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Research Article

Determination of Genotoxicity of Aqueous Extracts of *Flueggea leucopyrus* Willd. (Katupila) Using the Optimized "Alkaline Comet Assay"

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Abstract

Purpose: The comet assay is a simple, rapid, versatile, sensitive and cost effective method used to measure DNA damage caused by a suspected genotoxic agent. In this study, genotoxic potential of *Flueggea leucopyrus* Willd., which is used in traditional medicine to treat diseases, including cancers was evaluated using the optimized alkaline comet assay.

Methods: Aqueous extracts of *Flueggea leucopyrus* Willd. leaves (from two different samples isolated from Anuradhapura (FLA) and Maharagama (FLM) was used to evaluate genotoxicity on White Blood Cells (WBC) and Hepato cellular carcinoma cell line (HepG2) cells, using the comet assay. The parameter used to measure the level of genotoxicity in the above cell types was the % tail DNA after treatment with aqueous extract of *Flueggea leucopyrus* Willd. Different concentrations of FLA and FLM samples (0.001 mg ml^{-1}, 0.005 mg ml^{-1}, 0.01 mg ml^{-1}, 0.02 mg ml^{-1}) were prepared and the effect on WBC and HepG2 cells was evaluated separately, and compared to a negative control (HBSS for WBC and culture media for HepG2) and positive control (200 µM H_2O_2 for WBC and 50 µM H_2O_2 for HepG2). The results were analyzed by one way ANOVA followed by Dennett post hoc test using SPSS software.

Results: As the concentration of the aqueous extract was increased, the % tail DNA of both WBC and HepG2 cells increased gradually, compared to the negative controls (p<0.05).

Conclusion: Aqueous extracts of *Flueggea leucopyrus* Willd. appear to be moderately genotoxic at high concentrations, but the effect is insignificant at lower concentrations.

Keywords: *Flueggea leucopyrus* Willd, Single cell gel electrophoresis/comet assay, Genotoxicity, WBC (White Blood Cells), HepG2 (Hepato cellular carcinoma cell line)

Introduction

DNA is the heritable macromolecule, which carries information essential for life and has numerous functions such as long-term storage of information, control of cellular functions and carrying of the genetic code for protein synthesis. Failure of repair mechanisms of DNA plays a vital role in mutagenesis, carcinogenesis, aging and other chronic diseases.(1) There are several ways by which DNA can be damaged and include oxidative damage, DNA strand breaks due to exogenous and endogenous agents *etc.*(1),(2)

Oxidative DNA damage in living cells is a result of formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), due to metabolic and other biochemical reactions or due to external factors.(2) Any changes in the level of ROS, cellular antioxidants or efficiency of DNA damage repair causes modulations of steady-state level of oxidative DNA, which are especially abundant in the case of tumors.(2) Presence of high levels of antioxidants in plasma and a diet rich in antioxidants are supposed to reduce the risk of cancer and other chronic diseases(2) by its potential to scavenge free radicals, anti-cancer, anti-inflammatory and anti-aging activities.(3),(4) It has been found that fruit juices rich with antioxidants show a strong free radical scavenging capacity using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assay and β-carotene bleaching assay under *in vitro* conditions, but not under *in vivo* conditions.(6) Another assay has demonstrated that none of the antioxidant phytochemicals produce a clear decrease in the tumor promoting activity of 3,-3',4-,4'-tetrachlorobiphynyl in rats.(7)

Genotoxicity is the destructive effect on cell's genetic material (DNA and RNA) affecting its integrity, leading to mutations in different types of cells, that could eventually lead to cancer or a wide variety of diseases.(8) Genotoxins are substances that have genotoxic properties. Primarily, genotoxins are of three types, classified based on their effect on the genetic information of an organism: carcinogens (cancer causing agents), mutagens (mutation causing agents) and teratogens (birth defect causing agents). DNA damaging agents can be used as anti-cancer agents in the treatment of cancer.(8) One of the main targets in anti-cancer drugs is to arrest the cell cycle and prevent cell proliferation, by causing a high level of DNA damage. High level of DNA damage initiates cell cycle check points leading to arrest of cell cycle and cell death.(1) Some of the most often used DNA damaging agents are alkylating agents, platinum drugs, antimetabolites, topoisomerase inhibitors and ionizing radiation.(1) Therefore it is essential to measure the degree of various types of DNA damage to have a better understanding of the mechanism of action in the cell to respond to various substances, including genotoxic agents.

