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Radiation Cytogenetic Studies on Philippine Ashitaba (*Gynura nepalensis* DC)

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Abstract

Philippine Ashitaba (*Gynura nepalensis* DC.) at 100% and 50% leaf extract concentrations when administered to white mice (Mus musculus) before radiation treatment were found to reduce the incidence of chromosomal aberration (dicentrics, rings, breaks, gaps, fused, desperalized, chains, complex sticky and fragments) when compared with the somatic cells of white mice in the positive control, T+ (mice exposed to 2Gy at an exposure rate of 150 cGY per minute for 2.5 minutes Hence, the Gynura leaf extract induced to mice before radiation exposure possesses the potential to act as radioprotective agent.

Gynura leaf extract introduced to mice for 15 days after irradiation with the same exposure treatment did lower the occurrence of chromosomal aberrations as compared with the T+ (sacrificed 24 hours after irradiation) but there is a non-significant difference among the means of total chromosomal aberrations in T++ (irradiated and sacrificed after 15 days), T1 (100% Gynura leaf extract treatment for 15 days after irradiation), T2 (50% Gynura leaf extract treatment for 15 days after irradiation) and T0 (no irradiation), hence, it is not conclusive at this point that *Gynura* leaf extract possess potential against radiation's adverse effects. The reduction of chromosomal aberrations observed after irradiation may not be due solely to the effect of the leaf extract but may also possibly be due to the body's defense mechanisms in the mice that counteracted the negative effects of radiation.

Keywords: chromosomal aberration; chromosome repair damage potential, cytogenetics; radiation; radioprotective.

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1.0 Introduction

The ethno-pharmacological, anti-mutagenic and phytochemical properties of Philippine Ashitaba (*Gynura nepalensis* D.C.) has been established and contain alkaloids, catechic tannins, saponins and flavonoids [1,2,3] and has been widely promoted because of its wide array of medicinal potentials [1,4]. Secondary plant metabolites such as polyphenolic acids, flavonoids and other antioxidant components of other plants were reported to protect genetic material from ionizing radiation [5]. Flavonoids were found to have effects n biochemical pathways such as anti-inflamation, inhibition of cell proliferation, anti-oxidation, detoxification of mutagenic metabolites, apoptosis, and inhibition of angiogenesis [6] . Some phytochemicals such as alkaloids and phenolic compounds have the ability to participate in the body's repair and protective mechanisms, kill cancer cells, and scavenge free radicals from radiation exposure which can damage the genetic material, DNA or chromosome [7].

Humans are naturally exposed to ambient radiation but they may also be occasionally exposed to unnatural sources of ionizing radiation. Radiation may be acquired from the natural environment such as cosmic radiation or from man-made sources such as in medical diagnostics like X-ray, medical treatment, radiation therapy, or nuclear power plants used for industrial purposes. Although nuclear medicine has been proven to be beneficial to man both in diagnoses and treatment of diseases, Wolfe [8] cited adverse side effects in the form of modified biomolecules, mutant genes, cancer cells, non-functional gametes and cell death.

These side effects of ionizing radiation on the cell are a chain of reactions resulting from the formation of radicals and chemical modification and can be reflected on the chromosomal structure and behavior [9] and intercalation of DNA sequence inside the cells during treatment [10]. The formation of dicentric chromosome, ring chromosomes, fuse chromosome, gaps, breakage, and other structural changes are consequences of exposure to ionizing gamma radiation [11].

Hence, this study was conducted to determine the effects of *Gynura nepalensis* DC. leaf ethanol extract treatment in the somatic cells of white mice administered before and after exposure to ionizing gamma radiation.

2.0 Methodology

2.1. Research Design

This experimental method of research investigated the effects of *Gynura nepalensis* DC. Leaf extract on the somatic cells of 27 white mice (Mus musculus L.) weighing 20-28 gm, and randomly distributed to four and five treatments in the Gynura (50% extract and 100% extract) treatments before radiation exposure *(Study 1) and Gynura treatment after radiation exposure (Study 2)*, respectively. Three mice were used per treatment, each mouse representing a replicate. The non-irradiated mice served as the negative controls. The irradiated mice without Gynura treatment served as the positive controls.

