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Teratogenic and Anti-mutagenic Potentials of Aqueous Leaf Extract of *Momordica charantia* Linn.

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Abstract

Momordica charantia Linn. is an edible plant, used in various countries for the treatment of several diseases. For the purpose of this research, aqueous extract of air dried leaves of *M. charantia* was used. The aqueous leaf extract was administered (at 80mg/kg, 100mg/kg and 120mg/kg of body weight) to male albino rats for 90 days. After which, the bone marrow and sperm cells were observed for micronuclei, sperm count and sperm head abnormalities. The results from this study showed that increased dosage of the aqueous leaf extract produced a decrease in micronuclei observed among the groups, indicating that *M. charantia* has some anti-mutagenic potentials and it is able to inhibit chromosomal breakages. Contrary to its effects on the bone marrow cells, the results from the sperm analyses indicates that it has teratogenic potentials by reducing sperm production and altering sperm morphology.

Keywords: Anti-Mutagenic; Micronucleus; *Momordica charantia* Linn; Sperm Count; Sperm Head Abnormality; Teratogenic.

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1. Introduction

Momordica charantia Linn. belongs to the family Cucurbitaceae and it is commonly cultivated in the tropics and sub-tropics. Due to the characteristic bitter taste of *M. charantia*, it is known as Balsam pear, Bitter melon or Bitter gourd [1-2]. In addition to its use as an edible vegetable, various parts of the herb including the leaves and fruits are being used to treat various diseases [2-4]. In different communities, the leaves and fruits of *M. charantia* are used to treat and prevent diseases such as diabetes, liver diseases, ulcer, constipation, malaria, kidney stones, skin problems and so on [4-5]. Therefore, there is need to ascertain the genotoxic potentials of this plant. Though various studies are being done regularly to determine the genotoxic potentials of the fruits and seeds, yet not much has been done to determine that of the leaves. Hence, this research work was aimed at determining the mutagenic and teratogenic potentials of the leaves of *Momordica charantia* Linn.

2. Materials and methods

2.1. Plant material and extraction

Fresh leaves of *M. charantia* were collected during the wet season from Badagry, Lagos, Nigeria. The plant was identified at the Herbarium of the University of Lagos, Nigeria and a voucher number-LUH 6469 was assigned. The leaves were air-dried and blended to obtain a fine powder. The fine powder was boiled with distilled water in an uncovered beaker for about 15 minutes and filtered to obtain an aqueous decoction. The amount of dry extract in a ml of the decoction was ascertained, in order to determine the volume of extract to be administered to experimental animals.

2.2. Ethical approval

The research was approved by the Research Grant and Experimentation Ethics Committee of the College of Medicine, University of Lagos, South-West, Nigeria.

2.3. Experimental animals and procedure

24 male albino rats (*Rattus novergicus*- Sprague Dawley) weighing between 105.19-242.73g were used as experimental animals. The rats used were purchased from Komad Farm Limited, Sango Ota, Ogun State, Nigeria. The rats were housed in conventional plastic cages, at the Animal house of the department of Cell Biology and Genetics, University of Lagos; with 12hrs of light and dark cycle. The rats were fed with rat chow *ad libitum* and unlimited supply of water. Acclimatization of the rats to the new environment was carried out for 2 weeks before the onset of the research work. After determination of the lethal dosage (LD_{50}) of the aqueous leaf extract to be 140mg/kg, the male rats were placed in 4 groups (A, B, C, D). Group A being the control, were administered with distilled water alone; B: were administered with 80mg/kg body weight of each rat; C: were administered with 100mg/kg body weight; while D: were administered with 120mg/kg. Administration of extract was done using oral gavage method. The rats were exposed daily to the plant extract for 90 days. On day 91, sacrificing of the rats was carried out through the cervical dislocation method and the required organs removed for micronucleus and sperm cell analyses.

