Green Farming Vol. **7** (2) : 357-360 ; March-April, 2016 **Research Paper**

Anticlastogenic potential of pigeonpea (*Cajanus cajan* (L.) Millsp.) in white mice (*Mus musculus* L.)

G.C. SANCHEZ[a1], N.C. TANQUILUT[a2], M.R.C. TANQUILUT[a3], H.M. SORIANO Jr.[a4], M.G. MULA[b5*] and R.P. MULA[6]

[a] *Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines*

[b] *International Crop Res. Instt. for Semi-Arid Tropics, (ICRISAT) Patancheru- 502 324 Hyderabad (Andhra Pradesh,*

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ABSTRACT

This study investigated the anticlastogenic potential of International Crop Research Institute for Semi-Arid Tropics (ICRISAT) bred pigeonpea utilizing the micronucleus test using white mice models to determine the number of micronucleated polychromatic erythrocytes (MPCEs) in treated and non-treated white mice at the Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines. Furthermore, the study disclose if pigeonpea has detrimental effects on the vital signs and some vital organs such as lungs, heart, liver, kidney and intestine. Based on the study, pigeonpea ICPL 87051 leaves decoction have anticlastogenic effects. This might be attributed to the presence of flavonoids, tannins and stilbenes in pigeonpea leaves that has the ability to lower MPCEs in treated and non-treated white mice. In terms of its effects on the vital signs namely heart rate, respiratory rate and temperature, results showed that pigeonpea did not cause heart palpitation, tachycardia, hyperpnea and hypothermia. Treatments T+ (Positive control, TCN) and T_2 (Pigeonpea leaves extract of 0.5 per 20 kg body weight + TCN) have normal heart, liver, kidney, lungs and intestines.

Key words : *Anticlastogenic, Micronucleated polychromatic erythrocytes, Pigeonpea, White mice.*

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is both utilized as food crop (dried peas, flour, green vegetable peas) and as a forage crop. They contain high levels of protein and the important amino acids methionine, lysine, and tryptophan. In combination with cereals, pigeonpea make a well-balanced human food (Mula and Saxena, 2010). The dried peas may be sprouted briefly, then cooked, for a flavor different from the green or dried peas. Sprouting also enhances the digestibility of dried pigeonpeas via the reduction of indigestible sugars that would otherwise remain in the cooked dried peas.

In India, split pigeon peas (*toor dal*) are one of the most popular pulses, being an important source of protein in a mostly vegetarian diet. In regions where it grows, fresh young pods are eaten as a vegetable in dishes such as *sambar*. In Ethiopia, not only the pods, but also the young shoots and leaves are cooked and eaten. During the last few decades extensive studies have been carried out regarding the chemistry of *C. cajan* and considerable progress has been achieved regarding its biological activities and medicinal applications.

Chemical constituent investigations have indicated that C. *cajan* leaves are rich in flavonoids and stilbenes. They also contain saponins, conspicuous amount of tannins, and moderate quantities of reducing sugars, resins and terpenoids. Chemical studies reveal 2'-2' methyl cajanone, 2'-hydroxy genistein, isoflavones, cajanin , cahanones etc., which impart antioxidant properties. Roots are also found to possess genistein and genistin. It also contains hexadecanoic acid, a amyrin, ß-sitosterol, Pinostrobin, longistylin A and longistylin C which impart anticancer activity (Pal *et al.,* 2011a).

Cajanol, an isoflavanone from *C. cajan* roots is an important phytoalexin. The anticancer activity of cajanol towards MCF-7 human breast cancer cells was investigated.

[1] Professor & Director for Knowledge, [2] Associate Prof. and Dean
[3] Associate Professor and Director for Research & Development
[4] University President III, [5] Sr. Scientist *(m.mula@cgiar.org)
[6] Special Project Scientist

In order to explore the mechanism of cell growth inhibition of cajanol some parameters like cell cycle distribution, DNA fragmentation assay and morphological assessment of nuclear change, reactive oxygen species (ROS) generation, mitochondrial membrane potential disruption were investigated (Pal *et al.*, 2011).

