ANTI-MUTAGENIC POTENTIAL OF THE AQUEOUS EXTRACT FROM *Digitaria sanguinalis*

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ABSTRACT: *Antimutagenicity potential of the aqueous extract of Digitaria sanguinalis was evaluated using in vivo micronucleus test and in vitro Ames assay. Results indicated that the mice treated with aqueous extracts of D. sanguinalis showed no significant number of Micronucleated Polychromatic Erythrocytes (MNPCEs) relative to the positive control, methyl methanesulfonate (MMS). Mice pre-treated with the aqueous extract and subsequent treated with MMS showed suppressed MNPCEs formation, with the highest suppression observed in 10,000 ppm followed by 5,000 ppm and then 2,500 ppm, suggested that the plant extract has high antimutagenic capacity in vivo. The aqueous extract also did not cause significant number of revertant colonies of TA100 and TA104 strains of Salmonella typhimurium in Ames test, which indicated the aqueous extract's inability to induce mutation on both tester strains that reverted them from being histidine-dependent to histidine-independent. Moreover, pre-treatment with the aqueous extract and subsequent treatment with MMS showed a significant decrease in the number of revertant colonies in a concentration-dependent manner, which confirmed the antimutagenic activity of the extract. The overall results of the tests conducted have confirmed that, aside from being non-mutagenic, the aqueous extract of the aerial part of D. sanguinalis has very high antimutagenic potential. This finding made D. Sanguinalis a potential candidate for developing antimutagenic agent readily available from natural source.*

Keywords: Digitaria sanguinalis, Antimutagenicity, Ames assay, Micronucleus test

1. INTRODUCTION

Besides metabolic disorders, a spectrum of age related human diseases, including cancer, are caused by mutations [1] either inherited or through exposure to genotoxic agents [2,3]. Since cancer has become the number one cause of death, much attention has been focused on the chemoprevention of cancer and less attention has been given to substances in medicinal plants and herbal medicines that may serve to protect against chemical mutagens or carcinogens [4]. Many plant species are known to elicit antimutagenesis [5,6] and thus have a full range of prospective applications in human healthcare. The use of traditional herbal plants with chemopreventive actions could be helpful to improve life expectancy strategies due to low costs, little to no toxicity during long-term oral administration, and are relatively available at large scale. It has been suggested that regular consumption of natural anticarcinogens and antimutagens from edible and medicinal plants in the diet may be the most effective way of preventing human cancer [7]. This made search for novel antimutagens, acting in chemoprevention, a promising field in phytotherapy [8]. Herbal remedies and phytotherapy drugs, containing active principles are currently developed to protect against electrophile (e.g. free radical) attack on DNA and its widespread outcomes such as cancer. The occurrence rate of cancer is increasing worldwide and the determination of chemopreventive or chemoprophylaxis compound is important in the effort to reduce the risk of cancer. A plant extract indicating antimutagenicity is an indication that it could be a possible candidate for such purposes [9]. The importance of medicinal plants in solving health problems gains a lot of attention [10]. Its crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents.

Digitaria sanguinalis (Fig. 1) is locally known among the *Maranaos* in the Philippines as "Gemegeba". It is a species of grass known by the common name hairy crabgrass or large crabgrass. It is one of the better-known species of crabgrass, and one that is known nearly worldwide as a common weed [11]. This grass has light green sheaths, finely ribbed, shiny or dull, and hairy [12]. It produces seeds that are edible and have been used as a grain in Germany and especially in Poland, where it earned its name Polish Millet. Millets contains iron, protein, B vitamins, calcium, potassium, and fiber [11]. This weed has not been extensively explored for its biological and medicinal properties making it harder to source reported references on plant.

In this study, we reported herein the antimutagenic activity of the aqueous extract from the aerial part, except flowers and seeds, of *Digitaria Sanguinalis* confirmed by *in vitro* Ames assay and *in vivo* micronucleus test.

2. MATERIALS AND METHODS

2.1 Chemicals

Ethanol, hexane, ethyl acetate, Butanol, Distilled water, Methyl methanesulfonate (MMS), and Acridine orange were from Sigma. All chemicals are analytical grade reagents unless otherwise stated. Olympus stereomicroscope was used for scoring the coded slides in micronucleus assay.