Single cell gel electrophoresis assay/ comet assay is one of the techniques available to evaluate *in vivo* and *in vitro* genotoxicity and it is the most commonly used test to measure the degree of DNA damage, as it is rapid, sensitive, versatile and cost effective.(9),(10),(11) The comet assay was first introduced by two Swedish scientists, Ostling and Johanson in 1984 to detect a wide variety of DNA damage at the individual cell level in eukaryotes. Later in 1988, a modified version of the comet assay, the alkaline comet assay was introduced by Singh *et al*, which is the most widely performed version of the comet assay. This assay reveals the presence of alkali-labile sites, in addition to SSB (Single strand breaks) and DSB (Double strand breaks).(8),(10),(11),(12)

Plants produce phytochemicals as a secondary metabolites from primary metabolites, which mainly contain bioactive compounds such as flavonoids, alkaloids, terpenoids, phenolic compounds, steroids *etc*.(13) There are many different types of secondary metabolites in the plant kingdom and these play a vital role in the plant life cycle by mediating interactions with other organisms.(13) Thus plant secondary metabolites are suspected to be involved in interactions with plant pollinators, pathogens and as a form of self-defense

against plant herbivores.(13) Some of these phytochemicals have beneficial properties such as having anti-proliferative, anti-oxidative, anti-bacterial, and anti-fungal activities and powerful effects on humans and therefore used as medicines.(13),(14),(15)

Flueggea belongs to Euphorbaceae, a genus in the Kingdom Plantae and is mostly distributed in tropical and subtropical regions, with approximately 550 - 750 species.(16) Investigators have discovered bergenin as a constituent of *Flueggea*. It was shown to have antifungal properties against plant pathogenic fungi and anti-arrhythmic activity.(15),(17)

Flueggea leucopyrus Willd. (Katupila) is a bush weed and an erect shrub, which grows up to 1.5 – 4 m in height. Branches are cylindrical or obtusely angular in young plants. The stem and leaves of the plant exude a white substance.(13) The leaves are dark green and small in size while the flowers are small and light green. Mature fruits are white and small and the seeds are very small.(13),(18) *Flueggea leucopyrus* Willd. preparations have been used to treat many diseases including cancers, hay asthma, diarrhea, cough, constipation and the juice of the leaves is used to destroy maggots in sores in the traditional medicine system of Sri Lanka. It is also used as fish poison.(13),(14),(19),(20) The plant is found in the both wet and dry zones of Sri Lanka, Southern India, China, and other tropical and subtropical countries.(13),(14),(21)

This plant has recently gained popularity due to its application in malignancy.(13) The mature leaves and bark of the plant are normally used to prepare decoctions.(13) Many research groups have conducted studies on the chemical constituents and unique properties of *Flueggea leucopyrus* Willd(13). According to these studies, the plant contains alkaloids, unsaturated sterols and triterpenes, saponins, glycosides, phenolics, terpenoids, tannins, flavonoids and carbohydrates.(13) It is a potential candidate for commercial supply of bergenin on an industrial scale.(22) Extracts of leaves and fruits of the plant have also been successfully used to biosynthesize silver nano particles.(18) *Flueggea leucopyrus* Willd. has been shown to be anti-proliferative, anti-oxidative and anti-fungal, and also to have dose dependent cytotoxicity in HEp2, HepG2 and A2780 cell lines and to be lethal in brine shrimp lethality bio assay.(14),(19),(23) Phonetic variations of Katupila in different climatic zones have also been studied.(21)

As *Flueggea leucopyrus* Willd. is widely used as an anti-cancer drug in traditional medicine, its activity may be due to its genotoxic effects. This study was carried out with the objective of evaluating the degree of genotoxicity to both healthy cells (WBC) and tumor cells (HepG2 cells) by measuring the % tail DNA using the comet assay. The effectiveness of using *Flueggea leucopyrus* Willd. for anti-cancer treatment was evaluated by using aqueous extracts of the same concentration as used in traditional medicine. The extent of DNA damage was determined at various concentrations of the extract. Plants were also obtained from different geographical locations to determine if any differences in genotoxicities.

