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Research Article

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HEMATOPOIETIC TOXICITY OF *Loranthus europaeus* CHLOROFORM EXTRACT: *In-vivo* STUDY

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ABSTRACT

Methanolic extract of some mistletoe species exhibited moderate cytotoxicity against cultured human tumor cell line *in-vitro*. However, the genetic toxicity of *Loranthus europaeus* is not well defined. In this study, chloroform extract of *L. europaeus* was prepared and used for the investigation of the genotoxic effects of different doses, administered orally, on bone marrow and peripheral blood cells of mice. Results showed that treatment with large dose of the extract decreases the mitotic index and increase chromosomal aberration compared to vehicle treated animals and comparable to that produced by methotrexate. The high dose of the extract also significantly decreases the total and differential WBC count compared to both the lower dose and methotretxate. In conclusion, the chloroform extract of *L. europaeus* shows pronounced gene toxicity with increasing the dose when administered orally to mice.

Keywords: Mistletoe, gene toxicity, bone marrow, mice.

INTRODUCTION

Mistletoe is a semi-parasitic evergreen plant belongs to the family Loranthaceae, found growing on a host of evergreen and deciduous trees all year round, around the branches of the tree. It is an obligate parasite, obtaining part of its food from the host plant. It depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leathery, oblong leaves.[1] In many parts of the world, especially in Nigeria and some other parts of Africa, mistletoe has been used traditionally as antihypertensive and antidiabetic.[2,3] There have been reports on the phytochemical and antimicrobial properties of African mistletoe *Loranthus micranthus*.[4] However, information is scanty on the effects of the plant on biochemical parameters in experimental animals and on the possible risks associated with consumption of mistletoe extracts. Loranthus species in semi-parasitic plants are known to produce variety of bioactive compounds, including sesquiterpene lactones from *Loranthus parasiticus* that traditionally used for the treatment of schizophrenia[5] and (+)-catechin, 3,4-dimethoxycinnamyl alcohol and 3,4,5-trimethoxycinnamyl alcohol from *L. globosus* for the antimicrobial and antifungal properties.[6] Many other chemical components such as triterpenoids from *L. grewinkii*[7], and *L. falcatus*[8], flavonoids from the leaves of *L. kaoi*[9] and from *L. europaeus*[10], a cytotoxin from *L. parasiticus*[11], and phenolics from *L. longiflorus*[12] have been reported so far. Methanolic extract of a Korean species exhibited moderate cytotoxicity against cultured human tumor cell line *in-vitro*.[13] Mistletoe extracts are widely used in complementary and alternative cancer therapy in Europe; they also possess cytotoxic as well as immunostimulatory effect.[14] The activity principle of the mistletoe (*Viscum album* L.) phytotherapeutics could be considered as combined cytotoxic and biological response modifying activities (increasing host defense against cancer) that result from the activities of the plant lectins and the other biologically relevant substances.[15] The present study was designed to evaluate the potential cytotoxicity of *Loranthus europaeus* chloform extract in bone marrow and peripheral blood cells.

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MATERIALS AND METHODS

Preparation of chloroform extract

The plant was brought from the Iraqi market of Herbs and Medicinal Plants and authenticated by Dr Ali Al-Mossawy, Biology Department, College of Science, Baghdad University. The plant fruits were air dried and used for extraction of the chloroform fraction according to a standard procedure.[16]

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 (which accord with the NIH 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 8 to 10 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 (Dimethylsulfoxide, DMSO) and served as control; second group treated with methotrexate (20mg/kg) dissolved in DMSO; third and fourth groups are treated with 200 and 400mg/kg of dried chloroform extract of *Loranthus europeus* dissolved in DMSO respectively. All types of treatments administered as oral single daily doses for 7 consecutive days.

Evaluation of cytotoxicity in bone marrow and peripheral blood

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicine, and then 2 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.[17] 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.[18,19]

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

Gene toxicity of different concentrations of *L. europaeus* chloroform extract

In table **1**, 200mg/kg chloroform extract significantly increase mitotic index (85.4%; P<0.05), while increasing the dose to 400mg/kg significantly decrease this parameter (-25.7%) compared to control group; similar result obtained wit 20mg/kg methotrexate, where 58.5% decrease in mitotic index was achieved which is significantly different compared to vehicle treated animals. Concerning the effect on chromosomal aberration, the chloroform extract (in both doses) produced non-significant change (4.6% and 5.5%) in this marker compared to control (P<0.05) (Figures **1** and **2**). Meanwhile, 20mg/kg methotrexate significantly increases chromosomal aberration compared to control (165.2%), a result which significantly differ from that reported for the chloroform extract.

Table 1. Incidence of mitotic index and total chromosomal aberration in bone marrow of mice treated with methotrexate and different doses of *Loranthus europaeus* chloroform extract.

Treatment groups	Mitotic index	Total chromosomal aberration
DMSO (negative control)	5.1±0.15	0.11±0.08
Methotrexate (MTX) 20mg/kg	2.1±0.07^{*a}	0.31±0.2^{*a}
Chloroform extract 200mg/kg	9.4±0.27^{*b}	0.12±0.07^{b}
Chloroform extract 400mg/kg	3.8±0.21^{*c}	0.3±0.08^{a}

*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,c) among treatment groups are significantly different (P<0.05).*

Figure 1. Chromosomal aberration (acentric chromosome) in bone marrow of mice treated with *Loranthus europaeus* chloroform extract.

