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Research Article

HEMATOPOIETIC TOXICITY OF LORANTHUS EUROPAEUS: IN-VIVO STUDY

INTESAR T.NUMAN[1], MAHA N. HAMAD[2], ALI F. HASSAN[3]

[1]Department of Pharmacology & Toxicology, College of Pharmacy, University of Baghdad, Iraq,[2]Department of Pharmacognosy & Medicinal plants, College of pharmacy, University of Baghdad, Iraq,[3]Department of physiology and pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq , Email: ali_1371982@yahoo.com

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ABSTRACT

Iraqi species of mistletoe (*Loranthus europaeus*) have been used in the present study to evaluate genotoxicy of aqueous fraction in two doses (200mg/kg and 400mg/kg) on bone marrow cells in mice for seven successive days, comparing their effects with a (methotrexate). 200mg/kg of extract of L.europeaus significantly decrease mitotic index (P<0.05), while 400mg/kg none significantly increase this parameter compared to control group.

For chromosomal aberration, the aqueous fraction of L.europeaus (in both doses) produced significant increase at dose of 200mg/kg and none significantly increase at a dose 400mg/kg in this marker compared to control (P<0.05) Meanwhile, 20mg/kg methotrexate significantly increases chromosomal aberration compared to control. the extract at a dose 200 mg/kg showed significant decrease in total and differential white blood cells count when compared to water (negative control) (P<0.05); meanwhile increasing the dose to 400 mg/kg lead to none significant decrease in total and differential white blood cells when compared to water (P>0.05).

Keywords: genotoxicity, *loranthus europeaus*, mitotic index, total chromosomal aberration,

INTRODUCTION

Loranthus is a genus of parasitic plants that grow on the branches of woody trees. It belongs to the family Loranthaceae[1]. The European mistletoe is a green shrub with small, yellow flowers and white, sticky berries which are considered poisonous. It commonly seen on apple but only rarely on oak trees. Historically, mistletoe had been use in several cases like (swellings, tumours, epilepsy, hysteria, delirium, vertigo, antispasmodic, tonic and narcotic, diseases of spleen and liver, labour-pains, weakness of the heart' and oedema, eczema, ulcers of the feet, burns, and granulating wounds) [2]. Recent scientific research has confirmed the folklore with evidence that mistletoe extracts[1] induce apoptosis,[2] stimulate immunocompetent cells which slow the growth of cancer cells, and [3] protect the DNA of mononuclear cells[3]. Loranthus species are known to produce variety of bioactive compounds for examples kaempferol 3-*O*-α-D-rhamnoside, kaempferol 3, 7-di-*O*-β-D-glucoside, quercetin 3-*O*-α-D-rhamnoside and quercetin 3-*O*-β-D-glucoside from the leaves of L. kaoi and from L. europaeus [4].

Genotoxicity is a property possessed by some substances that makes them harmful to the genetic materials contained in organisms. A substance that has the property of genotoxicity is known as a *genotoxin*[5]. *Genotoxin may have* three primary effects on organisms, where they can be carcinogens (cancer-causing agents), mutagens (mutation-causing agents) or teratogens (birth defect-causing agents). Genotoxicity tests can be defined as *in vitro* and *in vivo* tests designed to detect compounds, which induce genetic damage directly or indirectly by various mechanisms. These tests should enable hazard identification with respect to damage to DNA and its fixation[6].

Chromosomal aberration can be defined as any change in either the normal structure of chromosome (called structural chromosomal aberration) or number of chromosomes (karyotype) called (numerical chromosomal aberration) [7].

Despite a relatively large variety of different kinds of chromosome aberrations each aberration usually results in one of three possible outcomes:

1. Reduction of chromosomal segments within a chromosome or between chromosomes that leads to a recombination of genes.
2. Loss of genetic material from the entire chromosome or part of it.
3. Gain of a whole chromosome(s) or a chromosomal segment[8].