Methods

Preparation of plant extract

Fresh mature leaves collected from Anuradhapura and Maharagama were washed with distilled water and dried under indirect sun light. Leaves (60 g) were boiled in 1920

ml of distilled water until the final volume was reduced to 240 ml under mild heat.(24) The extract was filtered and 10 ml aliquots were prepared and freeze dried. Stock solutions of the extract (5 mg ml^{-1}) were prepared by re-dissolving the freeze dried samples in HBSS and stored at -20 ˚C until use.

Cell line and cell culture

HepG2 cells were cultured in Eagles Minimum Essential Medium (EMEM) containing 10% heat inactivated Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, 1% L-glutamine and 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) at pH 7.4. Cells were maintained at 37 ˚C in a 5% CO_2 and 95 % humidified carbon dioxide incubator. Cells were passaged every 3-4 days after reaching 75% confluency. The harvested cells were treated with the plant extract.

Treatment of cells

Both white blood cells and HepG2 cells were selected for treatment with the plant extract.

Treatment of WBC: Blood (1 ml) was collected from volunteer blood donors and 20 µl was taken into a 1.5 ml eppendorf tube (approximately 2×10^4 Cells) and treated with varying concentrations of the freeze dried extract (0.001 mg ml^{-1}, 0.005 mg ml^{-1}, 0.01 mg ml^{-1}, and 0.02 mg ml^{-1}). Treatment was initiated within 2 hours of blood withdrawal to minimize the stress caused to cells, which can lead to overestimation of genotoxicity. For the positive control cells were treated with H_2O_2 (200 µM) and negative control contained cells treated with HBSS. Each trial was run with a positive and negative control. All steps after the withdrawal of blood was carried out in the dark to avoid light induced DNA damage. All tubes were incubated at 4 ˚C for 30 min.

Treatment of HepG2

After trypsinization of cells and cell counting, cells were centrifuged and re-suspended to contain 2×10^4 cells in 50 µl and 50 µl of the cell suspension was treated with 0.001 mg ml^{-1}, 0.005 mg ml^{-1}, 0.01 mg ml^{-1}, and 0.02 mg ml^{-1} concentrations of the extract separately. A positive control (cells treated with H_2O_2 (50 µM)) and negative control (cells treated with culture media) was included in the assay. All the tubes were incubated at 4 ˚C for 30 min. After the incubation period, cells were analyzed by the comet assay and also used for cytotoxicity testing.

Determination of cell viability of WBC and HepG2 cells by Trypan blue dye assay

After the incubation period the tubes were centrifuged at 2000 rpm for 5 min. The supernatant was discarded and cell pellet was re-dissolved in 100 µl of Hank's Balanced Sodium Salt Solution (Ca2+, Mg2+ free) (HBSS) and culture media for WBC and HepG2 respectively. An aliquot (20 µl) of the cell suspension was mixed with an equal volume of 0.4 % Trypan blue dye and kept for 3 min at room temperature. The mixture was diluted with equal volume of media and 10 µl of the mixture was loaded to a hemocytometer and observed under an inverted light microscope at 40X. The number of viable cells and the total number of cells were counted and cell viability was calculated using the following equation.

$$\% \text{ of viable cells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

All test concentrations including positive and negative controls gave cell viabilities above 80%.