2.2. Experimental Procedure

Ethanol Leaf extracts of *Gynura* at 100% and 50% were administered first to the mice prior to gamma irradiation in Study 1, while Study 2 exposed the mice to gamma irradiation first prior to treatment of leaf extract.

Chromosomal abnormalities on the bone marrow cells of the white mice were scored and statistically analyzed using Analysis of Variance and HSD test.

2.3. Plant Identification

A prepared live sample of *Gynura* plant was identified and authenticated by experts of the Philippine National Museum, Manila, Philippines.

2.4. Preparation of Plant Extract

The Gynura leaf extract was prepared by soaking first the dried, fine, cut leaves (air dried for 4 days) in 80% ethanol for 48 hours with the ratio of 1 kg:1 L. Then, the mixture was filtered and the filtrate was concentrated by using a rotary evaporator at the Biochemistry Laboratory of Angeles University Foundation. The ethanol extract was set aside in a refrigerator ready for treatment of animals. The 50% concentration was prepared by diluting 50 ml of plant extract into 50 ml distilled water while 100% concentration used the pure crude extract [12].

2.5 Acclimatization of the Test Animals

The 27 white mice (*Mus musculus* L.) were subjected to a 7-day acclimatization period to enable them to adapt to the laboratory environment. They were fed with commercial feed and distilled water *ad libitum* .

2.6 Administering the Treatment

Experiment No. 1. Gynura Treatment to Mice before Irradiation

The mice were treated with Gynura leaf ethanol extract for 15 days at 24-hour interval via oral gavage at the concentration of 0.5ml/20 grams body weight every 7:00 am. Standard mouse diet was given after 2 hours of extract administration (9:00 a.m.) for Treatments 1 (100%) and 2 (50%). However, the feed was removed at 4:00 in the afternoon to ensure that the gastric cavity was empty when the extract will be administered on the following day [13].

Twenty-four hours after the last treatment with Gynura, the mice were subjected for irradiation using radioisotope in the irradiating chamber facility (Gamma Cell 220 Co^{60}) of the Philippine Nuclear Research Institute in Diliman, Quezon City.

The white mice were exposed to the irradiation dose of 2 Grays at the exposure rate of 150 cGy per minute in 60 seconds once [14] to induce chromosomal aberrations in bone marrow cells at C- metaphase level. One group was not irradiated and set aside as negative control. Irradiated mice without Gynura treatment served as the positive control.

Experiment No. 2. Gynura treatment after irradiation

Two grays (2Gy) gamma radiation at the exposure rate of 150 cGy per minute [14] was administered once for 2.5 minutes to the mice. After irradiation treatment, the mice were given fifteen days treatment of Gynura leaf extract (0.5ml/20grams body weight) every 7:00 a.m. Standard mouse diet was given from 9:00 am – 4:00 p.m., however, the feeds were removed afterwards to ensure that the mice were fasted for the next administration [15].

The mice were sacrificed 24 hours from the 15th day of administration for cytogenetic screening. Another positive control was set aside from the irradiated mice without treatment of leaf extract and was sacrificed after 15 days.

2.7 Cytogenetic Techniques for White Mice

An improved cytogenetic technique modified by Gregorio and Medina [16] was used to obtain high quality C-metaphase spread from bone marrow cells of white mice.

The white mice were injected intraperitoneally with 0.025% of colchicine solution at 0.25 ml/kg body weight and were sacrificed one hour after colchicine treatment. The femur and testis were dissected out. Then, the femur was cut at both ends to allow flushing out of the bone marrow cells using a syringe in a micro test tube containing an isotonic solution (2.2% sodium citrate).

The bone marrow cell suspension was centrifuged for 5 min at 360 rpm. Immediately after, the samples were prepared for hypotonic solution treatment. Immediately after 5 minutes centrifugation, the supernatant was removed, while the pellets containing the cells were retained for breaking and stocking in the hypotonic solution (1.0% sodium citrate) for twelve minutes. Centrifugation at 1000rpm for 5 minutes followed after the hypotonic treatment.

