



Evaluation the possible Genotoxic Effect of Vitex Negundo Ethyl Acetate Fraction on Chromosomal Aberration in Mouse Spleen and Bone Marrow Cell

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ABSTRACT

Chromosome aberrations have long been recognized to be an important biomarker of human exposure to ionising radiation and genotoxic chemicals .many of plant in nature use for treatment of many diseases The present study is designed to evaluate the possible genotoxic effect of ethyl acetate fraction of vitex negundo (verbenaceae)on chromosomal integrity at two different doses (100mg/kg and 200mg/kg) on both bone marrow cells and spleen cells in mice for seven successive days, the results was compare their effects with methotrexate which was used as a positive control. The results have been showed that ethyl acetate fraction of vitex negundo at both doses showed a significant decrease in individual and total chromosomal aberration when compared with negative control (dimethylsulfoxide).

Keywords: *vitex negundo*, *bone marrow cells*, *spleen cells*, *ethyl acetate fraction*

INTRODUCTION

The use of plant products for the treatment, cure, and prevention of general disorders is one of the earliest forms of medical practice, and probably almost as old as the human species(1). Safety and toxicity studies of plants used in therapy are vital, due to its considerable range of applications and its widespread use in folk medicine, which, according to often represent the only therapy of many communities and ethnical groups(2).

Vitex negundo Linn. (Verbenaceae), commonly known as Chinese chaste tree, is a perennial plant that has great traditional medicinal values in different nations,(3) . It is traditionally used in parts of the Indian subcontinent to cure headache, fever, cold and cough (4).

It finds use as an antidote against snake venom (3). The plant has anti-hyperglycemic potential , hepatoprotective effects against liver damage induced by CCl₄ ,estrogen-like activity, antibacterial, antipyretic and antihistaminic agents (5) Its leaves are used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue(6) Phytochemical studies on *Vitex negundo* Linn revealed the presence of volatile oil, triterpenes, diterpenes, sesquiterpenes, lignan, flavonoids, flavones, glycosides, iridoid glycosides and stilbene derivative and the leave contain various chemical constituents as Friedelin, vitamin-C,

carotene, casticin, artemetin, terpinen-4-ol, α -terpineol, sabinene, globulol, spathulenol, β -farnesene, farnesol, bis (1,1dimethyl) methylphenol, α -pinene, β -pinene, linalool, terpinyl acetate, caryophyllene epoxide, caryophyllenol, vitexicarpin, viridiflorol, 4,4'-dimethoxy-trans-stilbene, 5,6,7,8,3',4',5'-heptamethoxy, 5-hydroxy-6,7,8,3',4'-pentamethoxy (5-Odesmethylnobiletin), 5-hydroxy-6,7,8,3',4',5'-hexamethoxy (gardeninA), 5-hydroxy-6,7,8,4'- tetramethoxy (gardeninB), 5-hydroxy-7,3',4',5'- tetramethoxyflavone (corymbosin), terpinen-4-ol, α -copaene, β -caryophyllene, β -elemene, camphene, α -thujene, α -pinene, sabinene, linalool, stearic acid and behenic acid (8), α - elemene, δ - elemene, β -elemene, β -eudesmol, camphor, camphene, careen, 1,8- cineol, 1-oceten-3-ol, γ - terpinine, α - phellendrene, β - phellendrene, α - guaiene, abieta-7,13-diene, neral, geranial, bornyl acetate, nerolidol, β - bisabolol, cedrol, 2'-p-hydroxybenzoyl mussaenosidic acid, agnuside, lagundinin, aucubin and nishindaside, viridiflorol, squalene, 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone, 5-hydroxy- 3,7,3',4' tetramethoxy flavones, 5,3-dihydroxy- 7,8,4-trimethoxy flavanone, p-hydroxybenzoic acid, 3,4 - dihydroxybenzoic acid, luteolin-7-glucoside, isoorientin, 3'- benzoyloxyhydroxy-3,6,7,4- tetramethoxyflavone, 5,3'-dibenzoyloxy-3,6,7,4- teramethoxyflavone, 5,3'-Dipropanyloxy-3,6,7,4'- tetramethoxyflavone, 5,3- Dibutanoyloxy3,6,7,4-tetramethoxyflavone, 5,3' Dipenty4enoyloxy-3,6,7,4' tetramethoxyflavone, 5,3- Dihexanoyl 3,6,7,4- tetramethoxyflavone, betulinic acid, ursolic acid, dimethoxyflavone, 5,3'-dihydroxy-7,8,4'- trimethoxyflavone, 7,8-Dimehylherbacetin-3-rhamnoside, vitegnoside, 1,4a,5,7a tetrahydro 1 β Dglucosyl (3',4'dihydroxybenzoyloxymethyl)-5-ketocyclopenta[c] pyran-4-carboxylic acid, luteolin-7-O- β -D-glucosid[28], 6'- phydroxy benzoylmussaenosidic acid(8).