Alkaline comet assay

As described above after the incubation period and centrifugation, the cell pellet was re-suspended in 100 µl of 1% low melting point agarose (LMPA) at 37 ˚C and immediately 50 µl of the solution was placed on microscopic slides precoated with 1% normal melting point agarose (NMPA) and a cover slip was immediately placed over the suspension, without introducing any air bubbles. Each treatment group was duplicated and microscopic slides were kept on petri plates resting on ice for 10 min in a dark room. Then coverslips were gently slipped off and another layer of 1% LMPA (50 µl) was placed on the gel and allowed to solidify, to obtain a layer of cells embedded in LMPA. The coverslip was gently removed and microscopic slides were gently slid into a pre-chilled coplin jar covered with aluminium foil and pre chilled lysis buffer was added to completely cover the slides. This was incubated for 90 min at 4 ˚C protected from light. The microscopic slides were then placed horizontally in a pre-chilled electrophoresis gel tank. Pre-chilled freshly prepared unwinding buffer was added to completely cover the slide upto a height of 1 - 2 mm above the slide and incubated at 4˚C for 30 min in the dark. The voltage gradient was then set to 1.15 V cm^{-1} and amperage to 300 mA. Electrophoresis was carried out for 30 min at 4 - 10 ˚C. The slides were then dipped in neutralization buffer at 4 ˚C for 5 min and same step was repeated twice. Slides were then air dried for 20 min. Dehydration of the gel was carried out by adding pre-chilled methanol drop wise on to the gel followed by air drying. They were then stored at 4 ˚C or visualized on the same day. The slides were visualized by adding 30 µl of 20 µg ml^{-1} ethidium bromide as the staining dye and observed under the 40X magnification using a fluorescent microscope. Comet images were captured using a digital microscopic eye piece camera (S/N 140129 – 319 CU 3.2M CMOS camera) fixed onto the eyepiece of the microscope. After visualization and image capturing, the coverslip was gently removed and the stain was removed by rinsing the microscopic slide with absolute methanol. Microscopic slides were then air dried and stored at 4 ˚C.

Quantification and data analysis

Tests were carried out in two triplicates with samples collected from different regions of the country (Anuradhapura and Maharagama) using WBC (in whole blood) and HepG2 cells. Each trial was inclusive of a positive and negative control and each treatment was carried out in duplicate on the same slide. For each slide 50 randomly selected comets were analyzed (25 comets per gel, two gels per slide). Images were analyzed using CaspLab software (casp_1.2.3b 1) and % tail DNA of the comet was selected as the parameter for image analysis. Mean, median and standard deviation was calculated and data was analyzed by one way ANOVA followed by Dennett post hoc test using SPSS software.

Results and Discussion

Results of cytotoxicity test

Dosage for each treatment group was determined after conducting cytotoxicity assays using the Trypan blue, to avoid overestimation of genotoxicity due to apoptotic cells. In the Trypan blue dye assay, viable cells appear clear while nonviable cells appear blue due to the uptake of dye by the damaged cell membrane. Based on the published guidelines to perform a genotoxicity assay, the cell viability should at least be 80%. Both human blood cells and

HepG2 cells used in the study had a viability of > 80%.

Table 1: Viability of WBC and HepG2 cells after treatment with *Flueggea leucopyrus* Willd. (30 min)

	Positive control	**Negative control**	**0.001 mg ml^{-1}**	**0.005 mg ml^{-1}**	**0.01 mg ml^{-1}**	**0.02 mg ml^{-1}**
Viability of WBC	96%	99%	99%	98%	98%	97%
Viability HepG2 of cells	87%	99%	99%	95%	97%	96%

WBC, White Blood Cells; HepG2, Hepato cellular carcinoma cell line

Result of the genotoxicity test

WBC (in whole blood) treated with different concentrations of the extract resulted in significant DNA damage compared to the negative control ($p<0.05$) and a dose dependence was observed (Table 2). Similarly HepG2 cells treated with the different concentrations of the extract resulted in significant DNA damage compared to the negative control (Table 3). However the extent of the damage in WBC and HepG2 at the same concentration of the extract were different. This is likely due to the nature of the cells where HepG2 is a tumor cell line. This has also been demonstrated in the positive control (treated with 50 µM H_2O_2) where the DNA damage in HepG2 was more extensive than WBC (Figure: 1 (A), Table 2 and Figure: 2 (A), Table 3). Mean values of % tail DNA in treated groups for all the test concentrations in both WBC and HepG2 were lower (20 - 50 %) compared to the positive controls. A gradual increase of DNA damage with increase in extract concentrations was also observed.