2.4. Sample collection and processing

2.4.1. Micronucleus test

The procedure utilized for the micronucleus test was according to standard protocol [6-7]. For each rat, both femurs were carefully removed and rinsed with physiological saline. The bone marrow was flushed out of the femur using Fetal Bovine Serum (Sigma Aldrich Cheme GmbH, Germany) into sterilized eppendoff tubes. The bone marrow was centrifuged at 2000rpm for 10 minutes. The cells were smeared on clean slides, followed by staining using May-Grunwald and Giemsa stains. Four slides were prepared per animal and a total of 1000 cells were evaluated per rat (at 250 cells per slide). The cells were viewed using an oil immersion lens (1000X); in order to microscopically score the number of micronuclei in polychromatic erythrocytes (PCE).

2.4.2. Sperm Count Analysis

The male rats were sacrificed and their epididymides carefully removed in accordance to the protocol of [8]. The contents of one epididymis for each rat, was gently emptied into a sterile specimen tube containing formol saline to a dilution of 1:10. The sperm cells were counted with a haemocytometer at 400X using a light microscope, after which the sperm concentration/count was evaluated for each rat using the guidelines of [9].

2.4.3. Sperm Morphology Assay

For each rat, the other epididymis was utilized for morphology assay following a modification of standard procedure [10-11]. The epididymis was finely minced in physiological saline and filtered, in order to remove tissues of the epididymis. Slides (4 slides per animal) were prepared by smearing the cell suspension on grease-free slides, followed by staining with Giemsa stain. 1000 cells per rat were evaluated for abnormalities, at 400X magnification with the use of a light microscope.

3. Results

3.1. Micronucleus

It is observed from Table 1, that increase in the dose of the treatment resulted in a decrease in the mean of micronuclei observed (Fig. 1). In addition, each treatment in Table 1 is denoted by a separate letter, thereby indicating that at $P < 0.05$, the treatment produced significantly different effects among the groups.

3.2. Sperm Count

Results from Table 1 shows that as the dosage of the treatment increased, there was a decrease in group mean for sperm count. An observation of the different letters used in denoting each treatment, indicates that increasing doses of the treatment, produced significant differences among the groups at $P < 0.05$.

3.3. Sperm Head Abnormality

The results shown in Table 1, reflects the effect of the treatment on sperm morphology. These results show that

increasing doses of the treatment had significant effects (at $P < 0.05$) on the morphology of the sperms. Thereby resulting to increase in the number of sperms with abnormal head observed (Figs. 3-6): pin-shaped head, sickled hook head, sperm heads without hook, sperm cells with knobbed head and skewed head when compared with the normal hooked head of the control group (Fig. 2).

Table 1: Showing the Mean and Standard Deviation for Micronucleus, Sperm Count and Sperm Head Abnormality.

TREATMENT	MNPCE (MEAN±SD)	SC ($X10^6$) (MEAN±SD)	SHA (MEAN±SD)
CONTROL	23.667±3.933d	114.667±4.457d	17.500±4.087a
80 mg/kg	14.667±1.506c	92.917±5.616c	126.167±5.269b
100 mg/kg	9.167±2.041b	77.167±4.719b	146.000±7.950c
120 mg/kg	3.500±1.517a	55.500±7.589a	203.333±13.604d
REMARK	Significant	Significant	Significant

MNPCE: Micronucleus in each Polychromatic Erythrocyte; SC: Sperm Count; SHA: Sperm Head Abnormality; SD: Standard Deviation; a: treatment with lowest figure; d: treatment with highest figure. Mean±SD followed by the same letter of alphabet are not significantly different at $P < 0.05$.