Structural abnormalities could be formed by chromosomal breakage or unequal crossing over, translocations, insertions and inversions, duplications could be led to for examples deletions, ring chromosomes acentric chromosomes or dicentric chromosome [9]

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 mitotic index can be worked out from a slide, even with light microscopy [10]. There is a direct relationship between cancer and the value of mitotic index, cancer cells have higher mitotic index because they have a mutation in the DNA, and so they reproduce uncontrollably and therefore divide faster; accordingly, they are mentioned as having a higher mitotic index. In a normal lung tissue, the percent of cells dividing is 5% while in a cancerous lung the percent of cells dividing is 25%. [11]

MATERIAL AND METHODS

Plants collection

The plant was brought from the Iraqi market and authenticated by Dr Ali Al-Moussawy, Department of biology, College of Science, and University of Baghdad. A voucher sample was kept at the department of pharmacognosy & medicinal plants, College of pharmacy/ University of Baghdad. The plant fruits were air-dried at room temperature and crashed by mortar and pistol to be extracted by reflux apparatus.

Preparation of aqueous fraction

Air dried fruits were rendered into gummy powder by triturating in a mortar, then 100 gram of the powder was introduced in a soxhlet apparatus and extracted with 750 ml of 80% ethanol . Extraction was conducted for 10 hours then cooled & filtered. The extract was concentrated under vacuum until all the ethanol was removed , then diluted with 100 ml water & transferred to a separatory funnel & extracted successively with 100ml x 3 times of petroleum ether (B.P 60-80^{o}C) , then with 100ml x 3 times of chloroform , & finally with 100ml x 3 times of ethyl acetate. Each fraction was dried over anhydrous sodium sulfate , filtered & evaporated to dryness under vacuum. The aqueous fraction was filtered & evaporated to dryness under vacuum after combining it with equal volume of ethanol. The yielding values were (5.1 grams for chloroform fraction) (5.5 grams for ethyl acetate fraction) & (11,5 gram for aqueous fraction).[12]

Phytochemical Investigation

Preliminary phytochemical investigation was carried out for aqueous fraction using Meyer's reagent , Dragendorff's spray reagent and 5% ethanolic KOH spray reagent

Animals and treatment protocols

Twenty-four albino Swiss mice, weighing 23-27 g, were used in this study in accordance with the guidelines of the Biochemical and Research Ethical Committee at College of Pharmacy, University of Baghdad (Canadian Council on Animal Care guidelines). Animals were purchased from the animal house of Biotechnology Research Centre, Al-Nahrain University. They were housed for 2 days under standard conditions (well ventilated, temperature 22±2°C, relative humidity 50–60% and 12 h day and night cycle). Food consisted of normal animal chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed from eight to ten a.m. All the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee. The animals were allocated into 4 groups (6 mice in each) and treated as follow: First group treated with the vehicle (distilled water) and served as negative control; second group treated with metho trexate (20mg/kg) dissolved in water as intra peritoneally single dose; third and fourth groups are treated with 200 and 400mg/kg of dried aqueous fraction of *Loranthus europaeus* dissolved in distilled water respectively. Negative control group and test groups' treatments administered as intra peritoneally daily doses for seven consecutive days.

Evaluation of cytotoxicity in bone marrow and blood

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicine, and then two hrs 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[13]. Blood samples were collected directly from the heart in heparinized tubes and used for evaluation of total white blood cells and differential count using hemocytometer [14].

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 of flavonoids & absence of alkaloids in the aqueous fraction.

Gene toxicity of different concentrations of *L. europaeus aqueous* fraction.

In table (1),dose 200mg/kg of aqueous fraction of L.europaeus significantly decrease mitotic index ($P<0.05$), while a none significant increase in this parameter compared to control group was seen on increasing the dose to 400mg/kg, while results obtained with 20mg/kg methotrexate, showed significant decrease in mitotic index compared to a vehicle treated animals. Concerning the effect on chromosomal aberration, the aqueous fraction of L.europaeus (in both doses) produced significant increase at dose of 200mg/kg and none significantly increase at a dose 400mg/kg in this marker compared to control ($P<0.05$)(Table 1), (Figures 1 and 2). Meanwhile, 20mg/kg methotrexate significantly increases chromosomal aberration compared to control.

