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ANTI-CLASTOGENIC EFFECTS OF *LAUNAEA TARAXACIFOLIA* LEAF EXTRACT ON CISPLATIN-INDUCED MICRONUCLEI IN BONE MARROW ERYTHROCYTES

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ABSTRACT

This study investigates the anti-genotoxicpotential of *Launaeataraxacifolia* (LT) aqueousleaf extract on cisplatin-induced micronuclei in bone marrow erythrocytes ofWistar rats**.** Thirty rats were randomly divided into 6 groups (I-VI) of 5 rats each. Groups III and IV were treated with100 mg and 400 mg of LT via oral administration respectively for 21days while Groups V and VI were similarly treated and then exposed to intraperitoneal (ip) administration of 10mg/kg body wtcisplatin on the 21st day. Group II was only administered ip 10mg/kg body wtcisplatin on day 21 and Group I which serves as control received water and food. The frequency of micronuclei polychromatic erythrocytes (MNPCEs) in exclusively cisplatin exposed Group II rats was significantly ($p<0.001$) higher when compared to control. The cytoprotective index (PCE: NCE) was also reduced in Group II rats while the PCE/NCE ratio increased significantly ($P<0.05$) in Group V and VI compared to group II rats.*Launeataraxacifolia*leaf extractprotect against cisplatin- induced genotoxic effect in bone marrow erythrocytes of rats.

Keywords: *Launeataraxacifolia*, cisplatin, genotoxic, erythrocytes and rats

Number of Figures: 3 **Number of References: 20**

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum, or cis-DDP) is a widely used chemotherapeutic drugs against a wide variety of animal tumors and human cancers (Calabresi and Parks, 1985). Parenrerally administered, Cisplatin undergoes intracellular hydrolysis and form cytotoxic complexes with either DNA; RNA; sulphur-containing enzymes and mitochondria. The results of these interactions are DNA damage, mutagenesis, carcinogenesis, or apoptotic cell death (Krishnaswamy and Dewey, 1993). Its toxic side effects particularly target highly meristematic tissues of bone marrow, gastrointestinal mucosa etc with the clinical consequence of severe nausea, vomiting and myelo supression (Guneri*et al*., 2001; Rudrama *et al*., 2010). The generation of free radicals is thought to be an important mechanism in the development of these cisplatin-induced toxicities (Baliga*et al*., 1998;Weijl*et al*., 1997).

Launaeataraxacifolia also known as wild lettuce belongs to the family Asteraceae. The leaf is of high nutritional and medicinal values among inhabitants of SouthWestern Nigeria in West Africa where it is called *efoyanrin* (Adebisi, 2000). *Launaeataraxacifolia* is nutritionally rich in vitamins, minerals, proteins, essential fatty acid**s,** fibre contents and flavonoids (Larson, 1988; Adinortey et al., 2012; Dickson et al., 2012; Gbadamosi et al., 2012). Despite a plethora of research works confirming phyto-antioxidants particularly polyphenols, flavonoids and phenolic acids as having chemopreventive activities (Grzegorczyk et al., 2007), there is no published research work on the protective roles of *L. taraxacifolia* against cisplatin induced micronuclei. This study then seeks to investigate this possibility. The results obtained may form the basis for the use of *Launaeataraxacifolia*as an ameliorating agent for cisplatin-induced toxicities.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The fresh leaves of *Launeataraxifolia* were harvested from the flower beds around Faculty of Basic Medical Sciences, University of Ibadan, Nigeria and authenticated by Prof. A.E Ayodele of the herbarium at the Department of Botany, University of Ibadan with voucher number UIH-22370.

Plant Extract preparation

The leaves (400 grams) were air-dried for 4 days, granulated and soaked in 2litres of distilled water heated at a sustained temperature of 60ºC for 24 hours after which they were filtered using filter papers. The filtrate was concentrated using a rotary evaporator to give 13.76g residue, a yield of 3.44%. The administered dose was then calculated per kilogram body weight of the rats.

Chemicals

The Cisplatin used was manufactured by Korea United Pharm. Inc. (Naojang, Chungnam, Korea) and procured from Kunle Ara pharmacy, a registered pharmaceuticals retail company. All other reagents were of analytic grade.