The search for non-toxic natural anticlastogens should be extended through the systematic screening of the unexplored rich diversity of pigeonpea. Hence, using micronucleus test which is an *in vivo* assay that detects the chromosome breaking effects of certain substance to the somatic cell was utilized in this study that aimed to determine the anticlastogenic property of pigeonpea in white mice.

MATERIALS AND METHODS

Experimental animals: Twenty (20) female white mice (*Mus musculus* L.) aged two months old weighing 20-30 grams were used in the study in 2013 at the Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines. They were placed individually in polypropylene plastic cages and were given two weeks adjustment period to acclimatize them to the laboratory environment. The experimental animals were fed with beef pro feeds while distilled water was provided fresh everyday. They were given individual clay feeders and beddings consisted of rice hull changed every two weeks.

The study was laid out in a Completely Randomized Design (CRD) and distributed randomly with five replicates per-treatment. The treatments are the following

T+ – (Positive control) - Tetracycline (TCN) at the rate of 0.50ml per 20 kg body weight;

T_0 – (Negative control) - Distilled water;

T_1 – ICPL 87051 pigeonpea leaves decoction (0.25 ml per 20 kg body weight) + TCN (0.50ml per 20 kg body weight)

T_2 – ICPL 87051 pigeonpea leaves decoction (0.50ml per 20 kg body weight) + TCN (0.50ml per 20 kg body weight)

Data gathered were recorded, tabulated and analyzed using the Analysis of variance (ANOVA). Treatment means were compared using the Least Significant Difference at 1% and 5% level of significance.

Preparation of pigeonpea leaves decoction : In the preparation of extract, the protocol of Sala and Sanchez (2006) was adopted in this study. Briefly, fresh young and mature leaves of pigeonpea (ICPL 87051) were collected. The volume of concentrated extract was expressed in gram/formula using the following formula:

$$\text{Extract concentration} = \frac{\text{Weight (g) pigeonpea extract}}{\text{Volume of the pigeonpea extract (ml)}}$$

Monitoring of feed, water intake, body weight and vital signs prior to the treatment of mice, the initial vital signs such as heart rate, respiratory rate and temperature were taken. Furthermore, the initial body weight was recorded. The vital signs such as temperature and heart rate were monitored on a weekly basis using digital rectal thermometer and stethoscope, respectively. The respiratory rate was obtained by counting the number of breaths per minute using a stop watch.

Micronucleus test proper : The protocol by Schmidt (1976) was utilized in this study. The micronucleus test was done to study the effects of tetracycline in combination with pigeonpea on the chromatin material of the bone marrow cells. Eight weeks post treatment, the mice were sacrificed by cervical dislocation six hours after the last treatment. Immediately after sacrificing the animals, both femora were removed by cutting through the pelvis and tibia. The bones were then freed from the muscles. The proximal end of the femur was shortened using sterile scissors until a small opening to the marrow became visible. The specific protocol of micronucleus test was then employed.

Staining was done a day after using 100% May-Gruenwald solution for 3 minutes then, transferred to a 50% May-Gruenwald solution for another 2 minutes. The slides were then washed with distilled water before they were stained for 10 minutes with 15% Aqueous Giemsa stain solution. After staining, the slides were rinsed with distilled water and blotted dry with a tissue paper before drying in air.

The stained slides were read. The staining of the mature erythrocytes (normocytes) must be red and a strong bluish tint in the young (polychromatic erythrocytes). One thousand cells per slide were counted using the Battlement method of counting giving a total of 3000 cells per rat. Polychromatic cells exhibiting micronuclei were scored. The numbers of MPCEs of the three slides per mouse was averaged with resulting figure recorded as the score of the animal.

To be considered positive for clastogenic effects, the number of MPCEs produced in the positive control must be twice or more than twice than those of the negative control.

