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Investigating genotoxicity of *Eleusine indica* by micronuclei assay in albino rats

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Abstract

Genotoxicity of *Eleusine indica* (Nkim enang: Efik) was investigated in the Wister strain albino rat (*Rattus novergicus*). Nine (9) male and nine (9) female rats were randomly assigned to three (3) groups, of which two were exposed to the aqueous extract of *E.indica* – Group A (control-no extract)), Group B (50 mg / kg BW of *E. indica*) and Group C (100 mg / kg BW of *E. indica*). This was administered to the rats by oral gavage for 14 days after which the peripheral blood from the tail tips were collected and assayed for the presence of micronuclei, following standard procedures. Proximate analysis and phytochemical screening of the herb extract was carried out. Results obtained showed that *E. indica* did not cause any significant ($P > 0.05$) increase in the incidence of micronucleated polychromatic erythrocytes in rat peripheral blood at any of the doses administered. The polychromatic: normochromatic erythrocyte (PCE: NCE) ratio was found to be in the range of 0.50 ± 0.11 to 0.55 ± 0.02. Also, the aqueous herb extract is rich in Carbohydrates (76.17%) and Tannins (21.76%). Mean body weights (MBW) of rats showed normal distribution throughout the duration of the investigation. The results of this study demonstrate that *E. indica* does not confer any genotoxicity in mammals. Further in-depth study on its efficacy is recommended.

Keywords: *Bull grass, Medicinal plants, Toxicology, Nucleated cells, Negative genotoxicity*

1. Introduction

Eleusine indica is primarily listed as an agricultural and environmental weed (Randall, 2012) and is considered a "serious weed" in at least 42 countries (Holm et al., 1979). It is a species of grass in the family *Poacaceo* with a small annual grass distributed throughout the warmer areas of the world to about 50^0 latitude. It is an invasive species in some areas. *E. indica* invades disturbed habitats in natural areas and the margins of natural forests and grassland, marshes, stream, banks and coastal areas. *Eleusine indicia* is called several names depending on where it is found. Such names include: Bull Grass, Crabgrass, Crowfoot grass, dog grass, clutch grass, Iran grass, Ox grass, Silver grass, Wild finger millet, Wire grass, and Yard grass (Holm et al., *1979*). In Nigeria, some of its common names are: Nkim-enang (Efik), Ichite (Igbo), Horki or Thiawa (Hausa) and Gbengin (Yoruba). It is a tufted annual grass, prostrated and spreading, or erect to about 40cm, depending on the density of vegetation but not usually rooting at the nodes (Plate 1). The whole plant,

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especially the root is considered diaphoretic and antipiuretic. Decoction of plant is used for treating convulsion in children. It is much used in liver complaints (Yusuf et al., 2009).

The plant is a component of the basic remedy in Vietnamese traditional medicine. It is also used in the treatment of influenza, hypertension, oliguria and retention of urine. The plant is applied externally to open wounds to stop bleeding. The whole plant is boiled with black sage for use in a sitting bath to treat fevers, colds, malaria, and for post-childbirth cleansing of a mother. The plant can be used fresh or dried, for de-worming, coughs, lungs trouble, dysentery, heart problems, bladder and kidney stones, spleen and liver complaints, and high blood pressure (Christensen and Kharazmi, 2001). The plant is also used for rituals in the local communities by Native doctors in Akwa Ibom, and Cross River State (Okokon et al., 2010). The grass, when tender, is eaten by cattle, goat, dogs, rats, chicken, etc., for abdominal disorder (Holm et al., 1979). Phytochemical studies of *Elusine indica* indicated the presence of sterol glucoside forms and C-glycosylflavone. It also contains cyanogenetic glucoside, triglochinin, ochratoxin A, a-amylase inhibitors, Albuminoids, starch and fatty oil. Other components are phenolic compounds and flavonoids (Ghani, 2003).

Plate 1: *Eleusine indica* (Field Work, 2017)

Apart from its medicinal properties and ethnobotany, *E. indica* is consumed as a vegetable. Hence the need to investigate the genotoxic effect(s) if any**,** of the herb on a mammalian model. The Albino rat (*Rattus norvegicus*) is used in this study.