After the hypotonic treatment and centrifugation, the supernatant was removed and the pellet containing the chromosome was retained and added with fixative solution (3:1 ethanol and glacial acetic acid). Fixation was done four times for the bone marrow cell suspension. After the cells have been fixed in the test tube with fixative solution, the cell suspensions were smeared in a new glass slide by using a Pasteur pipette and air dried for five hours. The prepared slides were stained in the Coplin jar containing 3% giemsia staining solution for five minutes.

2.8 Chromosome and Karyotype Analysis

With the aid of a binocular electric microscope, 100 C-metaphase mitotic cells per experimental animal were examined for chromosomal aberration. The chromosome spread was checked under LPO and HPO, and chromosomal aberrations such as breaks, gaps, dicentric, polycentric, desperalized, complex sticky and rings (centric and acentric) were analyzed under the oil immersion lens.

Photography using digital camera (Panasonic) was undertaken for analysis and documentation. The karyotype was prepared to assess further abnormalities.

3.0 Results and Discussion

Study 1. Gynura Leaf Extract Treatment Before Irradiation

The means and p-values of specific chromosomal abnormalities per treatment in bone marrow cells of white mice with pre-irradiation treatment showed a significant number of dicentric chromosome (Fig 2a), ring chromosome (Fig 2b), chromosome break (Fig 2c), chromosome gap (Fig 2c), fused chromosome (Fig 2f), chromatid break (Fig 2c), chromatid gap (Fig 2c), desperalized chromosome (Fig 2e), and fragment (Fig 2c). There are polycentric chromosome (Fig 2a) and complex sticky chromosome (Fig 2d), but the p-value indicates insignificance occurrence of the said aberration. Normal spread of chromosome and karyotype were also presented in Fig 1a and Fig 1b, respectively.

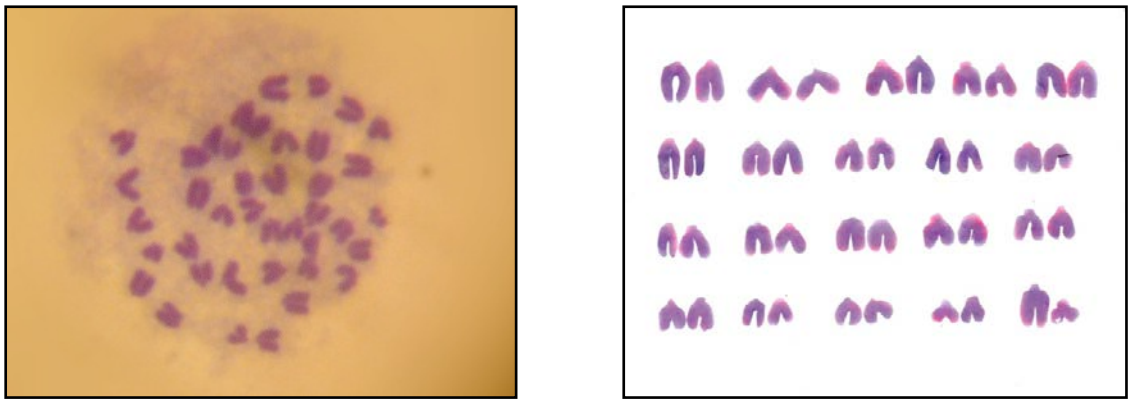
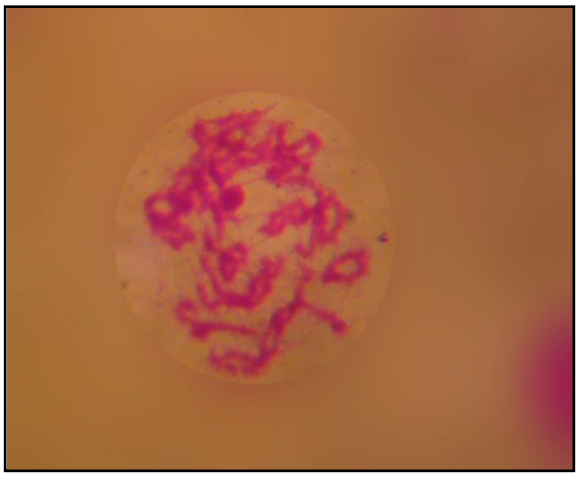
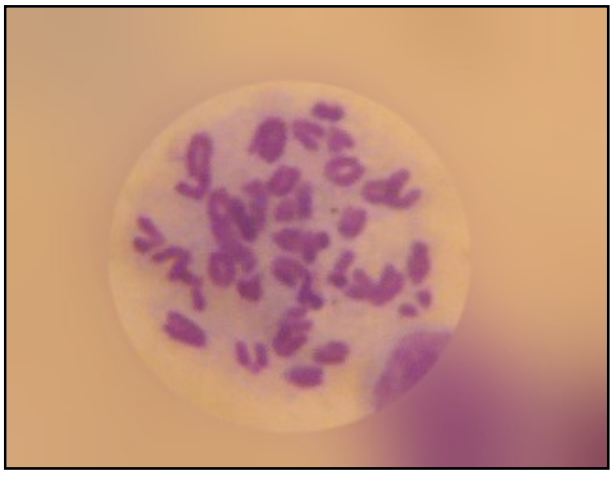


