotective Activity of Aqueous Methanolic Extract of Viola Odorata against Paracetamol-Induced Liver Injury in Mice." *Bangladesh Journal of Pharmacology* 9 (2): 198-202.

[30] Rathi, A., et al. 2008. "Hepatoprotective Potential of Fumaria Indica Pugsley Whole Plant Extracts, Fractions and an Isolated Alkaloid Protopine." *Phytomedicine* 15 (6-7): 470-7.

[31] Dollery, C. 1999. *Therapeutic Drugs*. London: London Churchill Livingstone Publishers.

[32] Majumdar, S., and Aggarwal, B. B. 2001. "Methotrexate Suppresses NF-κB Activation through Inhibition of IκBα Phosphorylation and Degradation." *The Journal of Immunology* 167 (5): 2911-20.

[33] Lee, D. M. A. W. 2001. "Rheumatoid Arthritis." *The Lancet* 358: 903-11.

[34] Cutolo, M., et al. 2001. "Anti-inflammatory Mechanisms of Methotrexate in Rheumatoid Arthritis." *Annals of the Rheumatic Diseases* 60 (8): 729-35.

[35] Genestier, L., et al. 2000. "Mechanisms of Action of Methotrexate." *Immunopharmacology* 47 (2-3): 247-57.