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GENOMIC INTEGRITY OF WHITE MICE BONE MARROW CELLS TREATED WITH ANTIBIOTICS AND PROVEN ANTIMICROBIAL PLANTS

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ABSTRACT

This study investigated the effect of antibiotics and proven antimicrobial plants on the genomic integrity of white mice bone marrow cells using DNA Fragmentation Assay. The study made use of 1% agarose gel and DNA extracts were run at 140V for 30 min. It was found that Co-amoxiclav induced the highest intensity of DNA fragmentation followed by tetracycline, and *Vitex negundo* decoction. *Pisidium guajava* decoction and clindamycin produced DNA smearing but *Psidium guajava* showed the higher intensity of DNA smearing. The results suggest that antibiotics can induce DNA damage possibly through the generation of free radicals that may promote DNA degradation. The absence of DNA smearing and DNA fragmentation in the bone marrow cells of mice treated with a decoction of proven antimicrobial plants may possibly be due to the presence of anti-mutagenic phytochemicals that counter the effects of radicals. Other molecular strategies such as comet assay and telomere probing on undifferentiated and highly differentiated cells must be considered to validate the results of this study.

Keywords: - *DNA fragmentation, DNA smearing, Free radicals, Antibiotics, Proven Antimicrobial Plant*

INTRODUCTION

Antibiotics have been widely used to treat diseases caused by bacterial infections in animals or human beings and their application has led to many benefits in medical science. However, in recent decades, the abuse of antibiotics has posed a threat to public health [1].

Tetracycline, co-amoxiclav, and clindamycin inhibit the synthesis of bacterial protein. In the case of tetracycline, the protein synthesis is inhibited by blocking the attachment of charged

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aminoacyl-tRNA to the A site on the ribosome. Tetracycline binds to the 30S subunit of microbial ribosomes preventing new amino acids to be introduced to the nascent peptide chain. The inhibition of protein synthesis by clindamycin is the result of inhibiting the translocation of the ribosome. This is achieved by binding the 50S subunit rRNA of the large bacterial ribosome subunit [2].

Plants have also been used as alternative therapies for diseases in many countries in the world.. *Psidium guajava* decoction has been used to control life changing conditions such as diabetes, hypertension, and obesity. In fact, the presence of phytochemicals such as tannins and flavonoids accounts for its medical and physiological activities [3]. The use of medicinal plants may, however, have associated risks because many of these plants contain toxic compounds. Therefore, the incorrect use of phytotherapy can produce severe damage to health [4].

Vitex negundo is a large native shrub that thrives in the Philippines. The Philippine Department of Health has conducted research and study regarding lagundi, local name of *V. negundo*, and has suggested that lagundi plant has many beneficial therapeutic values. *Vitex negundo* has been traditionally used as herbal medicine by Philippine folks. It was found that the essential oil is responsible for the relieving of sloughing wounds and ulcers. The leaves of *V. negundo* are reported to have pesticidal, antifungal and antibacterial properties [5].

Previous studies have shown that some antibiotics have cytotoxic and genotoxic [6, 7, 8] as well as ototoxic effects [9]. Antimicrobial toxicity of plant extracts has also been established in several studies [10, 11].

In this study, we report that co-amoxiclav, tetracycline, and *V. negundo* decoction induced DNA fragmentation. Clindamycin and guava decoction induced DNA smearing. The induction of DNA fragmentation could be due to the generation free radicals.

MATERIALS AND METHODS

Collection and preparation of plant leaves

Plant leaves were freshly collected from a local grower in Caloocan city. The leaves were weighed and 4.5g were used in the study. The leaves were washed with distilled water and chopped into smaller pieces. The extract was obtained using decoction method.

Preparation of antibiotic solutions

The antibiotics were purchased from a local pharmacy located in Caloocan City. The antibiotics were freshly prepared each day at a concentration of 30mg of tetracycline, 2 mg of clindamycin and 20 mg of Co-amoxiclav based on the body weight of the mice. 0.1 ml were administered to the mice for seven consecutive days.