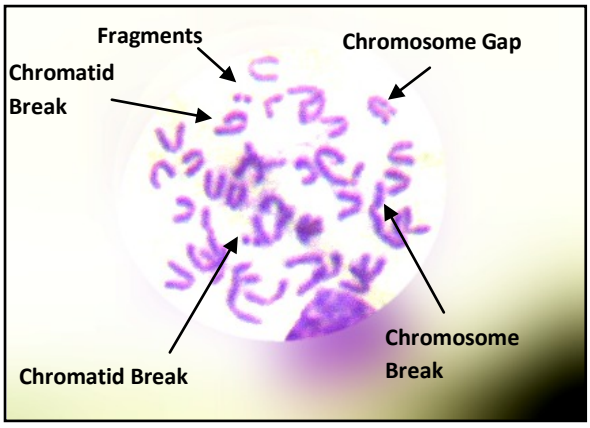
Fig 1. The normal chromosome spread and karyotype of the chromosomes of white mouse



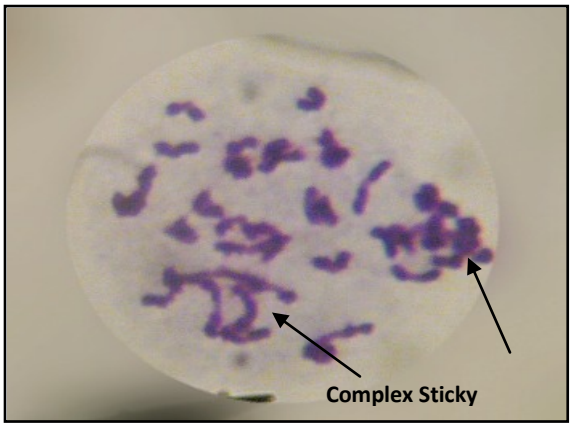
2a. Abnormal C-metaphase with dicentric, polycentric and ring chromosome



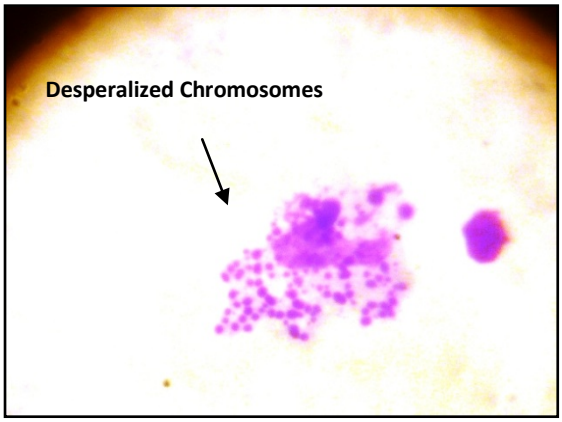
2b. Abnormal C-metaphase with ring and fragments