2. Materials and Methods

The study was carried out in the Animal House of the Biological Sciences Department, Cross River University of Technology (CRUTECH) Calabar, in Calabar South Local Government Area of Cross River State, Nigeria. Fresh leaves of *Eleusine indica* were collected from the botanical garden in Cross River University of Technology Calabar, Cross River State, Nigeria. The plants were identified and authenticated by Dr. Sam Udo, a Botanist in the Department of Biological Sciences. The fresh leaves (5 kg) of the plant were air dried on laboratory table under room temperature for three days and pulverized in a blender to powder one thousand grams (1000 g) of power were soaked in 500 ml 95% ethanol for 72 hours, and then filtered with a 0.2 mm mesh filter cloth. The liquid filtrate obtained was oven dried at 40^0C overnight (Etta et al., 2007). The paste was stored in a refrigerator at 4^0C until commencement of research.

A total of eighteen (18) mature (8 weeks old) albino rats of the wistar strain (nine males and nine females) were obtained from the Cross River University of Technology Animal House for this study. The rats were randomly housed in standard wooden cages with barbed wired tops, in groups of threes (3s). They were maintained on standard animal pellets and water *ad libitum* under standard temperature and relative humidity with a 12-h light / dark cycle and allowed to acclimatize for seven days. Permission and approval for animal studies was obtained from the College of Health Science, Animal Ethics Committee, Calabar.

The completely randomized design (CRD) was used as the experimental design for this study. The experimental animals were arranged into three groups viz A, B, and C with three (3) Male and three (3) female rats in each group.

Both Phytochemical Screening and Proximate Analysis of the extract were carried out, according to Soforowa (1993); Trease and Evans (1989), before the feeding of the rats commenced. The initial body weights of the animals were measured on commencement of the research and after administration of the test substance. The control animals were not treated with the plant extract while group B animals were treated orally with 50 mg / kg bw of the extract and group C with 100 mg / kg bw of the extract for fourteen days. 24 hours after the

last administration, peripheral blood samples were collected from the tail tips of both treated and untreated rats in heparinized bottles, for further analyses according to the OECD 474 protocol, 1997.

The micronucleus test is a comprehensive, quick and sensitive method for measuring DND damage – micronuclei (MNi). Microneuclei are chromatin-containing structures in cytoplasm surrounded by a membrane without any detectable link to the cell nucleus. They are formed by exclusion of whole chromosomes or chromatin fragments during cell division. MNi are scored specifically in once-divided binucleated cells. The micronuclei are biomarkers of chromosome breakage and / or whole chromosome loss. Scoring of micronuclei damage is from 5000 MNi, per cell.

2.1. Non-fluorescent Staining for Manual Microscopical Evaluation

The dry slides were stained with 5% Giemsa solution for 3-5 minutes, washed properly with water and dried at room temperature according to Fenech and Morley, 1985. The numbers of micronucleated normo chromatic erythrocytes (MNMCE) were determined by blind counting 2000 normo chromatic / polychromatic erythrocytes (NCE / PCE) per animal cell, under a microscope. Photo micrographs of analyzed slides were obtained using Canon 3050 camera.

3. Data Collection and Analyses

Data obtained from this investigation was analyzed statistically using Levenes' T- test and T-test for equality of means. Differences between the means were considered significant at 1% and 5% level of significance.

4. Results

Results of the proximate analysis and phytochemical screening of the aqueous extract of *Eleusine indica* are presented in Tables 1 and 2 respectively, with carbohydrate as the highest proximate component (76.17%) and fiber as the lowest (2%) and tannins (21.76%) and alkaloids(1.8%) as the highest and lowest phytochemicals.

Table 1: Proximate analysis and phytochemical screening of aqueous extract of *Eleusine indica*