Since chromosomes are a key component of the genetic code, any damage to a healthy cell's chromosome could have disastrous results, such as the development of cancer or a variety of heritable diseases, if the damage is not repaired.

Over a long period of time, a species' chromosome set remains largely constant. In fact, abnormalities involving the structure or number of chromosomes can be found within populations(9). These changes come about on their own as a result of mistakes in the cell's regular functions. In most cases, their effects are negative, leading to the birth of unwell or sterile people, but in a few rare instances, alterations offer fresh opportunities for adaptation that enable evolutionary change to take place. Chromosome structural rearrangements do happen, albeit less frequently, during the normal course of life. but, under the effect of mutagenic agents such as radiations and conditions of stress like very high temperature(10), chromosomes are more prone to these changes. Gene toxicity evaluation considered now a day is the most important protocol to evaluate the safety of the chemical compounds that have been used and to measure the ability of these compounds to reduce cancerous mass with minimum side effects on healthy cells(11). During normal course of life cycle, structural rearrangements in chromosomes do occur but less frequently but, under the effect of mutagenic agents such as radiations and conditions of stress like very high temperature, chromosomes are more prone to these changes(12)

The present study is designed to evaluate genotoxic effect of ethyl acetate fraction of vitex negundo (verbenaceae) on chromosomal integrity at two different doses (100mg/kg and 200mg/kg) on both bone marrow cells and spleen cells in mice for seven successive days

MATERIALS AND METHODS

Plant material

The plant had been collected from Baghdad alzawra public garden in August, washed thoroughly, chopped 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 (500gm) of the powdered plant was defatted by maceration in 1500 ml of hexane for 24hrs with occasional agitation then

filtered. the defatted plant materials was dried introduced in a thimble and extracted using soxhlet apparatus with 1500ml of ethyl acetate solvent (B.p.40-60oC) for 15 hours then cooled, filtered and evaporated under reduced pressure at 40 Co using rotary evaporator(13). The yield value for ethyl acetate fraction was calculated after evaporation of solvent.

Experimental model

Twenty four Albino Swiss mice (*Mus musculus*) were used for each experiment. They were supplied by National Center for Drug Control and Research. 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 (ad libitum). The animals were divided into four groups (six mice of each) as follow:

Group 1: Mice were treated with dimethylsulfoxide. This group was served as negative control the dose was given (I.P.) daily for seven successive days.

Group 2: Mice were treated with a single dose (20mg/kg) of methotrexate. This group was served as positive control.

Group 3: Mice were treated (oral) with (100mg/kg) of ethyl acetate extract of vitex negundo for seven successive days.

Group 4: 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

Evaluation of Genotoxicity in Bone marrow

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 processed using aseptic technique for evaluation of mitotic index and total chromosomal aberration as previously reported elsewhere.

Statistical Analysis

Data are expressed as Mean \pm 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

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

TABLE 1: Incidence of individual and total chromosomal aberration in bone marrow cell of albino mice treated with different doses of the ethyl acetate extract of vitex negundo compared to methotrexate and dimethylsulfoxide

Parameter BM Groups	Deletion % (mean±SD)	Dicentric % (mean±SD)	Acentric % (mean±SD)	Ring % (mean±SD)	Chromosome break (mean±SD)	Chromatid break (mean±SD)	Chromosome Gap (mean±SD)	Chromatid Gap (mean±SD)	Total % (mean±SD)
Control	AB 0.2335± 0.1415	AB 0.2553± 0.1201	AB 0.2489± 0.0982	BC 0.2594± 0.1289	AB 0.1462± 0.1174	AB 0.1423± 0.0921	B 0.1721± 0.1263	A 0.1721± 0.1263	B 1.5672± 0.2117
MTX	A 0.3127± 0.1309	A 0.3279± 0.0997	A 0.3442± 0.1421	A 0.4551± 0.1312	A 0.2721± 0.1172	A 0.2751± 0.1252	A 0.3397± 0.1465	A 0.1856± 0.1411	A 2.387± 0.229
Ethyl Acetate 100	AB 0.1684± 0.1252	AB 0.2069± 0.1462	ABC 0.2017± 0.1342	B 0.3025± 0.0682	AB 0.1657± 0.1159	B 0.1003± 0.0917	AB 0.2369± 0.0924	A 0.2369± 0.0924	B 1.486± 0.359
Ethyl Acetate 200	B 0.1253± 0.1281	AB 0.2309± 0.0929	BC 0.1336± 0.1412	BC 0.2292± 0.0859	AB 0.2309± 0.0929	B 0.1319± 0.1325	AB 0.2347± 0.1049	A 0.2347± 0.1049	B 1.4183± 0.2062