Table 1:Incidence of mitotic index and total chromosomal aberration in bone marrow of mice treated with methotrexate and different doses of aqueous fraction of *Loranthus europaeus*

Treatment groups	Mitotic index	Total chromosomal aberration
Water (negative control)	8.7±0.44	0.11±0.08
Methotrexate(MTX) 20mg/kg	$2.1\pm0.16^{*a}$	$0.31\pm0.2^{*a}$
Aqueous fraction 200mg/kg	$2.6\pm0.23^{*Aa}$	$0.21\pm0.2^{*Aa}$
Aqueous fraction 400mg/kg	8.8 ± 0.48^{Bb}	0.13 ± 0.1^{Ba}

Data for mitotic index are expressed as mean±S.D; *significantly different compared to negative control (P<0.05); values with non-identical superscripts (a,b) among treatment groups are significantly different (P<0.05), values with non-identical superscripts (A,B) between test groups are significantly different (P<0.05).

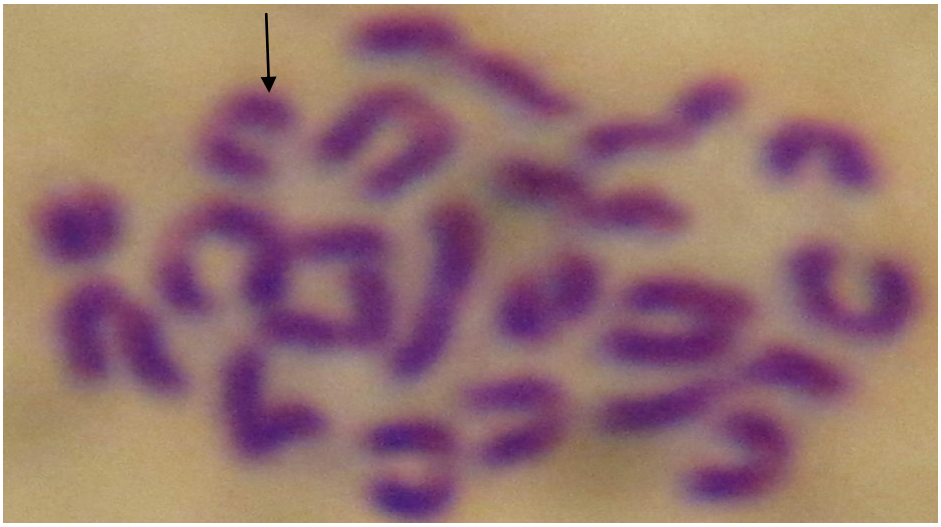

Figure 1:Chromosomal aberration (ring chromosome) in bone marrow of mice treated with *Loranthus europaeus* aqueous fraction

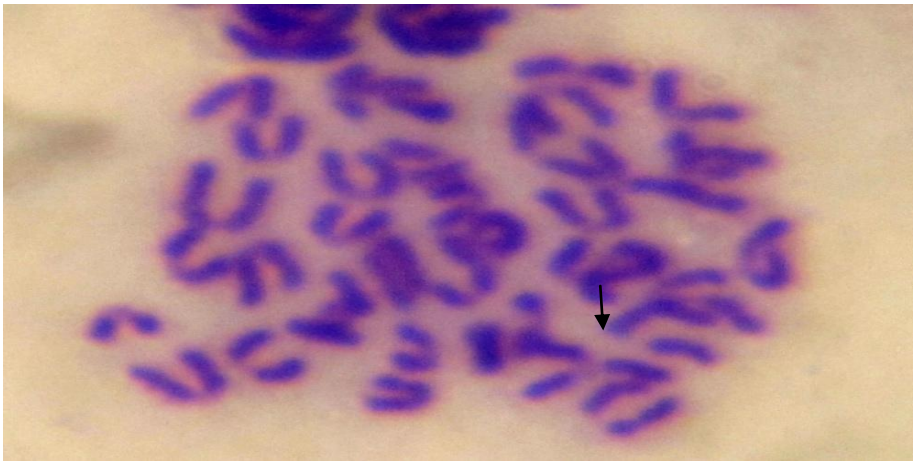

Figure 2:Chromosomal aberration (chromosome breaks) in bone marrow of mice treated with *Loranthus europaeus* aqueous fraction