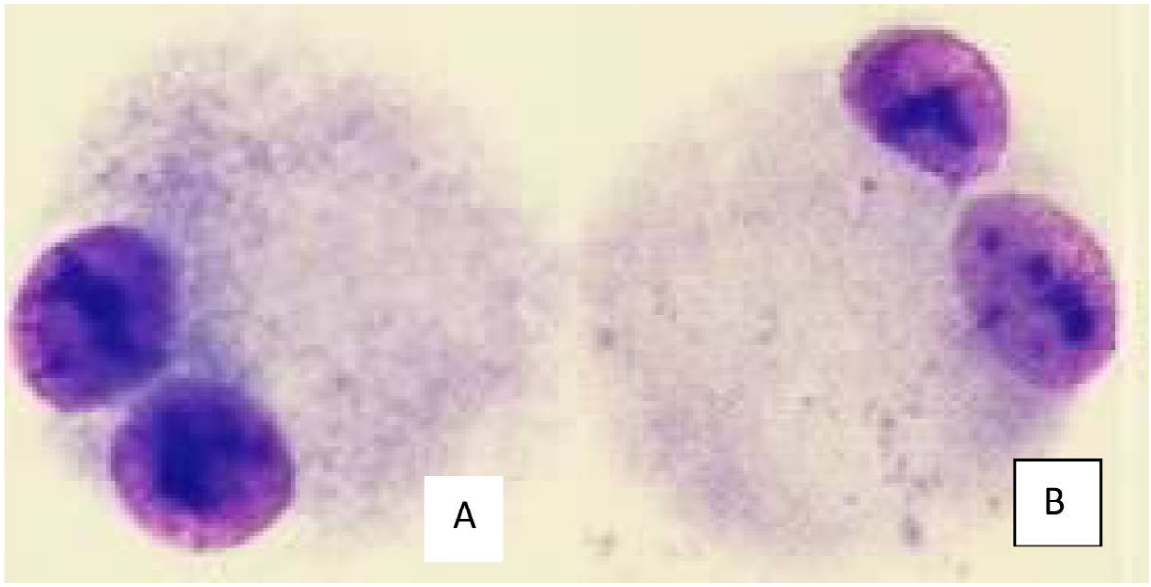
Components	Value (%)	Phytochemicals	Values(%)
Moisture content	48	Alkaloids	1.8
Ash content	10	Saponins	2.0
Fibre	2	Flavonoids	6.0
Fat	6	Tannins	21.76
Protein	6.8		
Carbohydrate	76.17		

Table 2: Mean ± SE of body weights of rats after administration of aqueous extract of *E. indica*

Parameter		N	Mean ±SE	SD	Sig.
Body weight:	Male	9	234.89±14.269	42.794	0.003
	Female	9	178.11±11.246	33.126	

The results of the Mean ± SD of body weights of rats fed aqueous extracts of *Eleusine indica* is presented in Table 2. The results present the mean body weight ± SE of the male rats as being higher than that of the female rats, 234.89±14.269 and 178.11±11.246 respectively.

Photomicrographs of the bi nucleated (BN) cells with or without micronuclei are presented on Plates 2-4. Results show maximum of two micronuclei formed in treated groups with none in the control group.



Pate 2: Control male (a) and female (b) showing bi nucleated cells with no micronuclei