While handling WBC, whole blood was used instead of isolated and cultured lymphocytes to avoid genotoxicity induced by sample handling and to provide *in vivo* conditions as much as possible. However a certain degree of DNA damage could also be observed in the negative controls, for both cell types (WBC and HepG2). This may be due to stress generated on samples under *in vitro* conditions.

In contrast to the positive control both cell types show a higher degree of damage in normal cells than malignant cells. A significant difference in the extent of damage was not observed for the different geographical isolates.

The generally consumed dose of *Flueggea leucopyrus* Willd. extracts in single component decoctions was estimated approximately to be 0.01 mg ml^{-1} and in poly herbal decoctions, 0.001 mg ml^{-1}. From the findings of this study, it was found that concentrations used for poly herbal decoctions do not appear to be significantly genotoxic whereas the concentration in single component decoction appears to be marginally genotoxic. Therefore long term consumption of high doses could result in a higher level of DNA damage, due to long term exposure and due to possible accumulation of components in the extract. *Flueggea leucopyrus* Willd. aqueous extract shows a higher level of % tail DNA in blood compared to HepG2. In that sense, plant extract has a higher potential to damage the healthy cells than malignant cells.

The anti-proliferative, antioxidant and cytotoxic properties of *F. leucopyrus* Willd. have been studied previously, using different methods such as MTT (3,4,5-(dimethylthiazol-2-yl)2-5-diphenyl tetrazolium bromide) and LDH (Lactate dehydrogenase) assays and protein synthesis with HEp-2 cells after a 24 hour exposure. DNA fragmentation and microscopic examination of cells stained with a mixture of ethidium bromide/acridine orange were used to visualize apoptosis in HEp-2 cells treated with *F. leucopyrus* Willd.(14) The results obtained show DNA fragmentation in HEp-2 cells after 24 hour exposure to the plant extract, indicating the ability to induce apoptosis, which is consistent with the results obtained in the comet assay. However the experiment was conducted only on malignant cells and therefore the effect on healthy cells cannot be compared.

Another study showed that the leaves and the bark of *F.leucopyrus* Willd. are rich in a number of important phytochemicals such as alkaloids, terpenoids, steroids, flavanoids, saponins, phenol and glycosides.(13) Flavonoids and phenolic compounds have been proven to have anti-cancer properties, which could be the reason behind the genotoxic properties of *F.leucopyrus* Willd. Further investigations are necessary to identify the individual components responsible for genotoxic properties of *F.leucopyrus* Willd.

Research related to the leaf phenolic composition of *F.leucopyrus* Willd. representing different climatic zones have clearly shown intra specific variations in morphology and the distribution of phenolics in leaves.(21) However a significant variation was not observed in this study in the samples obtained from different climatic zones. However a conclusion cannot be drawn in this regard, as only a limited number of samples were analyzed.

Table 2: Tail (%) DNA of WBC exposed to *Flueggea leucopyrus* Willd. (30 min).

		Mean ± SD					
		Positive control	**Negative control**	**0.001 mg ml^{-1}**	**0.005 mg ml^{-1}**	**0.01 mg ml^{-1}**	**0.02 mg ml^{-1}**
Sample FLA	**Trial 1**	50.44 ± 5.60	24.43 ± 4.12	25.68 ± 2.86	29.45 ± 3.57	36.56± 3.77	39.75± 4.33
	Trial 2	45.00± 4.83	22.40 ± 4.15	24.22± 3.60	27.20 ± 3.50	29.13± 3.80	31.52 ± 4.24
	Trial 3	43.14± 5.23	15.32± 3.16	19.16± 6.12	22.10± 5.23	27.43± 5.145	38.06± 5.00
Sample FLM	**Trial 1**	40.36± 4.64	14.45± 3.67	29.00± 4.34	30.53± 6.83	37.24± 8.47	39.00± 5.41
	Trial 2	42.80± 6.20	14.61± 3.97	22.39± 3.38	23.41± 8.35	24.32± 6.33	24.80± 5.45
	Trial 3	40.60± 5.40	22.50± 6.15	24.60± 6.90	31.26± 7.37	31.50±9 6.60	35.04± 5.85
Average	**FLA**	46.20± 5.22	20.71± 3.81	23.02± 4.20	26.24± 4.10	31.04± 4.24	36.44 ± 4.52
	FLM	41.25± 5.40	17.20± 4.60	25.33± 4.90	28.40± 7.52	31.01± 7.12	32.94 ± 5.57
	Both	43.72± 5.30	18.95± 4.20	24.20± 4.53	27.32± 5.80	31.03± 5.70	34.70± 5.04

