History of Medicine, 2023, 9(1): 1497–1501
DOI: 10.17720/2409-5834.v9.1.2023.181

Evaluation of the influence of the vitex negundo ethyl acetate fraction on the frequency of micronuclei and the mitotic index in mice

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Received: 20 January 2023 **Accepted:** 15 April 2023

Citation: jabber HM, Mutlag SH (2023) Evaluation of the influence of the vitex negundo ethyl acetate fraction on the frequency of micronuclei and the mitotic index in mice. History of Medicine 9(1): 1497−1501. https://doi.org/10.17720/2409-5834.v9.1.2023.181

Abstract

Vitex negundo L. belongs to the family verbenaceae. it has been reported to possess Anti-inflammatory, analgesic and anti-rheumatic activity, Antioxidant activity, Enzyme inhibitory activity,Antimicrobial Antinociceptive activity Anticonvulsant Antifungal activity Anti-tumor and anticancer activity Antidiabetic activity: Laxative. Hepatoprotective activity The present study designed to evaluate the genotoxicy of ethyl acetate fraction of vitex negundo , extract administered orally, in two different doses [100mg/kg and 200mg/kg] on both bone marrow and spleen cells in mice for seven successive days, and comparing their effects with methotrexate (positive control) and dimethylsulfoxide [DMSO](negative control). The results have been showed that ethyl acetate fraction of vitex negundo at a dose 100mg/kg and 200mg/kg showed a significant increase of mitotic index in bone marrow cells and spleen cells in comparison with DMSO, meanwhile it showed a significant decrease of micronucleus appearance in bone marrow cells, in conclusion ethyl acetate fraction of vitex negundo showed protective effect on mitotic index and micronucleus appearance in bone marrow cells and spleen cells in mice.

Keywords:

vitex negundo, Ethyl acetate, Mitotic index, micronucleus appearance

Most cells grow, perform the activities needed to survive, and divide to create new cells. These basic processes, known collectively as the cell cycle, are repeated throughout the life of a cell(1) .The cell division cycle plays a crucial role in the growth, development, repair and reproduction of living organisms in both normal and pathological conditions. Progression through the cell cycle requires faithful replication of the genome during S phase and equal partitioning of the replicated chromosomes to the two daughter cells during mitosis and cell division(2) The typical cell cycle in eukaryotes is composed of four phases including the G1, S, G2, and M phase. G1, S, and G2 together are called interphase. M phase is comprised of mitosis, in which the cell's nucleus divides, and cytokinesis, in which the cell's cytoplasm divides to form two daughter cells. Mitosis and cytokinesis are tightly coupled together. Mitosis is further divided into five subphases including prophase, prometaphase, metaphase, anaphase, and telophase. (3).

For many years, exogenous sources of damage have been thought to be the primary cause of DNA mutations leading to cancer. However, a study achieved on 2001 proposed that endogenous sources of DNA damage also contribute significantly to mutations that lead to malignancy as well as endogenous sources of DNA damage also contribute significantly to mutations that lead to malignancy (4). Mitotic index is a measure for the proliferation status of a cell population, and defined as the ratio between the number of cells in mitosis and the total number of

cells. The purpose of the mitotic index is to measure cellular proliferation (5) . The mitotic index is an important prognostic factor predicting both overall survival and response to chemotherapy in most types of cancer (6)

An elevated mitotic index indicates more cells are dividing and thus obvious in cancer cells, the mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals, as well as cellular repair the sire of an injury (7)

The decrease in the MI considered a delay in the cell proliferation kinetics and it may be suspected to be due to the cytotoxicity or genotoxicity of the drug at specific dose on mitosis (8) or by other different mechanisms for example by the effects of the drug on tubulin polymerization or due to the effects on tubulin associated proteins (9).

A micronucleus assay is an assay used in toxicological screening for potential genotoxic compounds (10). The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens (11). There are two major versions of this test, one in vivo and the other in vitro (12).

The in vivo test normally uses mouse bone marrow or mouse peripheral blood. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise anucleated cytoplasm (13). plants are the basic source of life of all the living organisms in the world. They produce a wide range of secondary metabolites like alkaloids, fatty acids, flavonoids, phenols, tannins, sterols and terpenes that can be used to treat different chronic and infectious diseases [14].

Vitex negundo belongs to family Verbenaceae and grows as small tree with thin grey bark. The plant is widely distributed and also has pharmacological actions against wide spectrum of diseases in traditional system of medicines. All parts of the plant especially its leaves contain numbers of secondary metabolites such as alkaloids, phenols, flavonoids, glycosidic irridoids, tannins and terpenes [15]. Because of the richness in phytochemicals, the plant is attributed to possess a number of therapeutic uses; antimicrobial, anti-inflammatory [16], anticancer and hepatoprotective [16] It is also used as insecticide and larvicidal [17]. Leaf extract is employed as. Anticonvulsant activity [19] laxative effect [20] Anti diabetic activity [21] CNC depressant [22].