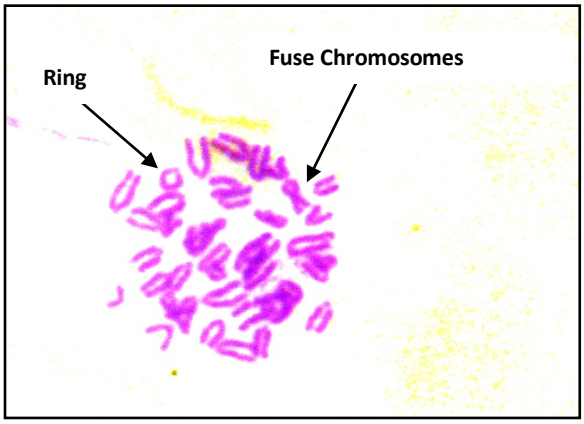
2c. Abnormal C-metaphase with chromosome break and gap, chromatid gap and break, and fragments



2d. Abnormal C-metaphase with complex sticky chromosomes



2e. Abnormal C-metaphase with desperalized Chromosomes



2f. Abnormal C-metaphase with fused and ring chromosomes

Fig 2. Chromosomal Abnormalities in Irradiated White Mice

Among the treatments in Study 1, T+ (positive control) was found to be more significant than T- negative control, T1 - 100%; and T2- 50% Gynura extract in terms of the chromosomal aberration such as production of dicentric, polycentric, ring, break, gap and fused chromosomes at 1% level of significance. The same trend was seen in chromatid type and other anomalies such as chromatid break, chromatid gap, desperalized and fragmented chromosomes. No significant differences among the T-, T1 and T2 were observed on the above abnormalities except for the occurrence of fused chromosomes wherein 50% extract imposed more significant difference than the 100% extract (Table 1). The results indicate the radioprotective property of *Gynura nepalensis* D.C. extract againts chromosomal aberrations imposed by radiation. Dicentric, ring, and breakage are noted to be the best indicators of radiation exposure [15], thus reduction of the said abnormalities support its radioprotective property. The radioprotective mechanism could be due to the ability of the flavonoids to block the

radiation penetration and scavenge the free radicals formed. Blocking action of the flavonoid is made possible due to the presence of a double bond on the Oxygen group. Radicals as a result of irradiation are stabilized by flavonoids by donating the H on their OH group [16].

Table 1. Mean number of specific chromosomal abnormalities in mitotic C-metaphase on white mice bone marrow cells with pre-irradiation treatment

Treatment	Chromosome Type					
	Dicentric	Polycentric	Ring	Break	Gap	Fuse
T-	0.00^{b}	0.00	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{c}
T1	0.00^{b}	0.00	8.33^{b}	4.67^{b}	1.67^{b}	$2.33b^{c}$
T2	0.33^{b}	0.00	9.00^{b}	1.67^{b}	1.00^{b}	7.67^{b}
T+	4.67^{a}	0.67	46.67^{a}	13.00^{a}	18.33^{a}	21.00^{a}
p-value	0.000046^{**}	0.444109^{ns}	0.001441^{**}	0.00051^{**}	0.001363^{**}	0.000056^{**}

Treatment	Chromatid type		Others		
	Break	Gap	Deperalized	Complex Sticky	Fragment
T-	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{b}
T1	4.67^{b}	0.33^{b}	0.00^{b}	0.33^{a}	10.67^{b}
T2	6.67^{b}	0.33^{b}	0.33^{b}	0.67^{a}	9.33^{b}
T+	29.67^{a}	6.67^{a}	3.00^{a}	1.67^{a}	31.67^{a}
p value	0.00022^{**}	0.000032^{**}	0.00536^{**}	0.35608^{ns}	0.00323^{**}

** Highly significant (1% level of significance)

ns - not significant

Means with the same letter have no significant difference

Study 2. Gynura Leaf Extract Treatment After Irradiation

The p-values in the means of chromosomal abnormalities such as dicentric, ring chromosome, chromosome break, chromosome gap, chromatid break, fused chromosomes, desperalized and chromosome fragments at post-irradiation treatment of *Gynura* on white mice showed a significant result . Polycentric and complex sticky chromosome have insignificant occurrence.