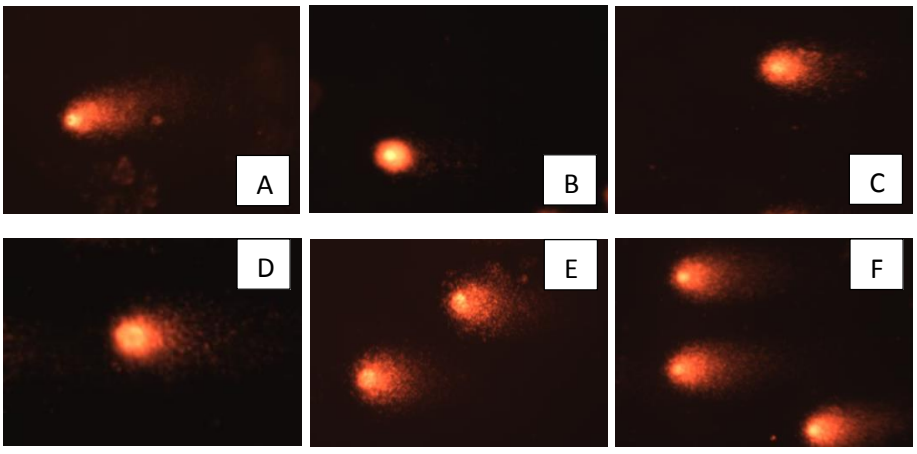
FLM, *Flueggea leucopyrus* Willd. leaves from Maharagama; FLA, *Flueggea leucopyrus* Willd. leaves from Anuradhapura; WBC, White Blood Cells

Table 3: Tail (%) DNA of HepG2 cells exposed to *Flueggea leucopyrus* Willd. (30 min)

		Mean ± SD					
		Positive control	**Negative control**	**0.001 mg ml^{-1}**	**0.005 mg ml^{-1}**	**0.01 mg ml^{-1}**	**0.02 mg ml^{-1}**
Sample FLA	**Trial 1**	62.80 ± 16.84	5.87 ± 1.94	8.73 ± 2.34	10.59± 2.63	12.07 ± 3.93	28.01 ± 5.061
	Trial 2	64.04 ± 15.10	8.50 ± 2.46	9.88 ± 2.66	11.16± 2.55	13.51 ± 3.61	25.63 ± 11.39
	Trial 3	52.73 ± 16.82	10.03 ± 2.95	11.14± 2.22	12.80 ± 2.90	14.50± 1.81	19.84 ± 3.31
Sample FLM	**Trial 1**	65.58 ± 18.67	5.21 ± 1.55	8.40 ± 2.10	11.23 ± 3.28	12.87 ± 2.43	25.38 ± 5.45
	Trial 2	66.10 ± 16.32	13.75 ± 3.67	14.05± 2.73	15.14 ± 4.83	18.70± 2.63	24.96 ± 5.00
	Trial 3	59.75 ± 16.74	14.94 ± 1.95	16.63± 2.76	18.90 ± 2.95	19.87 ± 4.10	29.21 ± 6.14
Average	**FLA**	59.86 ± 16.25	8.13 ± 2.45	9.92 ± 2.41	11.51 ± 2.70	13.36 ± 3.12	24.50 ± 6.60
	FLM	63.81 ± 17.24	11.30 ± 2.40	13.02 ± 2.53	15.10 ± 3.70	17.14± 3.05	26.52± 5.52
	Both	61.83 ± 16.75	9.72 ± 2.42	11.47± 2.47	13.30± 3.20	15.25± 3.08	25.50 ± 6.06