in table (1), ethyl acetate fraction of vitex negundo at a dose 100mg/kg and 200mg/kg showed non significant decrease in total chromosomal aberration, ring chromosome, deletion, acentric and chromatid break in spleen cells when compared to negative control ($p < 0.05$), other individual chromosomal aberration show not significant increase in spleen cells when compared to negative control ($p > 0.05$) at the same time ethyl acetate fraction of vitex negundo at a dose 100mg/kg and 200mg/kg showed significant decrease in total chromosomal aberration, chromatid break, chromatid gap, acentric chromosome, dicentric chromosome deletion and ring chromosomes in spleen cells when compared to positive control ($p < 0.05$), other types of individual chromosomal aberration showed not significant decrease in

table (2), ethyl acetate fraction of vitex negundo at a dose 100mg/kg and 200mg/kg showed non significant increase in total chromosomal aberration, ring chromosome, deletion, acentric, chromosome gap, chromatid gap and chromatid break in spleen cells when compared to negative control ($p < 0.05$), other individual chromosomal aberration show not significant decrease in spleen cells when compared to negative control ($p > 0.05$) at the same time ethyl acetate fraction of vitex negundo at a dose 100mg/kg and 200mg/kg showed significant decrease in total chromosomal aberration, chromatid break, chromatid gap, acentric chromosome, dicentric chromosome deletion and ring chromosomes in spleen cells when compared to positive control ($p < 0.05$), other types of individual chromosomal aberration showed not significant decrease

TABLE 2: Incidence of individual and total chromosomal aberration in spleen of albino mice treated with different doses of the ethyl acetate extract of vitex negundo compared to methotrexate and dimethylsulfoxide

Parameter Spleen Groups	Deletion % (mean±SD)	Dicentric % (mean±SD)	Acentric % (mean±SD)	Ring % (mean±SD)	Chromosome break (mean±SD)	Chromatid break (mean±SD)	Chromosome Gap (mean±SD)	Chromatid Gap (mean±SD)	Total % (mean±SD)
Control	AB 0.1439± 0.1049	AB 0.199±0 .0726	AB 0.195±0 .1279	B 0.2284± 0.0759	AB 0.1731± 0.069	B 0.0814± 0.0746	ABC 0.2322± 0.0897	AB 0.2322± 0.0897	B 1.367±0 .323
MTX	A 0.2715± 0.1063	A 0.3352± 0.1176	A 0.3057± 0.1013	A 0.4289± 0.1154	A 0.3128± 0.1131	A 0.2768± 0.126	A 0.3371± 0.1141	A 0.3371± 0.1141	A 2.545±0 .242
Ethyl Acetate 100	AB 0.2041± 0.0719	AB 0.2103± 0.1541	AB 0.1996± 0.1362	B 0.2731± 0.094	B 0.1368± 0.1476	AB 0.1683± 0.117	ABC 0.2401± 0.0979	AB 0.2401± 0.0979	B 1.6401± 0.1631
Ethyl Acetate 200	AB 0.1614± 0.1108	B 0.1322± 0.0748	B 0.1302± 0.0733	B 0.2263± 0.1429	AB 0.228±0 .0738	AB 0.2319± 0.0891	AB 0.268±0 .099	AB 0.268±0 .099	B 1.541±0 .442

We found in table 2 some type of chromosomal aberration increase when we increase the dose to 200 mg/kg as chromosome break, chromatid break, chromosome gap and chromatid gap while some type of chromosomal aberration decrease when we increase the dose as dicentric.

As we know vitex negundo contain many type of active compound as flavonoids, tannins, polyphenols, lignins, alkaloids glycoside, monoterpene, triterpenoid, and sesterterpenoid.

Polyphenols are a heterogeneous group of secondary metabolites. They have in common the presence in their structure of one or more phenol groups (14). It can be divided in flavonoids and non-flavonoids, such as coumarins and simple phenols. The presence of another type of active constituents like sterol and terpenoids according to the phytochemical study that done in the present study, these active constituents are reported to be antioxidant effects (15)

The ethyl acetate fraction contain simple phenols as phenolic acid like 4-parahydroxy benzoic acid where it contain 3.25 ug/ml which is more than the methanolic extract (16)

Also the ethyl acetate extract 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 (17).

One of the most important constituent of vitex negundo is vitexin 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 (18).

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/GSK3 β signaling pathway(18).

These results suggested that flavonoids not only the major contributor to the antioxidant effect of this plant, but there are other phytochemicals i.e. the non-flavonoidal compounds

such as caffeic acid, gallic acid, that reported in vitex negundo extract may contribute to this excellent activity since these compounds also exhibited a pronounced antioxidant effects(19)

The phytochemical screening revealed the existence of polyphenols as a major component in the extract, especially flavonoids and tannins, because of that the content of total phenols and flavonoids were determined. Phenolic compounds especially flavonoids have a notable antioxidant and free radicals scavenging activities. A structure activity relationship study of flavonoids such as quercetin indicates that the hydroxyl groups, the 3,4-catechol structure in the Bring, the 2-3 double bond and 4-oxo function which found in the quercetin structure are key factors for the antioxidant activity.(20)

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