Effects of radiation on the bone marrow cells of white mice was evident in T+ (irradiated mice sacrificed 24 hours after radiation treatment. However, those irradiated and treated with Gynura for 15 days revealed a comparable result among the treatments (T-, T1, T2 and T++) on the production of chromosomal aberrations but were significantly lower than T+ (positive control) as validated by HSD (Table 2).

Table 2. Mean number of specific chromosomal abnormalities in mitotic C-metaphase of white mouse bone marrow cells with post-irradiation treatment

	Chromosome Type of Aberration					
Treatment	Dicentric	Polycentric	Ring	Break	Gap	Fused
T-(Negative Control)	0.00^{b}	0.00^{a}	0.00^{b}	0.00^{b}	0.33^{b}	0.00^{b}
T1(irradiation+ 100% extract)	0.00^{b}	0.00^{a}	5.33^{b}	0.00^{b}	1.33^{b}	1.67^{b}
T2(irradiation + 50% extract)	0.00^{b}	0.00^{a}	7.00^{b}	0.33^{b}	1.33^{b}	1.00^{b}
T+(irradiated sacrificed after 24 hours)	4.67^{a}	0.67^{a}	46.67^{a}	13.00^{a}	18.33^{a}	21.00^{a}
T++(irradiated sacrificed after 15days)	0.00^{b}	0.00^{a}	5.33^{b}	1.33^{b}	0.67^{b}	2.67^{b}
p-value	$.00002^{**}$	0.49^{ns}	0.0003^{**}	0.00004^{**}	0.0003**	0.00**

Table 2. Mean Number……..Continuation

Treatment	Chromatid type of Aberrations		Other Aberrations'		
	Break	Gap	Deperalized	Complex Sticky	Fragment
T-(Negative Control)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{b}
T1(irradiation+ 100% extract)	3.33^{b}	0.67^{b}	0.00^{b}	0.33^{a}	3.67^{b}
T2(irradiation + 50% extract)	2.67^{b}	1.00^{b}	0.00^{b}	0.00^{a}	6.67^{b}
T+(irradiated sacrificed after 24 hours)	29.67^{a}	6.67^{a}	3.00^{a}	1.67^{a}	31.67^{a}
T++(irradiated sacrificed after15days)	3.33^{b}	2.67^{b}	0.00^{b}	0.33^{a}	7.33^{b}
p value	$.00001^{**}$	0.000066**	0.000024**	0.29ns	0.000007^{**}

**Highly Significant

ns- not significant

Means with the same letter have no significant difference

The non-significant difference in the reduction of chromosomal incidence in T1, T2 and T++ may not be conclusive that *Gynura* extracts administered after irradiation imposed sole positive effects against radiation adverse effects on the chromosomes of white mice. The fact that the incidence of chromosomal aberration in

the irradiated mice without Gynura treatment (T++) was reduced, other mechanisms may have occurred to repair the chromosome damage.

4.0 Summary and Conclusions

4.1. Summary

Cytogenetic analysis showed that the Gynura leaf extract is capable of reducing the occurrence of chromosomal aberration on the bone marrow cells of white mice with post irradiation exposure Thus, the said extract possess the potential to act as a radioprotective agent based on the chromosomal assessment.

Gynura leaf extract administered to mice after irradiation treatment did reduce the incidence of aberrant chromosomes. The same result was also observed in the irradiated mice treated with Gynura 15 days after irradiaton.

4.2. Conclusions

The *Gynura nepalensis* DC. leaf extract treatment at different concentrations (50% and 100%) possess potential to act as radioprotective agent against 2Gy gamma radiation as reflected in the decrease of the number of aberrant chromosomes observed. However, when administered after irradiation, it is not conclusive that it solely counteracted the adverse effect of radiation. Chromosome repair mechanisms or body defense mechanisms in the mice system could have acted on aberrant chromosomes induced by radiation treatment.

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