

Effect of sage (*Salvia officinalis*) aqueous extract on mitotic index in albino male mice

تأثير المستخلص المائي لنبات الميرامية على معامل الانقسام الخلوي في خلايا نقي العظم في ذكور الفئران البيض

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Abstract

This study aimed to evaluate the effect of aqueous extract of sage (*Salvia officinalis*) on bone marrow cells in albino male mice by using three doses (83.9, 167.8 or 251.7 mg/kg) and cytosar drug at dose of (1.54 mg/kg). The results showed that sage has the ability to increase the mitotic index in mice in comparing with the negative control and in mice treated with cytosar drug that caused reduction in mitotic index. The results of pre- and post-treatment with the ideal dose of aqueous extract of sage and cytosar drug showed the ability of sage to increase the mitotic index of bone marrow cells in mice in comparing with the negative controls.

المستخلص

تم دراسة تأثير المستخلص المائي لنبات الميرامية على خلايا نقي العظم في ذكور الفئران البيض باستخدام ثلاثة تراكيز للمستخلص (83.9 , 167.8 او 251.7) ملغم/كغم و عقار الساييتوسار بتركيز 1.54 ملغم/كغم . أظهرت النتائج قابلية نبات الميرامية على زيادة معامل الانقسام الخلوي في الفئران بالمقارنة مع السيطرة السالبة وفي الفئران المعاملة بعقار الساييتوسار والذي سبب نقصان في معامل الانقسام الخلوي . أظهرت نتائج المعاملة قبل و البعد بالجرعة المثلى للمستخلص المائي لنبات الميرامية وعقار الساييتوسار قابلية نبات الميرامية على زيادة معامل الانقسام الخلوي في خلايا نقي العظم للفئران بالمقارنة مع السيطرات السالبة .

Introduction

Herbs are plants or plant parts that are valued for their medicinal and savory qualities. They contain and can produce a variety of chemical substances that have different biological effects, with a special reference to their medicinal importance. Therefore, they are employed by herbalists of different cultures, anciently and recently, to remedy peoples of their sicknesses [1]. Therefore, the list of drugs that are of a herbal origin is increased, and the World Health Organization (WHO) has established that out of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways

that correlate directly with their traditional uses as plant medicines by native peoples [2]. Numerous species of the genus *Salvia* have been employed since ancient times in folkloric medicine and subjected to an extensive pharmacognostic research to identify their biologically active compounds. Sage has a very long history of effective medicinal uses and is an important domestic herbal remedy for disorders of the digestive system. Its antiseptic qualities make it an effective gargle for the mouth where it can heal sore throats and ulcers. Leaves applied to an aching tooth will often relieve the pain. The whole herb is antihydrotic, antiseptic, antispasmodic, astringent, carminative, stimulant, tonic and vasodilator. Sage is also used internally in the treatment of excessive lactation, night sweats, excessive salivation (as in Parkinson's disease), profuse perspiration (as in tuberculosis), anxiety, depression, female sterility and menopausal problems [3]. Chemical analyses of sage revealed that the plant is rich in the following constituents:

Essential oils and Hydroxycinnamic acid derivatives: Principally, they are presented by (caffeic acid dimmer and rosmarinic acid) and Phenolic diterpenes, Carnosic acid, Tricyclic diterpene, Triterpenes, Phenolic glycosides, Flavonoids, Polysaccharides and other Constituents [4].

The aetiology of cancer is multifactorial and an interaction between genetic and environmental factors is required to initiate the disease [6]. The environmental factors are able to induce a mutation [5] in the genetic material of the cell, and such event may promote the cell to undergo a carcinogenic transformation [7]. Many studies that determine the activity of immune system and the effect of different agents on it depend on the ability of lymphocytes to proliferate in lymphoid organs and/or depend on dividing cells of bone marrow because it is the source of all blood cells [8]. Mitotic index assay is defined as the ratio of the numbers of cells in a population undergoing mitosis to the total numbers of cells [9].



Fig (1): The plant sage (*S. officinalis*) [5]

Materials and Methods

Preparation of Plant Extracts

Plant leaves were done according to [10]. Leaves were air-dried, and then powdered using a coffee grinder. Fifty grams of the leaf powder was extracted for three hours in 250 ml of the solvent (distilled water) using the soxhlet apparatus and water bath 45°C. The leaf extract solution was then evaporated at 45°C using a rotary evaporator and the resultant crude extract was frozen at -20°C until use.