Test Organism

Four months old male mice (weight range 13-18 g) were purchased from Biocare and Breeding Center in Catmon, Malabon City. The mice were housed in a room with an air conditioner (turned on at 10:00 pm and turned off at 4:00 pm) and 12:12 h light: dark cycle was also maintained. Their beddings, food, and water intake were also checked three times each day. They were divided into three groups (n= 6, in each group). They were acclimatized for a period of 1 week. The treatment was done for a period of seven consecutive days.

DNA Fragmentation Assay [26]

After seven days of treatment, the mice were sacrificed by cervical dislocation. Bone marrow cells were obtained from both femora. The femur was cut at the proximal end until a small opening to the marrow is seen. The cells were flushed out using a syringe (Villasenor *et al*, 2002). 200 µl of 10% *Sodium dodecyl sulfate* (SDS) was added to the cells in a microtube and placed in water bath at 65^0C for 15 minutes. 200 µl cold isopropanol were added and the stringy DNA formed was transferred to a clean microtube. The microtube was centrifuged for 30 min at a speed of 3000 x g. When the DNA settled down, the supernatant was poured off. The DNA was air dried and stained with SYBR. The stained DNA was placed in the wells of gel electrophoresis and ran for 30 minutes at 140 V.

Statistical analysis

The number of fragmentation per treatment was subjected to one-way analysis of variance (ANOVA). The means per treatment were compared using HSD test. *P*-value < 0.05 was accepted as statistically significant.

RESULTS

A. Occurrence of DNA Fragmentation of White mice with different Treatments

To assess the occurrence of DNA fragmentation, the distances migrated by DNA fragments were measured and compared between groups. Co-amoxiclav administration significantly (p=0.0001) induced DNA fragmentation compared to the control indicating genotoxicity. As shown in Fig. 1, when Co-amoxiclav was compared with tetracycline, both were statistically insignificant (p=0.05) but when Co-amoxiclav was compared with *Vitex negundo* decoction, Co-amoxiclav showed extremely significant (p=0.0001) DNA fragmentation. The comparison was also made between them in terms of the intensity of the fragments. Co-amoxiclav produced the highest intensity of DNA fragment followed by *Vitex negundo* decoction which produced the second highest intensity of DNA fragment and tetracycline was the least among them.

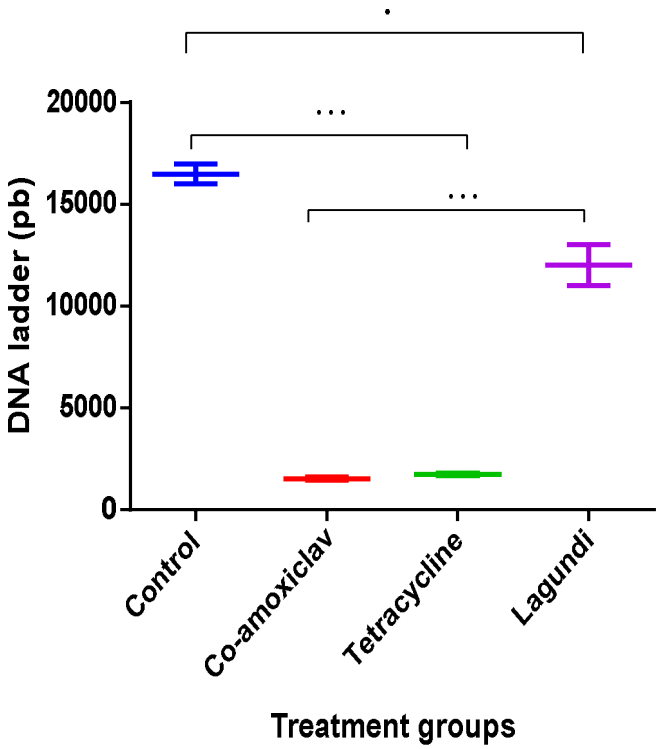

Fig 1: Effect of antibiotics and proven antimicrobial plants on the integrity of the DNA of Bone Marrow Cells of white mice. The larger the fragments the less distance they traveled. Both Co-amoxiclav and tetracycline traveled farthest distance due to small fragments induced by them.