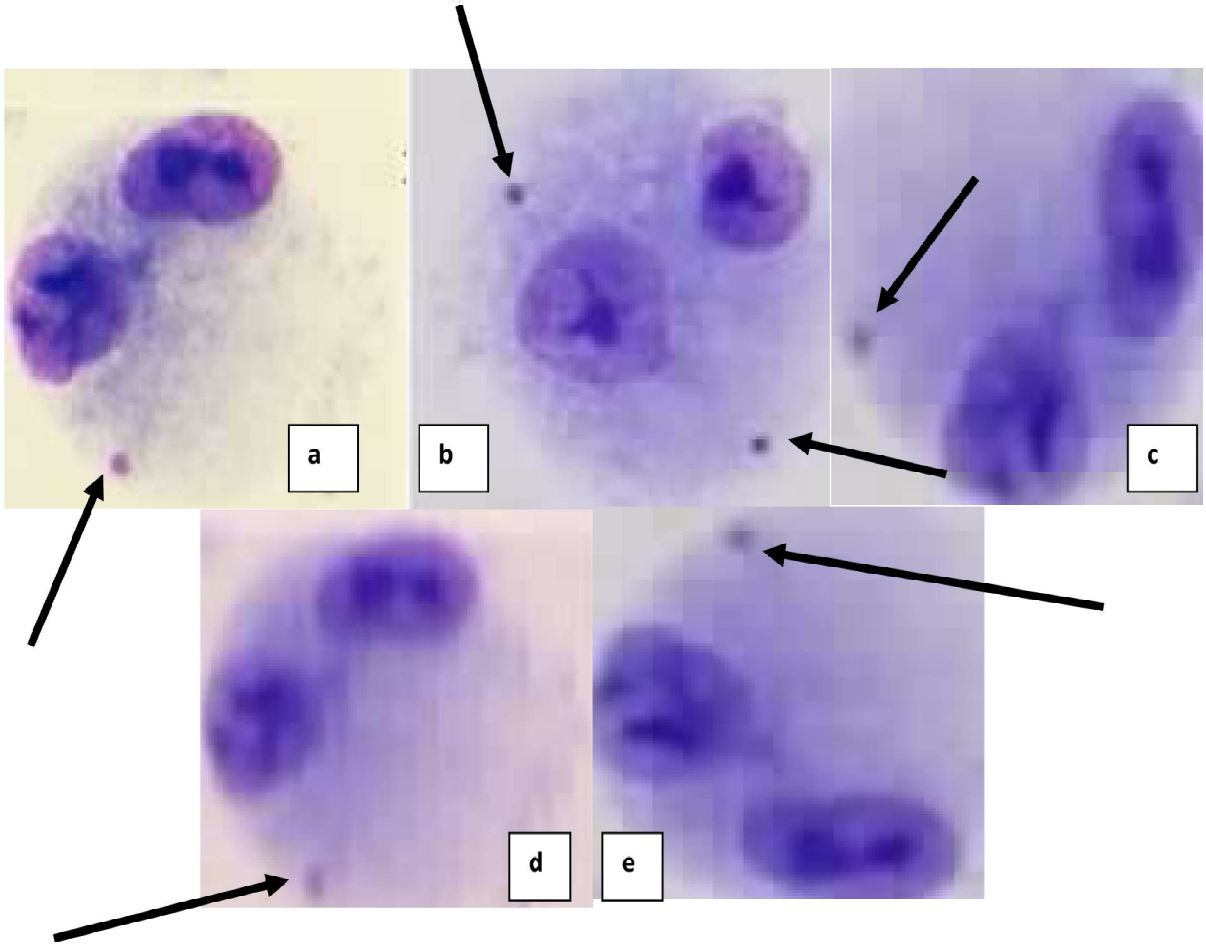


Plate 3: Group B Males and Females- a – binucleated cell with one MN; b – binucleated cells with two MN; c- bnucleated cell with one MN; d - binucleated cell with one MN and e - binucleated cell with one MN

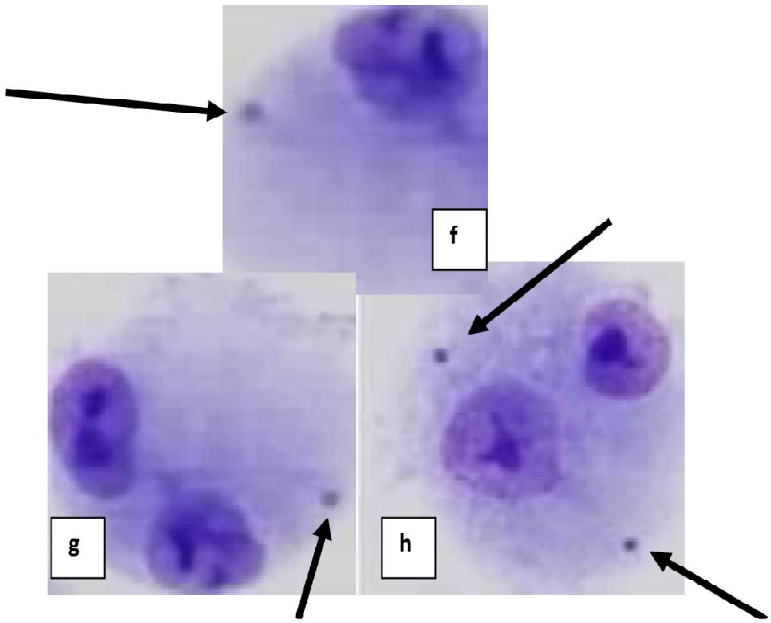


Plate 4: Group C males and females- f – cell with one MN; g – binucleated cells with one MN and h - binucleated cell with two MN