Materials And Methods

Plant material

The plant had been collected from Baghdad in april, from alzawra public garden, washed thoroughly, chapped into bits, and allowed to dry under shade. The dried plant was blended into fine powder using electric blender.

Preparation of extract

Five hundred grams of the powdered plant was defatted by maceration in 1500 ml of hexane for 24 hours with occasional agitation then filtered. The defatted plant materials were dried introduced in a thimble and extracted using soxhlet apparatus using 1500ml of ethyl acetate (B.p.40-60 °C) for 15 hours then cooled, filtered and evaporated under reduced pressure at 40 °C using rotary evaporator [23].

The yield values for ethyl acetate fraction have been obtained.

Experimental model

Twenty four Albino Swiss mice (Mus musculs) were used for each experiment. They were supplied by collage of pharmacy −university of baghdad. Their weights were 20-25 gram. They were divided into four groups; each was kept in a separate plastic cage. The animals were maintained at a temperature of 23 − 25°C, and they had free excess to food (standard pellets) and water.

The animal divided into 4 groups as below:

Group1: six mice were treated with dimethylsulfoxide (DMSO). This group was served as negative control the dose was given (I.P.) daily for seven successive days.

Group2: six mice were treated with a single dose (20mg/kg) of methotrexate (MTX). This group was served as positive control.

Group3: six mice were treated (oral) with (100mg/kg) of ethyl acetate extract of vitex negundo for seven successive days.

Group4: six mice were treated (oral) with (200mg/kg) of ethyl acetate extract of vitex negundo for seven successive days.

Mice were sacrificed by (spinal dislocation). Samples of bone marrow cells and spleen cells were taken and genotoxic analyses were carried out as described latter.

Phytochemical Investigation

Preliminary phytochemical investigation was carried out for ethyl acetate fraction using, 5% KOH Test/flavonoids ,1 % Lead actate test/tannins, Dragendorff Test/alkaloids, Vaniline/H2SO4 Test/steroidal, Ferric chloride Test/phenolic compound Benedict test glycoside

Chemical Test	Ethyl acetate Fraction
5% KOH Test/flavonoids	Positive+
1 % Lead actate test/tannins	Positive++
Dragendorff Test/alkaloids	Positive+
Vaniline/H2SO4 Test/steroidal	Positive+
Ferric chloride Test/phenolic compound	Positive+
Benedict test glycoside	Positive ++

Evaluation of mitotic index in Bone marrow cells and spleen cells

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicines, and then two hours later they are scarified by cervical dislocation. Bone marrow samples was aspirated from the femur bone and spleen cells have been collected processed using aseptic technique for evaluation of mitotic index as previously reported elsewhere[24].

Evaluation of micronucleus assay in Bone marrow cells

After chemical treatment, mice were killed and femoral marrow cells were smeared on clean glass slides, fixed with methanol for 5 min at room temperature, and stained with Giemsa [25]

Statistical Analysis

Data are expressed as Mean ± SD; unless otherwise indicated, statistical analyses were performed using unpaired t-test. If the overall F value was found statistically significant ($P<0.05$), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

Results And Discussion

Phytochemical Investigations

Phytochemical investigations revealed the presence flavonoids,tannins, alkaloids ,steroidal, phenolic compound and glycoside compounds. The yield value was (34 gram) of ethyl acetate extract

Mitotic index and micronucleus appearance of different concentrations of ethyl acetate fraction of vitex negundo In table 1 shows that, ethyl acetate extract of vitex negundo at both doses 100mg/kg and 200mg/kg caused increase of mitotic index in both bone marrow cells and spleen cells when compared to negative control (DMSO) ($P<0.05$), which is a parameter that give indication about cell division when compared to negative [DMSO], as well as , these two doses showincrese in mitotic index in both bone marrow cells and spleen cells when compared to positive control ($P<0.05$). Methotrexate caused significant decrease ($P<0.05$) of mitotic index compare to negative control and extract in bone marrow and spleen cell.

In Table 2, shows that, ethyl acetate extract of vitex negundo at both doses caused significant decrease in micronucleus appearance in bone marrow cells when compared to negative control (DMSO) ($P<0.05$). Methotrexate caused significant increase ($P<0.05$) in micronucleus appearance compare to negative control and extract in bone marrow.