A. The occurrence of DNA Smearing in White mice with different Treatments.

As shown in Fig. 2, DNA smearing was observed and comparison was made in terms of the intensity of DNA smearing of bone marrow cells. Clindamycin and *Psidium guajava* decoction were able to produce smearing in both replicates while *Vitex negundo* decoction produced smearing only in the first replicate of the study. The intensity of smearing produced by *Psidium guajava* decoction is higher than the ones produced by clindamycin and *Vitex negundo* decoction but clindamycin produced smearing with farther distance migrated than *Psidium guajava* decoction and *Vitex negundo* decoction.

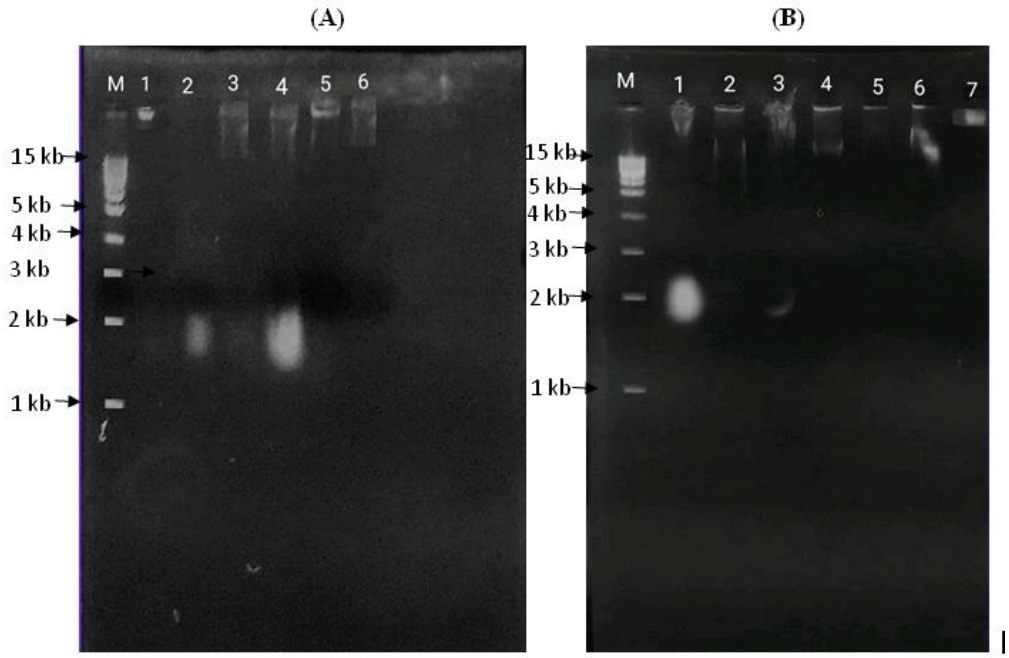

Fig.2. (A) 1% agarose gel electrophoresis of DNA obtained from white mice bone marrow, where M = DNA ladder, 1 = control, 2 = tetracycline, 3 = clindamycin, 4 = Co-amoxiclav, 5 = *Psidium guajava* decoction and 6 = *Vitex negundo* decoction. (B) M = DNA ladder, 1 = co-amoxiclav, 2 = clindamycin, 3 = tetracycline, 4 = *Psidium guajava*, 5 = negative control and 6 = *Vitex negundo* decocton and 7= normal control. Co-amoxiclav and tetracycline induced DNA damage whereas *Vitex negundo* decoction produced DNA damage slightly. Clindamycin and *Psidium guajava* decoction produced DNA damage marked by the smearing of DNA.