2.2 Plant Sample Extraction

Plant sample of *D. sanguinalis* (crabgrass) was obtained from Pala-o, Iligan City. The aerial part except flowers and seeds of *D. sanguinalis* were airdried at room temperature (26°C) for two (2) weeks, after which they were ground to a uniform powder with the use of an electric blender. The ethanol extract were prepared by soaking fifty grams (50 g) of the ground plant material with 90 % ethanol at room temperature for 72 h. The obtained extract were then filtered and

concentrated using rotary evaporator with the water bath set at 40°C. The concentrated ethanol extract obtained were further subjected to sequential solvent partitioning using hexane, ethyl acetate and butanol. The ethanol extract was extracted first with n-hexane, three times, to give a fraction containing nonpolar compounds. The same procedure was also done to the residue with ethyl acetate and butanol respectively. All serial extracts of hexane, ethyl acetate, and butanol were evaporated to dryness under reduced pressure and stored in the refrigerator for future usage while the aqueous extract was freeze-dried and the dried samples were used for further testing [13].

2.3 Ethic Statement

This study was conducted in strict accordance with the recommendations for animal care and handling according to the guidelines set by the National Ethical Guidelines for Health Research of the Philippine National Health Research System, abiding RA number 8485-Animal Welfare Act of 1998 and its implementing Rules and Regulations (DA Administrative Order number 40 series of 1998 and the Code of Practice for the Care and Use of Laboratory Animals in the Philippines, 2nd edition, 2002 developed by the Philippine Association for Laboratory Animal Science (PALAS)).

Fig. 1: The Photograph of *Digitaria sanguinalis* plant English name: Crabgrass; Local name: Gemegeba. [11].

2.4 Experimental Animals

Swiss albino mice, about 7 week-old, of both sexes, weighing 10-20 grams were used as test animals in the Micronucleus test. All mice were placed in plastic cages at an ambient room temperature, with sawdust bedding in a small group according to feeding conditions. They were provided with standard food pellets and distilled water *ad libitum*. The animals were given a week to get acclimatized with the laboratory condition until the day of experiment.

2.5 Maximum Tolerated Dose Determination

The Maximum Tolerable Dose (MTD) of the aqueous extract from the aerial part except flowers and seeds of *D. sanguinalis* was initially determined by preparing a saturated solution of the extract. Three (3) Swiss albino mice were treated orally, using gavage needle, twice at 24-hours intervals, and were observed for any signs of intoxication. If there was, the dose concentration was lowered until no permanent undesirable effects were observed. Earlier to each treatment, test animals were fasted for 16 h and were extended to two (2) hours after every treatment.

2.6 Micronucleus Test

The method was according to Hayashi et al., (2000) [14] with modifications. Briefly, the test animals were divided into eight (8) groups which composed of five (5) members in a group. The first group was given with distilled water to serve as negative control. The second group received 2000 ppm MMS, which served as positive control. The third, fourth and fifth groups received the three different concentrations of the test samples (10,000 ppm, 5,000 ppm and 2,500 ppm), 0.1 mL/20 g body weight of experimental mice. Finally the sixth, seventh and eighth groups were given with the three different concentrations of the test samples and subsequently treated with MMS.

2.6.1 Peripheral Blood Cell Preparations, Staining and Scoring

Test animals blood samples were collected between 24-30 hours after the second treatment. Blood samples were collected through making a small incision on the fore skin of the mouse tail and a drop of blood was smeared on a pre-cleaned and coded glass slides. The blood smears were fixed in methanol then air dried and stained with acridine orange (AO). The micronucleated polychromatic erythrocytes were then scored using 100x magnification stereomicroscope. Five slides per mouse were prepared and scoring was done from 1000 cells for each slide [14].