Animal Protocol

Adult male Wistar rats weighing between 200 and 210 g, obtained from the Experimental Animal Unit of Faculty of Veterinary medicine, University of Ibadan, Nigeria, were housed in well-ventilated plastic cages, provided with rat pellets and water ad libitum. All the experimental animals were given humane care according to criteria provided in the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Science published by the National Institutes of Health. The ethical regulations of the *University of Ibadan Ethical Committee for the protection of animal welfare during experiments* were strictly followed.

Experimental design

Thirty healthy adult male Wistar rats were randomly divided to six groups of five rats each as follows:

I. **Control**: received saline (5 ml/kg body weight) and water (5 ml/kg body weight) intraperitoneally.

II. **Cisplatin(CIS)**: were given a single dose of cisplatin (CIS) intraperitoneally at 10 mg/kg body wt. on the 21st day

III. ***Launeataraxacifolia*** **(**LT) **100mg**: given orally administered 100 mg of *Launeataraxacifolia* for 21 days.

IV. **IV.LT 400mg:** were given orally administered 400 mg of *Launeataraxacifolia* for 21 days.

V. **V.LT 100mg + CIS:**orally received 100 mg of *Launeataraxacifolia* for 21 days plus single dose of 10 mg/kg body wt. of cisplatin intra peritoneally on the 21st day.

VI. **LT 400mg + CIS:** were given 400 mg of *Launeataraxacifolia* for 21 days plus single dose of 10 mg/kg body wt. of cisplatin intraperitoneally on the 21st day. The rats were sacrificed by cervical dislocation on the 5th day after the administration of CIS and femoral bone was dissected out for the evaluation of micronuclei polychromatic erythrocytes (MNPCE) variations in bone marrow.

Micronucleus assay

Bone marrow cells within femurs were flushed with 1ml of foetal bovine serum into a centrifuge tube, the suspension was centrifuged at 2200 rpm for 10 min, the supernatant was discarded and smears were prepared from the sediments on glass slides. The air-dried slides were fixed in methanol and stained with May-Grünwald and Giemsa. A total of 2000 polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCE) were screened per animal for scoring the frequency of PCEs containing micronuclei and the ratio of PCEs to NCE was also determined.

Statistical Analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) and the groups were compared using Turkey post hoc test for multiple comparisons. The level of significance was $p<0.05$. While the results were expressed as group mean ± standard error of mean (SEM).