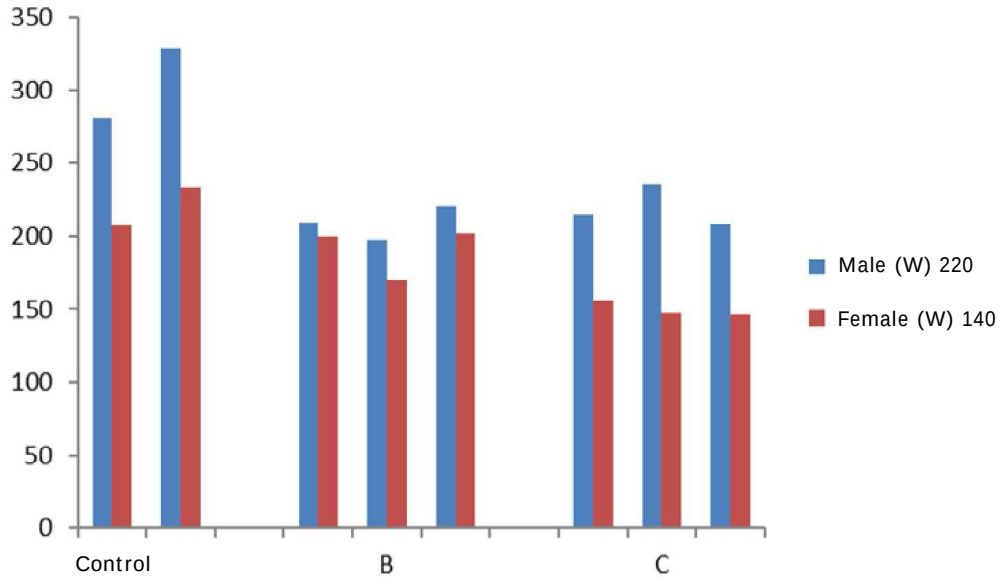


Figure 1: Bar chart for the distribution of male and female albino rats in the study

Comparative binomial test for categories and Kolmogorov – smirnor tests were carried out to compare the effect of the herb extract, if any, on the sexes and results are presented in Table 3.

Table 3: Effect of extract on sexes

Sex	Test	Sig.	Decision
Male: Female	One sample Binomial	1.000	Sex does not affect the effect of the plant on the rats.
Male: Female	One sample Kolmogorov – smirnor	0.709	Sex does not affect the effect of the plant on the rats.

5. Discussion

5.1. Proximate Analysis and Phytochemical Screening of aqueous extract of Eleusine indica

The proximate analysis from this study showed that *E. indica* is rich in carbohydrates (76.17%). This result is higher than that reported by Shodhganga (2012), where the carbohydrate content was 72.5%. The Carbohydrate content in this study is almost the same as the carbohydrate content in rice (78.2%) (Shodhganga, 2012). High-fiber carbohydrates improve bowel transit time and reduce risk of colorectal cancer. Carbohydrates are not essential in the human diet, but because foods rich in carbohydrate are abundant and cheap, compared with fats and protein, they naturally form a major part of the diet in most of the world. The four-fifth's of the world's population relies mainly on plant food. Carbohydrates provide at least 70% and often up to 90% of the total caloric intake. They are the most abundant biomolecules produced on earth. Regmi et al. (2004) reported crude protein and Ash content of *E. indica* as 12.4%and 35.8% respectively. In this study, ash content was 10% and protein content 6.8%, values quite lower than those reported by Regmi et al. (2004), but in agreement with some other studies where the protein content was recorded as 8.06% (Shodhganga, 2012). Proteins are an essential component of the diet needed for survival of animals and humans. The basic function of proteins in nutrition is to supply adequate amounts of needed amino acids. According to Umezawa (1989), the protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption and minimal obligatory rates of oxidation. Cereals and millets are moderate sources of protein as they contain only about 10% protein (Gopalan et al., 1989). Hence, the protein content of *E. indica* in our study is acceptable. Madibela and Modiakgotla (2004) recorded 15.1% ash and 8.29% crude protein in one accession of finger millet; these values are slightly higher than what is reported here. *Eleusine indica,* in a research by Shodhganga (2012) contained moisture-12.04%; protein-8.06%; fat-2.48%; carbohydrate-72.6 %; crudefiber-4.26%; and energy-345 kcal. In his study, the moisture content was 48%, fat 6% and fiber 2%. The fiber content in food is important because it protects the body against duodenal ulcers (Aldoori et al., 1997), cancer and may affect intestinal immune function (Terry et al., 2001). Trowel (1985) submitted that dietary fiber is the skeletal remains of plant cells that are resistant to digestion by man's enzymes. Fats are a chemically diverse group of compounds that are insoluble in water and have a variety of functions. Oils and fats are the principle stored forms of energy in many organisms, (Lehninger et al., 1993). The phytochemical screening in this study showed the presence of alkaloids (1.8%), saponins (2.0%), flavonoids (6.0%) and tannins (21.76%). Homburger (1989) reported that the ethanolic extract of the herb was confirmed to have a negligible acute toxicity and contains all the phytochemicals reported in this study. Tannins were recorded as the highest phytochemicals (21.76%), in this study. The presence of flavonoids indicates the presence of polyphenolic secondary metabolites in the extract. The low value of the alkaloids confirms the selective antiviral activity reported by Abdul et al. (1996). Saponnins, as reported in this study, are known to be antifungal (Cowan, 1999).