Sage Doses and Concentrations

Aqueous plant extract was assessed in a mammalian model (albino mouse; *in vivo*). Three doses of sage extract were used (83.9, 167.8 or 251.7 mg/kg). These doses were correspondent to 10, 20 and 30%, respectively of the LD₅₀ dose in mice (83.9 mg/kg) of the same genus (*Saliva*) but for a different species (*S. libanotica*) [11].

Laboratory Animals

Albino Swiss male mice (*Mus musculus*) were used in the experiments. They were supplied by the Biotechnology Research Centre (Al-Nahrain University). Their age at the start of experiments was 8-10 weeks, and their weight was 23-27 gram. They were divided 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 a temperature of 23 – 25°C, and they had free excess to food (standard pellets) and water.

Animals Groups

First Stage

Effects of three doses (83.9, 167.8 and 251.7) mg/kg of sage extract (aqueous) and cytosar were investigated. Therefore, the animals were divided into three groups:

Group I: Animals which were treated with distilled water (negative controls = 8 animals).

Group II: Animals which were treated with cytosar at a dose of 1.54 mg/kg (positive controls = 8 animals).

Group III: Animals which were treated with three doses of the aqueous extract (83.9, 167.8 or 251.7) mg/kg (24 animals).

The tested materials were injected subcutaneously as a single dose (0.1 ml) per a day and for 7 days. Then the mice were sacrificed in day 8 for laboratory assessments.

Second Stage

In this stage, interactions (pre- and post-treatments) between the ideal dose of sage extract 83.9 mg/ kg and cytosar (1.54 mg/kg) were carried out.

In pre-treatment interaction, the plant extract (aqueous) was given for 6 days (single dose/day), while cytosar was given in day 7, and then animals were sacrificed in day 8 for laboratory assessments. The material was given subcutaneously.

In post-treatment interaction, the animals was given cytosar on day one, while the plant extract (aqueous) was given in day 2 till day7 (single dose/day), and then animals were sacrificed in day 8 for laboratory assessments. The material was given subcutaneously (0.1 ml).

Methods of Mitotic Index

Metaphase index was determined for cells obtained from bone marrow following the procedure of [12]. Each animal was injected intraperitoneally with 0.25 ml of colchicine solution, and after 1.5 – 2 hours, the animal was sacrificed by cervical dislocation and then dissected to obtain femur bone. The femur bone was cut from both ends, and its cellular contents were collected in a test tube using a disposable insulin syringe (1 ml) and normal physiological saline (5 ml). The cell of organ was manipulated as follows:

1. Cells were gently suspended using Pasteur pipette, and the tubes were centrifuged (2000 rpm/min) for 10 minutes
2. The supernatant was discarded, and the cell deposit was suspended in 5 ml of a warm 37°C hypotonic solution (KCl; 0.075 M). The tubes were incubated in a water bath 37°C for 30 min with a gentle shaking every 5 minutes.
3. The tubes were centrifuged at 2000 rpm/min for 10 minute, and the supernatant was discarded.
4. Five ml of the fixative solution was added a drop-wise to the cell deposit with a gentle and a continuous mixing to make a homogeneous cell suspension. Then, the tubes were incubated in the refrigerator (0°C) for 30 minutes.
5. The tubes were centrifuged (2000 rpm/min) for 10 minutes, and step 4 was repeated two times.
6. The cell deposit was well-suspended in 2 ml of the fixative, and 4-5 drops of the cell suspension were dropped on a clean slide from a height of about two feet.
7. 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.
8. The slide was examined under oil emersion lens (100X), and at least 1000 cells were examined. The percentage of metaphase cells (metaphase index) was recorded using the following equation:

$$\text{Metaphase Index} = \left(\frac{\text{Number of Metaphase Cells}}{\text{Total Count}} \right) \times 100$$

Results

Bone Marrow Cells

The metaphase index of bone marrow cells in distilled water negative control was 3.00%, and such index was significantly decreased (2.35%) when mice was treated with cytosar (positive control). Treatment of animals with the three doses of aqueous extract was

increased the percentage of metaphase index, especially the dose 251.7 mg/kg (5.80%), in which the treatment efficiency was 93.3%. Figure (2) Table (1).