DISCUSSION

Antibiotics have been used to treat infectious diseases and their effects on DNA have been reported in several studies (7, 8, 12). The effect of antibiotics is not limited to the DNA, but they also cause apoptosis [13, 14. 15, 16]. Apoptosis which is a programmed cell death usually happens when the cell is in stress due to oxidative agents and aging.

Antimicrobials from plant origins are used as alternatives to antibiotics because they are considered to be safer; however, several studies have reported that certain antimicrobials from plant origin can cause cytotoxicity [17] as well as DNA damage [18].

Co-amoxiclav is a combination of amoxicillin and clavulanic acid induced DNA damage in vivo (bone marrow cells of white mice) after seven consecutive days of treatment. This study supports a study conducted by [19] which reported that amoxicillin induced DNA damage in mammalian cells. DNA damage induced by amoxicillin was a result of the generation of reactive oxygen species (ROS). ROS is highly reactive and unstable. The oxidative stress ruptures the DNA and causes the degradation of DNA. On the other hand, antioxidants can prevent the effect of ROS.

A previous study reported that amoxicillin induced DNA damage via the generation of ROS in human lymphocyte and gastric mucosa cells [7]. This clearly indicates that there is a higher possibility of ROS involvement in Co-amoxiclav-induce DNA damage in this study.

Co-amoxiclav is also reported to frequently cause liver damage via oxidative stress [21] and, as already noted in previous studies [7,19], amoxicillin showed oxidative stress that induced DNA damage. This confirms that there is higher chance that Co-amoxiclav induced DNA damage via oxidative stress.

Tetracycline was also evaluated in terms of its effect on genomic integrity. Tetracycline induced DNA damage. The induction of DNA damage could be mediated by oxidative stress because when tetracycline was exposed to *Chlamydomonas reinhardtii* in another study, it showed an increase in the level of antioxidant enzymes [20]. The increase in this enzyme clearly showed oxidative stress. Liver failure can result from the production ROS and tetracycline has been associated with hepatotoxicity and nephrotoxicity [22].

Vitex negundo is the only antimicrobial from plant origin to induced little DNA damage ($p=0.05$) in this study. The cause of the damage induced by *V. negundo* is not known in this study. However, in another study it was shown that a compound from *Vitex negundo* known as poly methoxy flavones showed weak to moderate cytotoxicity against human HepG2 and rat C6 cell lines [23].

Clindamycin did not induce DNA fragmentation but it is observed to induce smearing. The smearing shown in (Fig. 2) produced by clindamycin could be a result of necrosis. The digestion of chromatin by proteases and endonucleases into smear pattern is associated with necrosis while the ladder pattern is associated with apoptosis [24]. The picture in (Fig. 2) shows smearing of DNA fragments due to non-specific DNA fragmentation which could be due to necrosis. However, clindamycin is reported to cause hepatotoxicity in rare cases [25].

Psidium guajava also did not induce DNA fragmentation but only produced DNA smearing. The reason guava did not induce DNA fragmentation could be due to the presence of several antioxidant compounds in *Psidium guajava* leaves such as 1,8 cineole and trans-caryophyllene, nerolidol, caryophyllene, beta bisabolene, p-selinene, aromadendrene, beta-sitosterol, leucocyanidin, and triterpenoids. Antioxidants have been proven to reduce free radicals which cause oxidative DNA damage [26].

CONCLUSIONS

It can be concluded that occurrence of DNA shearing in sample antibiotics is significantly

higher and more intense as compared to proven anti-microbial plants as the DNA fragments of antibiotically treated mouse traveled far as compared to the DNA of mouse treated with proven antimicrobial plants. Indeed, in this study genomic integrity of the bone marrow is more stable on mice treated proven antimicrobial plants than of antibiotic treated mice.

The cause of DNA fragmentation on the bone marrow cell of white mice is possibly because of radical formation. The less fragmentation and smearing in proven anti-microbial plants may be attributed to the phytochemicals that have antimutagenic and radical scavenging activity which is present in lagundi and guava leaves.

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