In table (2), aqueous fraction of *Loranthus europaeus* at a dose 200 mg/kg showed significant decrease in total and differential white blood cells count when compared to water (negative control) (P<0.05); meanwhile increasing the dose of aqueous fraction of *Loranthus europaeus* to 400 mg/kg lead to none significant decrease in total and differential white blood cells when compared to water (P>0.05). Both doses of *Loranthus europaeus* showed to be significantly higher when compare to methotrexate (positive control).

Table 2:Total and differential white blood cells count in mice treated with methotrexate or different doses of aqueous fraction of *Loranthus europaeus*.

Treatment groups	Total WBC count(Cell ×10³/mm³)	Lymphocyte count (Cell/mm³)	Neutrophil Count (Cell/mm³)	monocyte Count (Cell/mm³)
Distilled Water (negative control)	106.8±2.3	5572.4± 141	4661±467	319.4±70.1
Methotrexate 20mg/kg	51.8±3.3^{*a}	2623.2± 281.3^{a}	2302.4±241.2^{*a}	152.2±65.4^{*a}
Aqueous fraction (200mg/kg)	76.6±4.66^{*Ab}	4036.2±259.1^{*Ab}	3187.2±219^{*Ab}	145.2±160.7^{*Aa}
Aqueous fraction (400mg/kg)	102.8±5.4^{Bb}	5596.4±630.3^{*Bb}	4356.8±503.9^{Bb}	301±4.3^{Bb}

Data are expressed as mean ± SD; n=6 animals in each group; *significant different compared to negative control (P<0.05); values with non-identical super scribes (a,b) among treatment groups are considered significantly different (P<0.05) values with non-identical superscripts (A,B) between test groups are significantly different (P<0.05).

DISCUSSION

Phyotochemical investigation showed that this fraction contain several types of flavonoids. The flavonoids have properties including antioxidant, anti-mutagenic, anti-oestrogenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of the genome. However not all flavonoids and not all actions of individual flavonoids are necessarily beneficial. Some have mutagenic and/or pro-oxidant effects, as well as interfering with essential biochemical pathways including topoisomerase enzyme activities, prostanoid biosynthesis and signal transduction [15]. Alkaloids didn't detect in aqueous fraction of L.europaeus.

In the present study, aqueous fraction of *Loranthus europaeus* at a dose (200mg/kg) showed significant increase in total chromosomal aberration when compared with negative control . These effects were reflected on cell mitosis (significant decrease in mitotic index when compared with negative control). It has been reported that any damage to genetic material lead to activation of DNA repair system and/or activation of apoptosis mechanism [16]. On the other hand any compound interfere with DNA synthesis by different mechanism lead to decrease in DNA synthesis process which occur in S phase in cell cycle and the repair of the damaged DNA on G_2 phase this lead to decrease in cell division and proliferation [17].

The significant decrease in mitotic index at a dose 200mg/kg for aqueous fraction of L.europaeus could be pronounced in total white blood cells counts which is also significantly decrease when compared with negative control . The same situation could be seen in lymphocytes counts, neutrophil counts and monocytes counts which significantly decreased when compared with negative control. When a dose of aqueous fraction of *Loranthus europeaus* have been increased to 400mg/kg more flavonoids have been introduce to each animal. Flavonoids reported to have anti-clastogenic effect, and protect against the chromosomes clastogenic compounds, several studies have been done with different types of flavonoids and found that they significantly decrease total chromosomal aberration [18]. According to this anti-clastogenic effect of flovonoid lead to non significant decrease in total chromosomal aberration when compared to negative control and significantly lower when compared to lower dose (200mg/kg). Also the anti-clastogenic effect of flavonoids leads to restore bone marrow cells division and this could be seen obviously in mitotic index in which there were no significant changes when compared to negative control.

The total white blood cells counts of aqueous fraction of *Loranthus europaeus* at a dose (400mg/kg) showed no significant decrease when compared to negative control (p >0.05) .

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