2.7 Ames test

Method outlined in Mortelmans and Zeiger (2000) [15] was followed in this study with minor modification. The tester strains TA100 and TA104 were used. Three trials having three plates in each were made for each of the three different concentrations of the test samples (10,000 ppm, 5,000 ppm, and 2,500 ppm) along with 100 ppm MMS and sterilized distilled water, as the positive and negative controls, respectively. The following reagents were added to the sterile 20 mL test tubes: 0.5 mL of phosphate buffer, 0.1 mL bacterial culture, and 0.05 mL of test solution (for anti-mutagenicity: 0.05 mL MMS + 0.005 mL test solution). The mixture was mixed using a vortex. The prepared mixture was then pre-incubated for 20 minutes at 37°C. After incubation, 2 mL of top agar (with 0.5 mM histidine/biotin solution) maintained at 43-48°C was added and then mixed using vortex at low speed for 3 seconds. It was then poured onto the surface of minimal-glucose agar plates then tilted and rotated for even distribution of the mixture. The top agar was set aside to harden for an hour after which the plates were inverted and placed in an incubator at 37°C for 48-72 h and the number of visible colonies per plate was counted.

3. RESULTS AND DISCUSSION

3.1. Maximum Tolerable Dose Determination

The maximum tolerable dose (MTD) was determined to be 10,000 ppm. At this concentration, no signs of intoxications were observed from the mice treated with the saturated solution of the aqueous extract from *D. sanguinalis.* Hence, the concentration of this solution (10,000 ppm) was used as the Maximum Tolerable Dosage (MTD) of the test sample. Half and quarter concentrations of the MTD; 5,000 ppm and 2,500 ppm, respectively, were also used in the antimutagenicity and mcronucleus assays.

3.2 *In vivo* Micronucleus Test

In order to determine if the aqueous extract of *D. sanguinalis* is mutagenic, mice were treated with 10,000 ppm, 5,000 ppm, and 2,500 ppm of the extract and the presence of micronucleated polychromatic erythrocytes (MPCEs) were assessed with respect to the MMS and distilled water. After scoring, results revealed that, although significantly higher than the negative control, the micronucleus observed in mice treated with the extract were significantly lower than that of the micronucleus observed in MMS treated mice, with 2,500 ppm having MPCEs comparable to the negative control (Fig. 2). Generally, the presence of MPCEs in extract treated mice is a dose-dependent manner, hence it can be inferred that the aqueous extract of *D. sanguinalis* has no mutagenic effect *in vivo*.

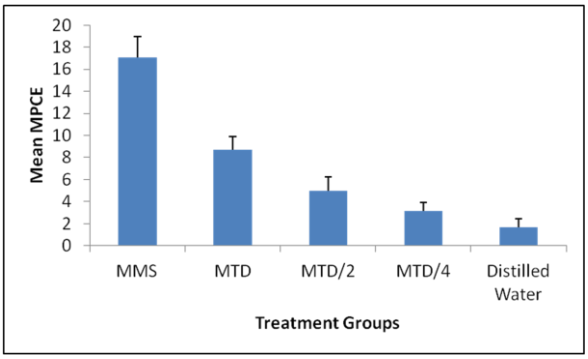

Figure 2: Non-mutagenic activity of *D. Sanguinalis* showing the number of micronucleated polychromatic erythrocytes (MPCEs) induced by the MMS and the extract of different concentrations. MTD means 10,000 ppm extract, MTD/2 = 5,000 ppm extract, and MTD/4 = 2,500 ppm extract. Values presented are the averages of the different treatment groups. Error bar means standard deviation of the trials (n=25).

The antimutagenic effect of the extract was also tested to determine if the extract will have synergistic or antagonistic effect on the mutagenicity of MMS. Groups of mice were pre-treated with different concentrations of the extracts (10,000 ppm, 5,000 ppm, and 2,500 ppm) and then subsequently treated with MMS. Results shown in Fig. 3 indicated a significant decrease in MPCEs when mice were pre-treated with the extract compared to the untreated group [16]. Noticeably, the extract's ability to suppress MPCE formation, induced by MMS, is proportional to the dosed concentration. It seems likely that the anti-mutagenic activity of the extract is at its peak at higher concentration and this trend in MPCE results is the opposite to what was found to be the activity of the extract once administered alone, shown in Fig. 2.