RESULTS AND DISCUSSION

Number of Micronucleated Polychromatic Erythrocytes (MPCEs): Results of this study revealed that pigeonpea leaves were capable to reduce significantly ($P<0.01$) the number of MPCEs in clastogen-induced mice as shown in **Table 1 and Plate 1.** The negative control (T_0),

Table 1. Effects of pigeonpea on the chromosome-breaking potential of tetracycline in bone marrow cells of mice based on the number of micronucleated polychromatic erythrocytes (MPCEs)

Treatment	Mean number of MPCEs
T+	12.78a
T_0	1.75c
T_1	4.47b
T_2	3.08b

Means having the same letter are not significantly different at (P< 0.05)

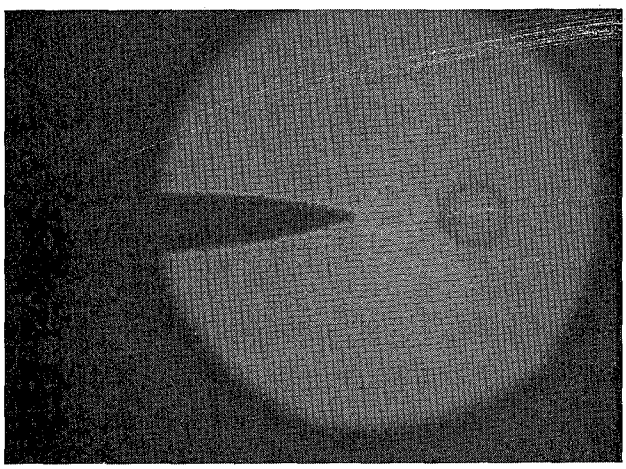

Plate 1. Micronucleated polychromatic erythrocytes (MPCEs) from bone marrow cells at 200x magnification

which received only distilled water, had 1.75 and was followed by T_2 and T_1 with 3.08 and 4.47 mean values of MPCEs, respectively. The positive control however, had more than thrice the number of MPCEs at 12.78.

The known mutagen used in the study was tetracycline (TCN) which acts by blocking the attachment of aminoacylB transfer ribonucleic acid (RNA) to messenger RNA complex, thereby, interfering with protein synthesis, as cited by Sylianco (1990). It acts as clastogen by alkylating the bases of deoxyribonucleic acid, thus, fragmenting the chromatin material resulting in numerous number of MPCEs as shown in **Fig. 1**. Chemical constituent investigations have indicated that *C. cajan* leaves are rich in flavonoids and stilbenes. They also contain saponins, conspicuous amount of tannins, and moderate quantities of reducing sugars, resins and terpenoids (Pal *et al.*, 2011a). This might be one of the reasons why there was reduction in the number of MPCEs on treated mice which supports the claim of Zhang *et al.* (2010) that tannins shown to have powerful antioxidant activity which protect cells against damage.