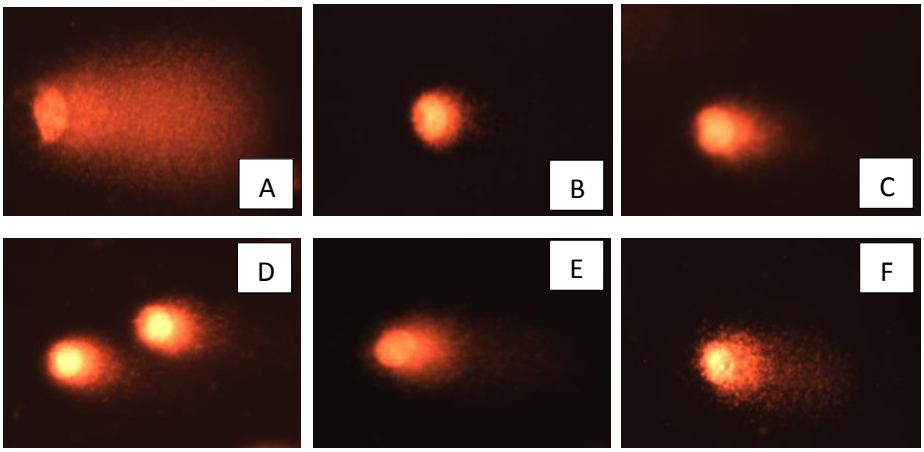
FLM, of *Flueggea leucopyrus* Willd. Leaves from Maharagama; FLA, of Flueggea leucopyrus Willd. leaves from Anuradhapura; WBC

Figure 1: Results of images of the comet assay for WBC exposed to *Flueggea leucopyrus* Willd.(30 min). (40X).



A: WBC exposed to 200 µM H_2O_2 (positive control)
B: WBC exposed to HBSS (negative control)
C: WBC exposed to *Flueggea leucopyrus* Willd. 0.001 mg ml^{-1}
D: WBC exposed to *Flueggea leucopyrus* Willd. 0.005 mg ml^{-1}
E: WBC exposed to *Flueggea leucopyrus* Willd. 0.01 mg ml^{-1}
F: WBC exposed to *Flueggea leucopyrus* Willd. 0.02 mg ml^{-1}

Figure 2: Results of images of the comet assay for HepG2 cells exposed to *Flueggea leucopyrus* Willd. (30 min) (40X)



A: HepG2 cells exposed to 50 µM H_2O_2 (positive control)
B: HepG2 cells exposed to complete media (negative control)
C: HepG2 cells exposed to *Flueggea leucopyrus* Willd. 0.001 mg ml^{-1}
D: HepG2 cells exposed to *Flueggea leucopyrus* Willd. 0.005 mg ml^{-1}
E: HepG2 cells exposed to *Flueggea leucopyrus* Willd. 0.01 mg ml^{-1}
F: HepG2 cells exposed to *Flueggea leucopyrus* Willd. 0.02 mg ml^{-1}

Conclusion

At all concentrations tested extracts of *F.leucopyrus* Willd. was genotoxic to WBC (in whole blood) and HepG2 cells. An increase in genotoxicity with increasing concentration of the extract was observed. The effect was more pronounced in WBC than HepG2. At doses generally consumed the extract appeared to be marginally genotoxic.

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References

1. Swift LH, Golsteyn RM. Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells. Int J Mol Sci. 2014;15:3403–31.
2. Bont R De, Larebeke N Van. Endogenous DNA damage in humans : a review of quantitative data 1. Mutagenesis. 2004;19(3):169–85.
3. Zhang Y, Gan R, Li S, Zhou Y, Li A, Xu D. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules. 2015;20:21138–56.
4. Wang S, Meckling KA, Marcone MF, Kakuda Y, Tsao R. Can phytochemical antioxidant rich foods act as anti-cancer agents. FRIN. 2011;44(9):2545–54.
5. Li P, Zhang H, Chen J, Shi Y, Cai J, Yang J, et al. Level and risk of gastric cancer. Int J Cancer. 2014;1–10.
6. Kulisic-Bilusic T, Schnabele K, Schmoller I, Dragrovic-Uzelac V, Krisko A,

Dejanovic B, Mladen Milos GP. Antioxidant activity versus cytotoxic and nuclear factor kappa B regulatory activities on HT-29 cells by natural fruit juices. Eur Food Res Technol. 2009;228:417–24.