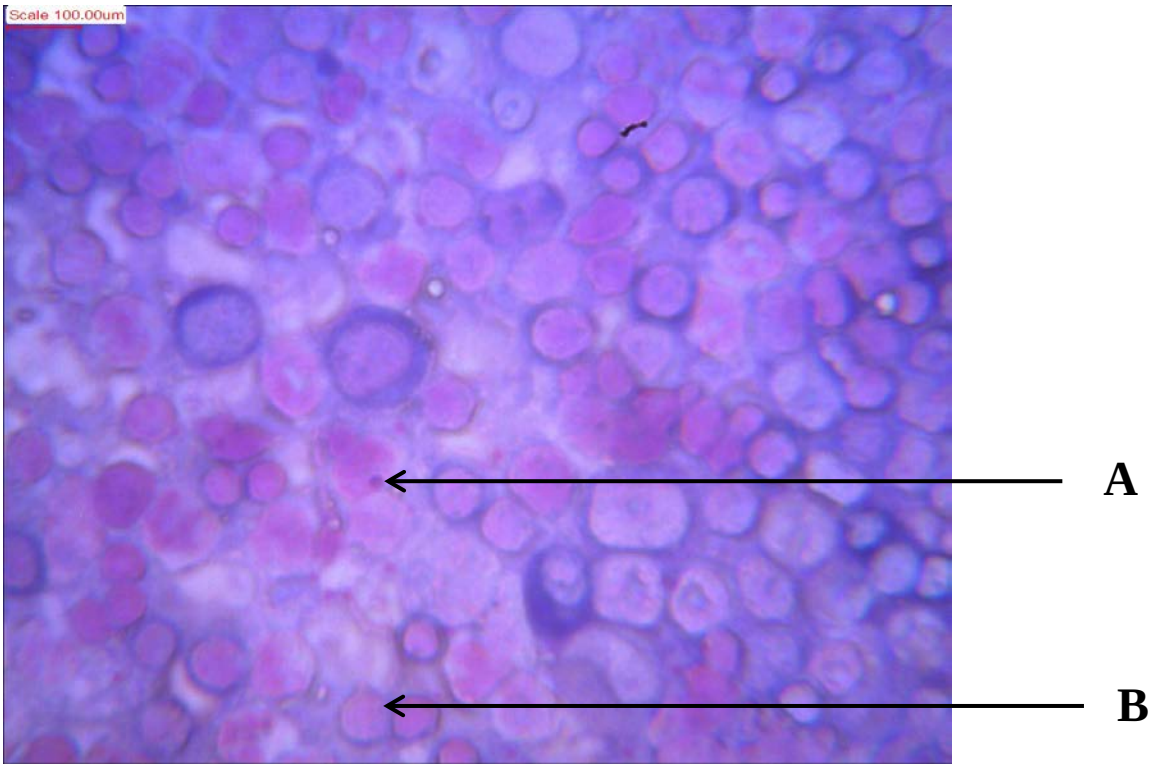

Figure 1: A - An Immature Erythrocyte with Micronucleus, B – A Normal Immature Erythrocyte.