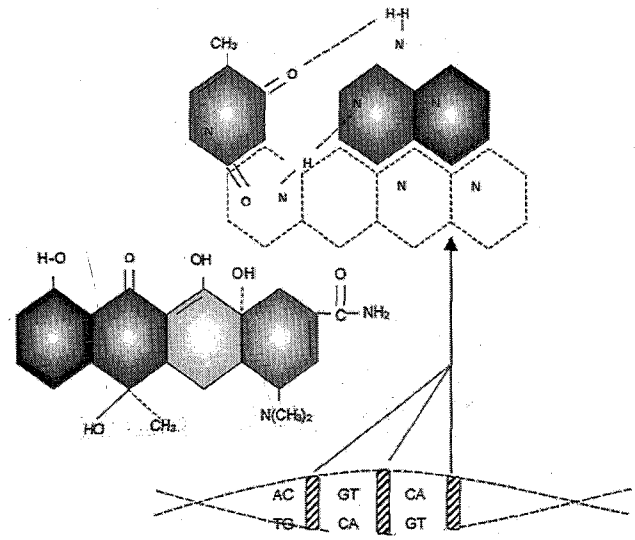

Fig. 1. Intercalating activity of Tetracycline in the DNA structure

Vital signs (Heart rate, respiratory rate and temperature) : In terms of its effects on the vital signs such as heart rate, respiratory rate and body temperature, results are within the normal as revealed in **Tables 2, 3 and 4.** Pigeonpea did not produce adverse effects on the vital signs of mice. The findings documents absence of adverse effects in the body such as heart palpitation, hyperpnea or increased rate of breathing, and or hyperthermia or hypothermia. Thus, has been shown to be safe and effective to use at this dosage level. The respiratory rates are a little higher for those that are active and have undergone exercise however, after taking the breaths per minute at rest, results revealed that they are within the normal values.

Table 2. Effects of pigeonpea on heart rate in clastogen-induced mice (beat/minute)

Treatment	Total	Mean
T+	3500	700
T_0	3550	710
T_1	3500	700
T_2	3500	700

Reference value Heart rate : 450-750

Table 3. Effects of pigeonpea on respiratory rate in clastogen-induced mice (breath/minute)

Treatment	Rest	Exercise/Active
T+	218	296
T_0	216	294
T_1	218	295
T_2	217	295

Reference value Respiratory rate : 200-220/minute

Table 4. Effects of pigeonpea on temperature in clastogen-induced mice (°C)

Treatment	Temperature (°C)
T+	37.5
T_0	37.5
T_1	37.5
T_2	37.5

Reference value Temperature rate: 37.5 (°C)

Effects on gross appearance of organs of mice that were sacrificed post treatment: Table 5 presents the gross appearance of vital organs post treatment of tetracycline (T+); distilled water alone (T_0) ; and T_1 and T_2 pigeonpea treatments at 0.25ml and 0.50ml per 20 g body weight, respectively. As revealed, T+ and T_2 have normal heart, liver, kidney, lungs and intestines. Pale liver, lungs and intestines were observed in the negative control (T_0) while T_1 have pale lungs observed in three mice.

For the tetracycline-induced mice, the absence of pathological gross lesions could be attributed to the action of the antibiotic while for the T_2 or those mice treated with 0.50ml per 20 gram body weight, the action of pigeonpea can be attributed to coumarin cajanuslactone. Luo *et al.* (2010) claimed that a new natural coumarin cajanuslactone has been isolated from the leaves of *C. cajan* which is a potential antibacterial agent against Gram-positive micro-organisms.

Table 5. Gross appearance of different organs post treatment

Organs	Treatment			
	T+	T-	T_1	T_2
Heart	Normal appearance	Normal appearance	Normal appearance	Normal appearance
Kidney	Normal appearance	Normal appearance	Normal appearance	Normal appearance
Liver	Normal appearance	Pale	Normal appearance	Normal appearance
Lungs	Normal appearance	Pale	Pale	Normal appearance
Intestine	Normal appearance	Pale	Normal appearance	Normal appearance

CONCLUSION

The research showed that pigeonpea leaves have anticlastogenic effects. This might be attributed to the presence of flavonoids and tannins that has the ability to lower MPCEs in clastogen-induced bone marrow cells. In terms of its effects on the vital signs namely: heart rate, respiratory rate and temperature; pigeonpea leaves decoction showed no adverse effects and does not cause heart palpitation, tachycardia, hyperpnea and hypothermia. The absence of abnormal gross lesions for T+ (Tetracycline) could be attributed to the action of antibiotic tetracycline. While for T_2 (0.50ml per 20 kg body weight) + TCN, coumarin cajanuslactone which is a potential antibiotic for gram positive organisms, respectively. T_1 (pigeonpea leaves extract of 0.25ml per 20 kg body weight + TCN) which has lower dosage rate can explain for the presence of pale lungs that might have been infected with respiratory organisms. Moreover, it is possible that there are chemical substances in pigeonpea that can shield the interaction of TCN with base pairs in DNA, however, this needs further investigation.

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