Table 1: Incidence of mitotic index in bone marrow and spleen cells of albino mice treated with different doses of the ethyl acetate extract of vitex negundo compared to methotrexate and dimethylsulfoxide

Treatment Groups	Mitotic Index	
	Bone Marrow Cells	Spleen Cells
Dimethylsulfoxide (DMSO) (Negative control)	5.884±0.578	4.354±0.488
Methotrexate (MTX) (positive control) 20mg/kg	2.656±0.36*a	1.897±0.36*a
Ethyl acetate extract 100mg/kg	6.51± 0.0.618 Aa	5.027±0.575*Ab
Ethyl acetate extract 200mg/kg	6.706±0.435* Ab	5.099±0.434*Ab

Table 2: Incidence of micronucleus appearance in bone marrow cells of albino mice treated with different doses of the ethyl acetate extract of vitex negundo compared to methotrexate and dimethylsulfoxide figure 1

Micronucleous appearance	
Treatment group	Bone Marrow Cells
Dimethylsulfoxide (DMSO) (Negative control)	6.454±0.437
Methotrexate (MTX) (positive control) 20mg/kg	28.75±3.37*a
Ethyl acetate extract 100mg/kg	5.736± 0.534 Ba
Ethyl acetate extract 200mg/kg	5.35±0.606 B a

For table 1 and table 2 Data are expressed as mean±S.D; n=6 animals in each group; -*significantly different compared to DMSO (negative control) (P<0.05); -Values with non-identical small letters superscripts (a,b) consider significant different when compared between groups (P<0.05); - Values with non-identical capital letters superscripts (A,B) consider significant different when compared among tests doses (P<0.05).

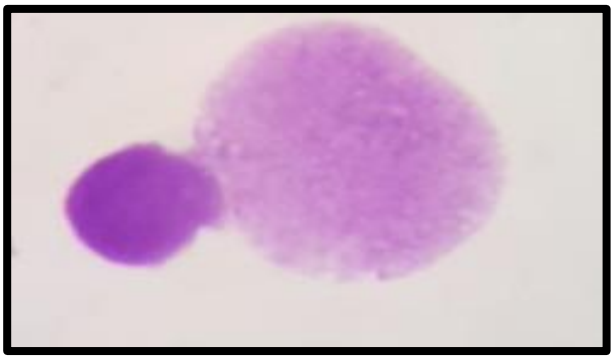

Figure 1: Micronucleus appeared after treatment with ethyl acetate extract of vitex negundo

In Tables 1 and 2 indicate that, methotrexate effects on mitotic index cells was significantly lower when compared to ethyl acetate extract in both doses 100mg/kg and 200 mg/kg. Meanwhile they showed significant increase in micronucleus appearance in both doses as compare to extract.

Regarding to the results obtained the explanations that ethyl acetate fraction of vitex negundo contain poly phenolic compounds ,Polyphenols are a heterogeneous group of secondary metabolites[26]. They have in common the presence in their structure of one or more phenol groups It can be divided in flavonoids and non-flavonoids, such as coumarins and simple phenols as phenolic acid like 4-parahydroxy benzoic acid where the it contain 3.25 ug/ml which is more than the methanolic ectraxt [27].

Phenolic compounds especially flavonoids have a notable antioxidant and free radicals scavenging activities [28]

Also the ahtyl acetate extrac contain Two new chromone derivatives methyl 3-(2-(5-hydroxy- 6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoic acid were isolated from V. negundo these two chromones ameliorated the irritant-induced nociceptive behavior and paw edema, therefore suggestive of analgesic and anti-inflammatory propensities by interaction with cyclooxygenases [29] .

One of the most important constituent of vitex negundo is vitixin with their derivatives such as isovitexin, rhamnopyranosyl-vitexin, methylvitexin (isoembigenin), vitexin-2-O-rhamnoside (VOR), and vitexin-2-O-xyloside

Vitexin has been proven capable of donating electrons and has acted as a good radical scavenger. It has a better antioxidant activity than apigenin, since the presence of C-8 glucoside in vitexin causes a reduction of its bond dissociation enthalpy compared to aglycone apigenin. The most stable radical order of vitexin after reaction with reactive oxygen species (ROS) was reported as 4′-OH, 7-OH, and 5-OH, respectively[30] .

Also vitixin increase cell viability of PC-12 cells against neurotoxicity of isoflurane and reduce inflammatory cytokines (TNF-α, Il-6) and ROS and increase glutathione (GSH) and superoxide dismutase (SOD). Vitexin also reduced apoptosis in both PC-12 cells and hippocampus neurons and increased expression mir-409 in both models. Vitexin has protective effects against oxidative stress and inflammation induced by isoflurane and the underlying mechanism is probably through activation AMPK/GSK3β signaling pathway (30). β- Sitosterol is one of phytosterols (plant sterols) with chemical structures similar to that of cholesterol. They are hydrophobic solvents like chloroform and soluble in alcohols. Several studies have been focus on the effect of this compound on chromosomal integrity. It has been found that β-sitosterol has anti-clastogenic effect on chromosome, several studies shown that; β-sitosterol has anti-oxidant effect by working as free radical scavenger [31]

Conclusion

The Ethyl acetate fraction of vitex negundo L showed protective effect on mitotic index and micronucleus appearance in bone marrow and spleen cells in mice.

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