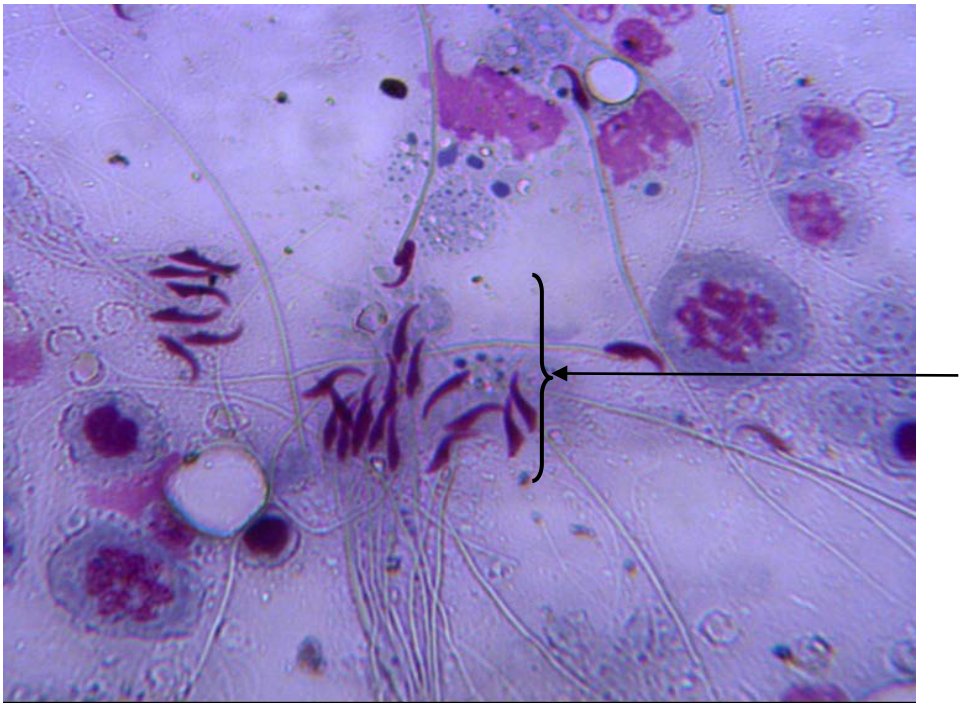

Figure 2: Arrow Showing Cells with Normal Sperm Head and hook.

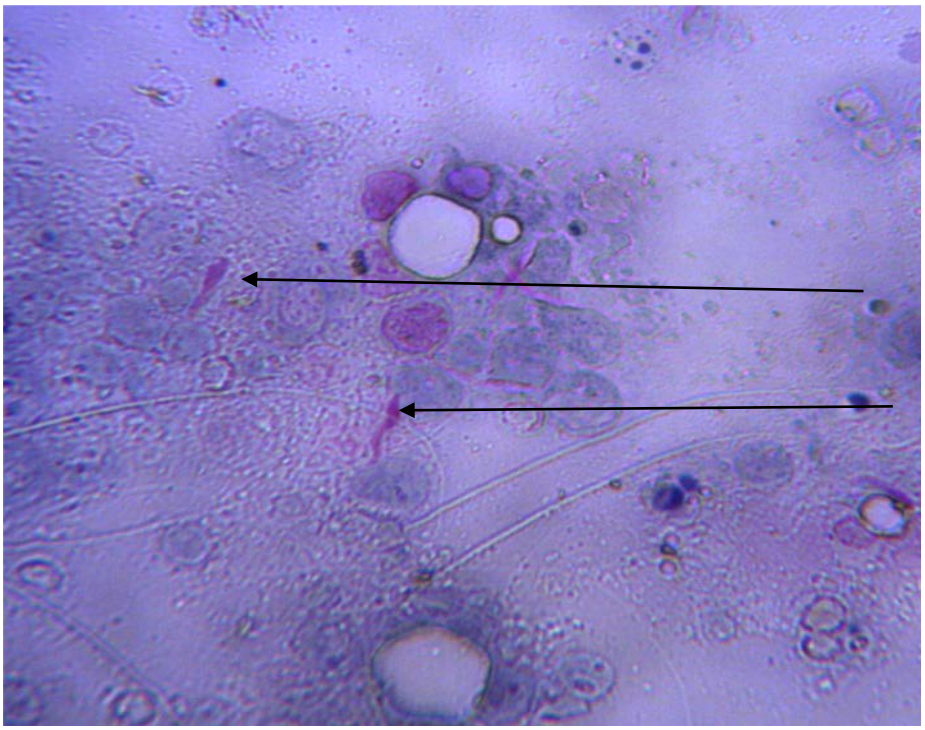

Figure 3: Arrows Showing Sperm Cells with Abnormally Skewed Head and Hooked at wrong angle.