3.3. In vitro Ames assay

The mutagenic potential of *D. sanguinalis* aqueous extract and its effect on the mutagenicity of methyl methanesulfonate (MMS) was determined on the number of revertant colonies of the *S. typhimurium* tester strains TA100 and TA104. Different concentrations of the extracts introduced into the tester strain TA100 showed a significantly lower number of revertant colonies compared to the positive control (MMS) as shown in Fig. 4. This could indicate that the aqueous extract of *D. sanguinalis* is not mutagenic, with 5,000 ppm and 2,500 ppm concentrations of the extracts showed no significant difference. On the other hand, bacterial suspension pre-treated with the extracts and consequently exposed to MMS showed concentration-dependent decrease in the number of revertant colonies, with 2,500 ppm showed slight decrease in the number of revertants. The results also suggested that the strongest concentration of the extract produced lesser number of revertants while the lowest tested concentration of the extract only slightly decreased the revertants with respect to the MMS [17]. The same trend of results was also observed

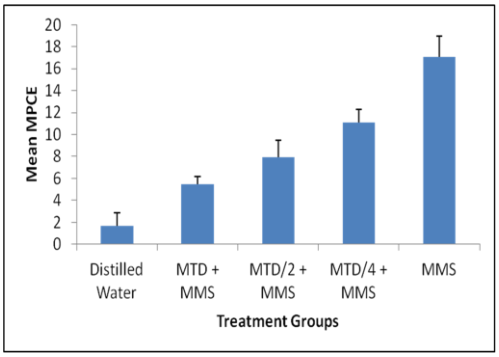

Fig. 3: Anti-mutagenic activity of *D. Sanguinalis* showing its inhibitory activity against the formation of micronucleated polychromatic erythrocytes (MPCEs) induced by MMS. MTD+MMS means 10,000 ppm extract + 2000 ppm MMS, MTD/2+MMS means 5,000 ppm extract + 2000 ppm MMS, and MTD/4+MMS means 2,500 ppm extract + 2000 ppm MMS. Values presented are the averages of the different treatment groups.

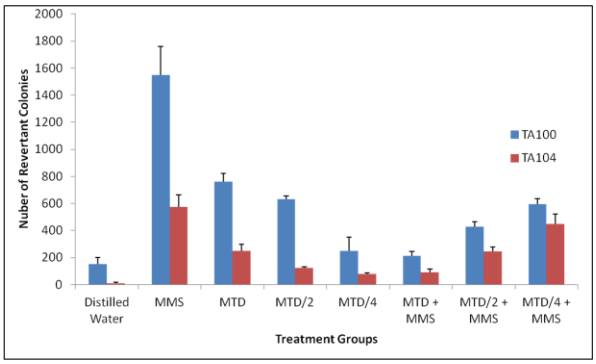

Fig. 4: Reversion of *Salmonella typhimurium* tester strains TA100 and TA104 by the *D. sanguinalis* aqueous extract and their positive and negative control groups by plate incorporation method. *D. sanguinalis* aqueous extract showed less number of revertant colonies alone, while in the presence of MMS the extract was able to decrease the number of revertant colonies affirming its anti-mutagenic activity. MTD means 10,000 ppm extract, MTD/2 = 5,000 ppm extract, and MTD/4 = 2,500 ppm extract. MTD+MMS means 10,000 ppm extract + 100 ppm MMS, MTD/2+MMS means 5,000 ppm extract + 100 ppm MMS, and MTD/4+MMS means 2,500 ppm extract + 100 ppm MMS. Values presented are the averages of the different treatment groups. Error bar means standard deviation of the trials (n=9).

in the TA 104 strain. Noticeable inhibition of the mutagenic activity of the MMS was exemplified in bacterial culture pre-treated with the extracts, thereby giving clear trend in suppressing the mutagenic effect of MMS.

4. CONCLUSION

This study demonstrated the efficacy of the aqueous extract of *D. sanguinalis* as antimutagenic. *In vivo* micronucleus test and *in vitro* Ames Assay confirmed that the aqueous extract did not cause spontaneous mutations. The aqueous extract of *D. sanguinalis* also suppressed mutations caused by MMS by reducing the number of MPCEs in mice and the number of revertant bacterial colonies of *S. Typhimurium* in a concentration-dependent manner. In both *in vivo* and *in vitro* tests, it was established that the extract showed higher antimutagenic activity at higher concentration. The increased concentration of the extract decreased the mutation caused by MMS, while doses of extracts alone generally didn't show any significant mutations towards the test organisms. This finding made *D. Sanguinalis* a potential candidate for developing antimutagenic agent readily available from natural source.

5. ACKNOWLEDGEMENT

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