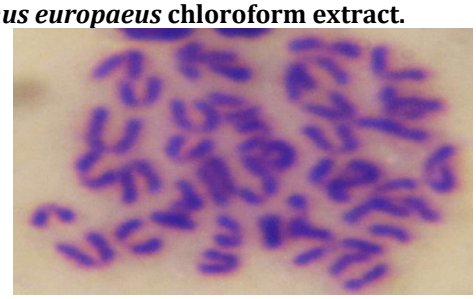
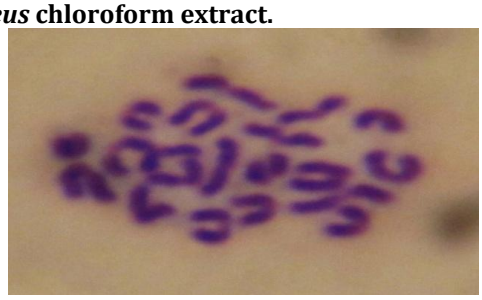


Figure 2. Chromosomal aberration (chromatid gaps) in bone marrow of mice treated with *Loranthus europaeus* chloroform extract.



Effects on Total and Differential WBC count

In table **2**, 200mg/kg of the chloroform extract significantly increases total WBC count (123.3%) compared to control, while doubling the dose of the same extract reveal non-significant elevation of the count (5.3%). Methotrexate also did not significantly affecting total WBC count compared to control group. Table **2** also showed that the low dose of the extract significantly decrease the lymphocytes count (20%), while the larger dose increase this count (5%) compared to control group; meanwhile, methotrexate did not significantly affect lymphocytes count. In case of the effects on neutrophils count, both doses of the chloroform extract increase the count, but only the lower dose (200mg/kg) reveal significant difference compared to control (21%, P<0.05). However, methotrexate shows no significant changes in this respect (0.76%, P>0.05).

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

Treatment group	Total WBC count (Cell x 10^3/mm^3)	Lymphocyte count (Cell/mm^3)	Neutrophil Count (Cell/mm^3)
DMSO (control)	52.4±4.0	2693.8±401	2285±334.1
Methotrexate 20mg/kg	51.5±3.3^{a}	2623.2±281.3^{a}	2302.4±241.2^{a}
Chloroform extract 200mg/kg	117±4.3^{*b}	3234.6±293.6^{*b}	2763.6±211.8^{*b}
Chloroform extract 400mg/kg	55.2±4.2^{a}	2819.6±303.1^{*a}	2426.6±250^{a}

*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).*

Previous chemical and pharmacological studies on some species of the Loranthaceae family have indicated the presence of several chemical compounds, including flavonoids and tyramine[20] alkaloids, lectins and viscotoxins[21], arginine and polysaccharides[22] in certain members of the family, while the property of preventing carcinogenesis has been reported in many plant extracts.[23] In the present study, 200mg/kg of the chloroform extract

significantly increases mitotic index while 400mg/kg dose decreases it. Phytochemical evaluation of this fraction revealed that it contains mainly alkaloids and flavonoids, and the reported gene toxicity may be attributed to its alkaloid content.[24] The reported difference in the effects of the 2 doses can be explained on the bases that in the lower dose the alkaloidal content may not be enough to decrease mitotic index, while such effect only appear when the dose of the extract was doubled, which may lead to the formation of variable adducts with DNA, proteins and other macromolecules and consequently affecting cell divisions by either prolongation of S and/or G2 phase in duration.[25] In this respect, methotrexate, the standard comparator drug used in the study, decreases the mitotic index which is an expected effect for this compound that has a cytostatic effect in variety of test systems.[26]

These results are confirmed by the reported hematological data, where the lower dose of the extract caused significant increase in total WBCs counts compared controls, while in a dose of 400mg/kg, the chloroform fraction did not show any change in the count. D-galactose- and/or N-acetyl-D-galactosamine-specific lectins are considered to be major active components in European mistletoe and have molecular masses between 50 and 60 kDa.[27] Among the mistletoe components, the cytotoxic and immunological properties of mistletoe preparation are considered to be linked to lectins which are focus of modern biomedical research. The sugar binding B chain of lectins is able to bind galactoside residues on the cell membrane preferring certain confirmations.[28] In low (not cytotoxic) doses, the B chain is responsible for the enhancing effect of lectins on the pro-inflammatory activity of natural immune system. The presented data also showed that chloroform extract of *Loranthus europaeus* (at both doses) caused a non-significant increase in total chromosomal aberration in bone marrow cells and spleen cells, but such effect was relatively higher with the large dose. The chloroform fraction contains alkaloids and flavonoids; the later was reported to have different pharmacological activities including anti-clastogenic effect, which may protect chromosomes from clastogenic compounds; several studies showed that flavonoids significantly decrease total chromosomal aberration[29], and have apoptotic effect on cancer cells, and several studies focused on the ability of the flavonoids to induce programmed cell death.[30] In Indonesia, *Scurrula* sp. (syn. *Loranthus* sp.) are one of the five components of Benalu Teh used as an infusion in fatigue and in cancer pathologies. Previous studies on *S. ferruginea* Danser exhibited attractive cytotoxic activity on U251 glioblastoma cancer cell line.[31]

Another study showed that loranthus extract significantly inhibited the tumor growth induced by Dalton's lymphoma ascites (DLA) and Ehrlich ascites (EA) tumor cell. In short, apart from its remarkable cytotoxic (tissue culture) response, the partially purified compound was found to inhibit the peritoneal tumors induced by DLA and EA tumor cell lines.[32]

CONCLUSION

The chloroform extract of *Loranthus europaeus* shows pronounced gene toxicity with increasing the dose when administered orally to mice.

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