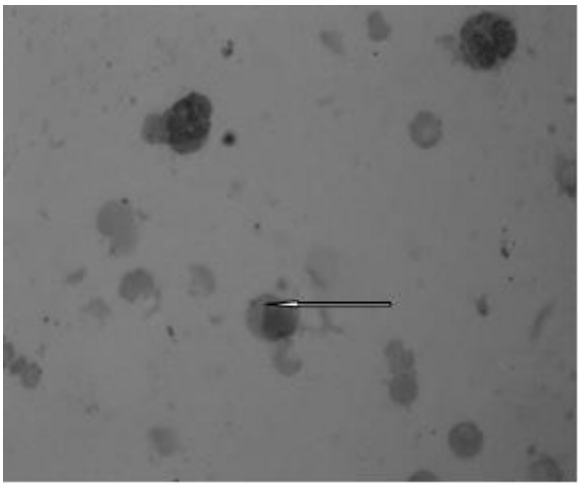

Fig 1*: The presence of micronucleus (white arrow) in cisplatintreated animals*

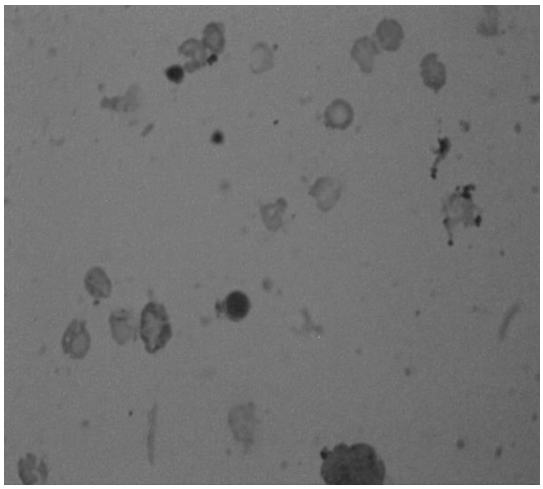

Fig2: *The absence of micronucleus in Launeataraxacifolia-treated rats.*

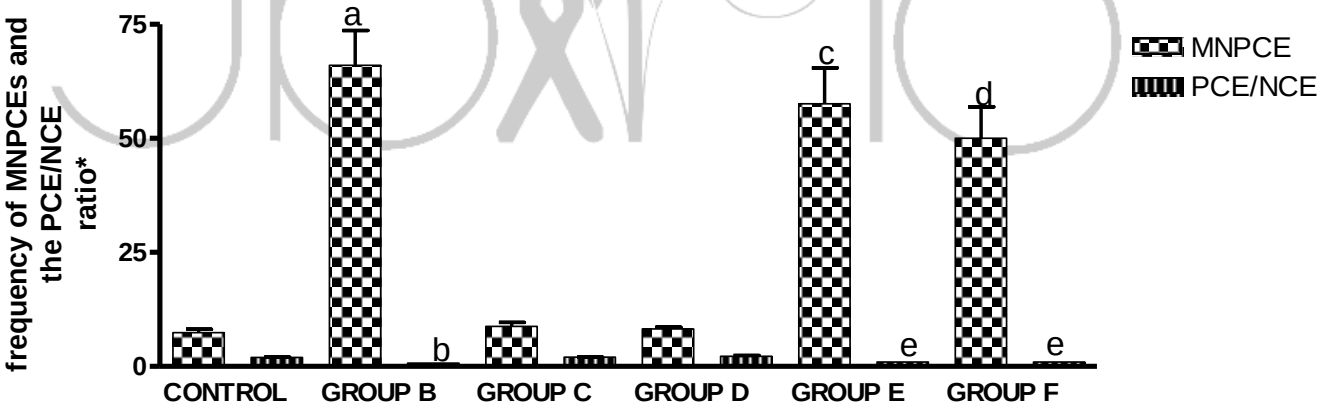

Fig 3: *Effect of aqueous extract of Launeataraxacifolia on the frequency of MNPCEs and the PCE/NCE ratio in cisplatin treated rats. Bars with different letters are significantly different; p-values: a<0.001, b<0.01-CIS compared with normal control; c<0.05, d<0.01, e<0.05 - experimental groups compared with CIS*

RESULT AND DISCUSSION

The MNPCEs frequency in figure 3 increased significantly ($p<0.001$) with concomitant decreased PCEs to NCEs ratio(an index of cyto protection) in cisplatin exposed Group II rats compared to control group. This data shows that cisplatin is genotoxic and this further corroborates its widely reported genotoxic property (Barbara et al., 1996; Giri et al., 1998). The latter has been attributed to DNA damage and successive DNA fragmentation, chromosomal breaks, and micronucleus formation causing genomic instability and eventually ends in mutagenesis, carcinogenesis, or apoptotic cell death. Pretreatment with 100 and 400 mg of *L. taraxacifolia*in Group V and VI significantly ($p < 0.05$; $p< 0.01$ respectively) reduced the frequency of MNPCE and increased significantly ($p< 0.05$) the PCE/NCE ratio in a dose dependent fashion relative to cisplatin exposed Group II rats (Figure 3). These observations are in consonance with documentation on the beneficial effect of natural compounds in medicinal and dietary plants in reducing micronuclei formation and improving cyto-protective index as previously reported for *MurrayaKoenigi* (Rudrama et al., 2010), *Cyamopsistetragonoloba* (Yadav et al., 2013) and *Salvia officinalis* leaves(Alkan et al., 2011).

CONCLUSION

This study shows that *Launeataraxacifolia* leaf extract protect against cisplatin- induced genotoxic effect in bone marrow erythrocytes of rats. *Launeataraxacifolia* supplementation is therefore strongly recommended before cancer treatment.

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