The mean body weights of the experimental animals from this study show that the males tolerate the extract to a larger extent than the females. It can thus be assumed that *E. indica* aqueous extract did not confer any systemic toxicity at the doses administered. The Levene's test for equality of variances and the independent *t*-test both confirmed these assumptions. The rich reserve of oily saponins may have also aided the weight increase in the treated rats as saponins are known to suppress ammonia production in the body, hence relieving metabolism and increasing the feed efficiency in animals (Westendarp, 2005; and Aregheore, 2005).

Further tests, binomial test for categories refined by group (male and female) and Kolmogorov–Smirnor test were carried out to confirm the non-toxicity of the extract on the body weight of the treated animals (Table 3). Independent sample median tests, to establish the effect of the extract on the sexes of treated animals yielded a non-significant ($P>0.05$) result, confirming that observed effect on the sexes was non-sex dependent. The result of the micronucleus analysis is as presented on Plates 2, 3 and 4. A micronucleus test is a test used in toxicological screening for potential genotoxic compounds (Wikipaedia, 2015). In this study, control animals had no micronuclei formed in their erythrocytes. On the other hand, treated rats had 1,000 or 2,000 micronuclei formed in their erythrocytes. However, scoring of micronuclei damage is from 5,000 MNi/cell (OECD, 1997). Hence, there was no genotoxicity conferred on the treated animals by the aqueous extract of *E. indica* (L) . That notwithstanding, according to French et al. (1999), the presence of 1000 and 2000 MNis indicate the presence of acentric chromosome fragments or extruding whole chromosomes, from the main nucleus of the cell. Daiji et al. (1989) recorded similar results in mice fed irradiated diets. Reddy et al. (1981) also reported lack of micronucleus formation in bone-marrow cells of mice fed on irradiated diet for 7 days. Our results corroborate the reports of Amorin (1995). Vijayalaxmi and Sadasivan (1975), on the other hand, reported an increase in

chromosomal anomalies in bone-marrow cells in rats which were fed on irradiated wheat. From the bar chart (Figure 1), it was established that the mean distribution of the sexes, by 3 replications for the control (A) B, and C treatment groups, is considered normal. This is an indication that the effect of the herb extract on the sexes of treated animals was not significant.

6. Conclusion

In conclusion, this investigation revealed the safety of *E. indica* (L) as food for both human and animal consumption. The aqueous extract conferred no adverse effect on the body weight of treated animals and did not induce any genotoxic effects in the blood cells of treated animals. Hence, the herb is safe as food or food supplement. Similarly, it is all right for medicinal purposes, especially as famine food. However, its use as a medicinal plant should be with the usual caution accorded medicinal plants to avoid overdosing and the subsequent adverse effects.

References

Abul farah, M., Ateeq, B, Niamat Ali, M., Ahmad, W. (1996). Introduction of Micronuclei and erythrocyte alterations in the catfish clarias acid and butacheior. *Mutat Res*. 518, 135-144.

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. *Crit Rev Oral Biol Med*. 13, 184-196.