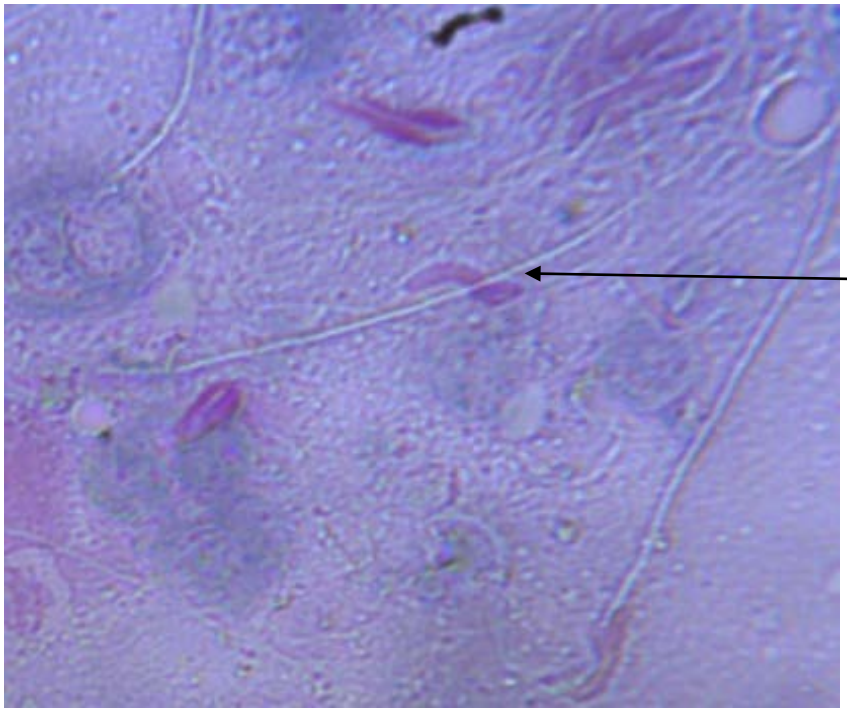

Figure 4: Arrow showing Sperm Cell with a Knobbed Head.

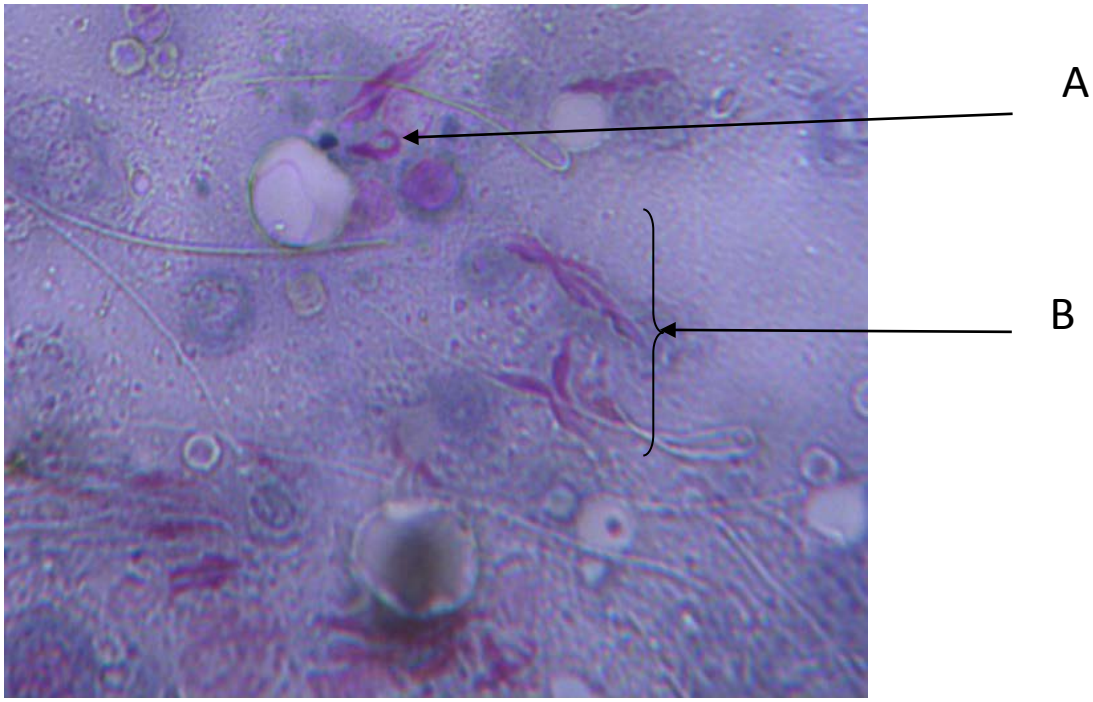

Figure 5: A - Sperm Cell with a sickled hook, B - Sperm Cells without hook.