7. Tharappel JC, Lehmler H, Srinivasan C, Robertson LW, Spear BT, Glauert HP. Effect of antioxidant phytochemicals on the hepatic tumor promoting activity. Food Chem Toxicol. 2008;46(11):3467–74.
8. Shah SU. Importance of genotoxicoty & S2 A guideline for genotoxicity testing for pharmaceuticals: Cardiovascular disease Cancer Genetic diseases. IOSR J Pharm Biol Sci. 2012;1(2):43–54.
9. Collins AR. The Comet Assay for DNA Damage and Repair. Mol Biotechnol. 2004;26.
10. Azqueta A, Collins AR. The essential comet assay : a comprehensive guide to measuring DNA damage and repair. Springer. 2013;1984.
11. Kang SH, Kwon JY, Lee JK, Seo YR. Recent Advances in In Vivo Genotoxicity Testing : Prediction of Carcinogenic Potential Using Comet and Micronucleus Assay in Animal Models. J Cancer Prev. 2013;18.
12. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen. 2000;35:206–21.
13. Bulugahapitiya VP, Munasinghe AB, Hettihewa M. Investigation of chemical composition of *Flueggea leucopyru*s Willd . World J Pharm Pharm Sci. 2014;3:79–94.
14. Soysa P, De Silva IS, Wijayabandara J. Evaluation of antioxidant and antiproliferative activity of *Flueggea leucopyrus* Willd. (katupila). BMC Complement Altern Med. 2014;14:274.
15. Prithiviraj B, Singh UP, Manickam M, Srivastava JS, Ray AB. Antifungal activity of bergenin , a constituent of *Flueggea microcarpa*. Plant Pathol. 2000;46:224–8.
16. Summanen JO. A Chemical and Ethnopharmalogical Study on *Phyllanthus emblica* (Euphorbiaceae). 1999. 15-17 p.
17. Gan L, Fan C, Yang S, Wu Y, Lin L. Flueggenines A and B , Two Novel C , C-Linked Dimeric Indolizidine Alkaloids from *Flueggea virosa*. Org Lett. 2006;8:2285–8.
18. Donda MR, Rao K, Alwala J, Miryala A, Sreedhar B, Pratap MP. Synthesis of silver nanoparticles using extracts of *Securinega leucopyrus* and evaluation of its antibacterial activity. Int J Curr Sci. 2013;1–8.
19. Hettihewa, LM, Munasinghe, MMAB, Bulugahapitiya, VP KN. Dose dependent anti proliferative and cytotoxic effects of *Flueggea leucopyrus* willd. against human ovarian carcinoma; mts and human telomerase enzyme inhibition. Eur J Biomed Pharm Sci. 2015;2:14–8.
20. Solangaarachchi SM. Floristic composition and medicinally important plants. J Natn Sci Coun Sn Lanka. 1993;21:209–26.
21. Priyadarshan A.I.S, Senanayake SP, Jayatileke MP R. Intraspecific variation of *Flueggea leucopyrus* Willd. grown in Sri Lanka and establishing a suitable propagation system. J Sci. 2014;9:15–25.
22. Wijayabandara MDJ, Choudhary MI, Wijayabandara MDLO. Isolation of bergenin from the leaves of *Flueggea leucopyrus* willd (katupila) – a novel method of obtaining bergenin . Pharm J Sri Lanka 2015. 2015;10–5.
23. Bulugahapitya, VP, Maduranga, S and Paranamana N. Investig cytotoxity *Fluggea leucopyrus* using Brine Shrimp

Cytotoxity Assay. Proceedings of the 9 th Ruhuna Science Symposium. 2013;13.
24. Thero GSD. Kashaya Sangrahaya. B.A.D.Manoris Appuhamy; 1927.