Bisht and Mukai. (2002). Genome organization and Polypoid evolution in the genus *Eleusine* (Poaceae). *Plant Systematic and Evolution*. 233 (3 / 4), 243-258.

Christensen, S.B., Kharazmi, A. (2001). Antimalarial natural products. Isolation, characterization and biological properties. In *Bioactive compounds from natural sources: Isolation, characterization and biological properties.* Ed. Tringali, C. London, Taylor & Francis. 379-432.

Cihak, R. (1979). Evaluation of benzidine by the micronucleus test, *Mutation Research*. 11 (6), 212-230.

Cummings, R. W. (2016). *Eleusine indica*. Natural Resources Conservation Service PLANT Database USDA. *Retrieved, 2016.*

Cole, R.J., Taylor, N., Cole, J., Arlett, C.F. (1981). Short-term tests for transplacentally active carcinogens. 1. Micronucleus formation in fetal and maternal mouse erythroblasts, *Mutat Res.*. 80 (1), 141-157.

Condori-Penaloza, E. M., Costa, S. S. (2016). Characterization of Main Phenolic Compounds in *Eleusine indica* L. (Poaceae) by HPLC-DAD and 1H NMR. World Academy of Science, Engineering and Technical. *International Journal of Agricultural and Biosystems Engineering*. 3 (6).

Fenech, M., Morley, A. A. (1985). Measurement of micronuclei in lymphocytes. *Mutat Res*. 147. 29-36.

Heddle, J.A., Hite, M., Kurkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program, *Mutation Research*. 123, 61-118.

Hossan Md. Shahadat, Abu Hanif, Bipasha Agarwala, Md. Shahnawaz Sarwar, Masud Karim, M. Taufiq-Ur Rahman, Rownak Jahan and Mohammed Rahmatullah (2010). Traditional use of medical plant in Bangladesh to treat urinary tract infections and sexually transmitted diseases. *Ethnobotany Research and Applications*. 8, 61-74.

Homburger, F. (1989). *In vivo* testing in the study of toxicity and safety evaluation. *In: A guide to General Toxicology*. Marquis, J. K. (Ed). 2nd E. EdnKarger, New York.

Kliesch, U., Danford, N., Adler, I.D. (1981). Micronucleus test and bone-marrow chromosome analysis. A comparison of 2 methods in vivo for evaluating chemically induced chromosomal alterations, *Mutation Research*. 80, 321–332 .

Lorenzi ., Jeffery (1987). *Weeds of the United States and their control*. New York, USA; Van Nostrand Reinhold Co. Ltd. 355, 152-154.

OECD Protocol (1997). Safety Assessment of new food result of an OECD survey of serum banks for all engenicity testing and used of databases. Paris Organization for Economic cooperation and Development (OECD). http: / / www.oils.oecd.org / oils.1997doc.nsf.link to NTooooc6A / SFILE / JT00121603 pdf

Okokon, J.E., Odomena, C.S., Imabong, E., Obot J., Udobang J.A. (2010). Department of Pharmacology and Toxicology. Faculty of Pharmacy, University of Uyo, Uyo. Akwalbom State, Nigeria.

Regmi, P.R. , Devkota, N.R., Jagadish Timsina. (2004). Re-growth and Nutritional Potentials of Eleusine indica (L.) Gaertn. (Goose Grass). Acoustics, Speech, and Signal Processing Newsletter, *IEEE*. 25DOI: 10.3126/jiaas.v25i0.387

Recio, L., Bauer, A., Faiola, B. (2005). Use of genetically modified mouse models to assess pathways of benzene-induced bone marrow cytotoxicity and genotoxicity. *Chemico-Biological Interactions*, 153-154, 159-164.

Salamone, M. F., Mavourin, K. H. (1994). Bone marrow micronucleus assay: A review of the mouse stocks used and their published mean spontaneous micronucleus frequencies. *Reprod Toxicol*. 3, 115–125.

Sato, S., Tomita, I. (2001). Short-term screening method for the prediction of carcinogenicity or chemical substances: Current status and problems of an *in vivo* rodent Micronucleus Assay. *Journal of Health Science*, 47 (1), 1–8.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa.* 2nd ed. Spectrum Books Limited, Ibadan, Nigeria.

Sturbell *et al*. (2010). Food security: the challenge of feeding 9 billion people. World Health Organization (2003).

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