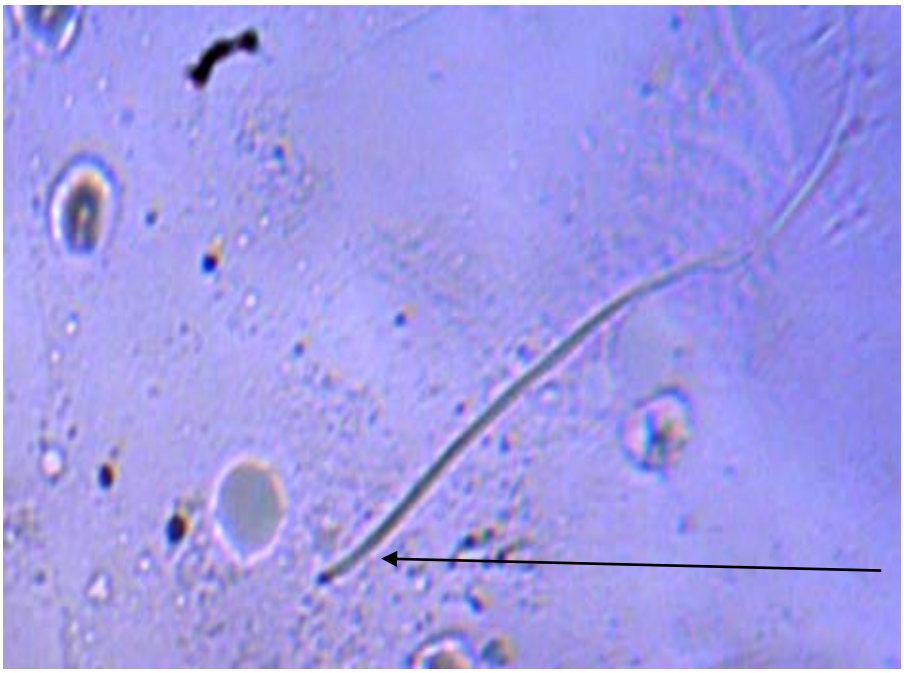

Figure 6: Abnormal Sperm Cell with a Pin-Shaped Head.

4. Conclusion

To determine the genotoxicity of a substance several assays are employed, some of which are the micronucleus test, sperm count and sperm head abnormality assays. The micronucleus test is used to determine the mutagenic potential of a substance, while the sperm cells are used to ascertain its teratogenic potential. Therefore, these assays were utilized in determining the mutagenic and teratogenic potentials of aqueous extract of *M. charantia* leaves. An observation of the results for the micronucleus test showed that the rats administered with aqueous leaf extract of *M. charantia*, possessed fewer number of MNPCE. It is also observed that as the dosage increased from 80mg/kg to 120mg/kg, there was a significant decrease in the mean MNPCE per treatment (Table 1). This indicates that *M. charantia* inhibits chromosomal breakages and prevents the development of micronuclei in PCE. Thereby, establishing the fact that *M. charantia* possesses anti-mutagenic potentials as stated in a previous study conducted using aqueous fruit extract [12]. Upon observing the sperm cells of these rats, it is observed that the mean sperm count decreases with increased dosage (Table 1); with 120mg/kg having the lowest sperm count. This implies that the aqueous leaf extract of *M. charantia* inhibits sperm production; similar to the results obtained from a study conducted on the sperm cells using methanolic seed extract [13]. Likewise, the results of the sperm head morphology (Table 1), shows that aqueous leaf extract of *M. charantia* induced an increase in the production of sperms with abnormally shaped heads (Figs. 3-6); as the dosage increases [14]. The results on the sperm cells invariably imply that the leaves of *M. charantia*, has teratogenic potentials. The results from this study and those of previous studies, imply that the consumption of *M. charantia* can induce male infertility. Therefore, caution should be applied in the consumption of this plant and its use in the treatment of diseases.

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