Fig (2): Metaphase preparation of a bone marrow cell in mouse treated with the first dose (83.9 mg/kg) of sage's aqueous extract (100X).

Metaphase Index of Bone Marrow Cells after Interaction

The metaphase index of bone marrow cells was significantly increased as a consequence of pre-treatment (4.42 vs. 2.01%), as well as, post-treatment (3.63 vs. 1.37%) with the aqueous extract as compared to the corresponding controls. Table (2).

Table (1): Mitotic index of bone marrow cells (mean $\pm$ standard error) in albino male micetreated with aqueous extract of sage, distilled water and cytosar drug (positive control).

Groups		Dose (mg/kg)	Mean of mitotic index $\pm$ Standard Error (%)		Treatment Efficiency (%)
Sage Extract	Positive Control (Cytosar Drug)	1.54	2.35 $\pm$ 0.04	a	-21.7
	Negative Control (Distilled Water)	0.00	3.00 $\pm$ 0.14	b	
	Aqueous	83.9	3.43 $\pm$ 0.92	b	14.3
	Aqueous	167.8	3.84 $\pm$ 0.16	bc	28.0
	Aqueous	251.7	5.80 $\pm$ 0.15	c	93.3

Different letters in the same column: significant difference ($P \leq 0.05$) between means.

Table (2): Mitotic index of bone marrow cells (mean $\pm$ standard error) in albino male mice after interactions (pre- and post-treatments) between the ideal dose (83.9 mg/kg) of aqueous extrats of sage leaves and cytosar drug.

Groups	Mean of mitotic index $\pm$ Standard Error (%)		Treatment Efficiency (%)	
	Pre-treatment	Post-treatment	Pre-treatment	post-treatment
Control I (Distilled Water-Cytosar)	2.01 $\pm$ 0.16 a	1.37 $\pm$ 0.11 a		
Aqueous Plant Extract (Ideal Dose)-Cytosar	4.42 $\pm$ 0.16 b	3.63 $\pm$ 0.30 b	125.0	165.0

Different letters in the same column: significant difference ($P \leq 0.05$) between means.

Discussion

Plants are a source of different chemical compounds, which make them of a medicinal importance. These compounds are divided into two types (inert and active constituents) depending on their activity. The inert constituents are defined as compounds that have no medicinal or physiological effects; for instance cellulose, lignin and subrine, while active constituents have these effects. The active constituents in turn are divided into other types (alkaloids, tannins, carbohydrates, volatile oils, saponines, steroids and flavonoids) depending on their chemical and physical characteristics [13] and the chemical analysis of sage (*S. officinalis*) extract (aqueous) revealed some of these constituents (steroids, tanins, glycosides, flavonoids, saponines and terpens). A water-soluble polysaccharides complex from *S.officinalis* composed of galactose, glucose, mannose, xylose, and fructose have shown an immunomodulatory activity in the comitogenic thymocyte test which is interpreted as being an *in vitro* correlate of adjuvant activity in addition to their mitogenic activity [14]. These polysaccharides from *S.officinalis* have previously shown to stimulate the immune function of bone marrow cells and in this regard several European demostic herbs have been analysed biologically for active polysaccharides components and reported that these polysaccharides are good modulators of the immune system (anti-cancer, anti-inflamantory, anti-ulcer, complement activating potency, macrophage phagocytosis stimulation and induction of cytokines) [15]. These augmentations of humoral and cellular immune responses involve mainly four immune cells (neurophils, macrophages and T- and B-lymphocytes), and the effect of sage extracts on these cells numerically or functionally can be explained in the light of active constituents that act either separately or synergistically in enhancing the responsiveness of these cells directly or indirectly [16].

The study also demonstrated that the anti-leukemia drug cytosar which caused reduction of metaphase index, and such findings suggest that such drug is a mutagen, as with other anti-cancer drugs. In this regard, [17] have demonstrated that anticancer drugs may cause abnormalities in lymphocyte receptors involved in mitogen recognition. Such effect may result in an inhibition of blastogenic index, mitotic index, and increase micronucleus formation and chromosomal aberrations. The action of these drugs may act on the repair systems inside the cells, and as a result cells loss the ability to repair the damaged DNA [18].

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