



GENOTOXICITY AND REPRODUCTIVE EFFECTS OF CHRONIC CONSUMPTION OF AQUAOUS BITTER LEAF EXTRACT IN ALBINO RATS



M.O. Yakubu^{1*}, I.A. Taiwo¹, J.I. Otabor²

^{1*}Department of Biological Sciences, Federal University Wukari, Taraba State, Nigeria

¹Department of Cell Biology and Genetics, University of Lagos, Lagos State, Nigeria

²Department of Biological Sciences, Federal University Wukari, Taraba State, Nigeria

*Corresponding author: moyakubu@fuwukari.edu.ng

Abstract: *Vernonia amygdalina* is a very important medicinal plant that is extensively used in Nigeria for treating various illnesses. Despite this, there is a need to assess this plant for genotoxicity potential. The present study was carried out to determine the genotoxicity and reproductive effects of *V. amygdalina* aqueous extract in albino rats using sperm head abnormality assay. Three groups consisting of eight male albino rats per group were used. The first group (control) was treated with distilled water for forty-two days while the second and third groups received 1000 mg/kg body weight (b. wt.) and 10,000 mg/kg b. wt. of *V. amygdalina* leaf extracts respectively, for the same period. Administration of the extract to the animals was by oral gavage. The mean sperm count (cell/mm³) result for control group was: 23500 ± 4200 while *V. amygdalina* 1000 mg/kg b.wt. treated rats gave: 16500 ± 2300 and *V. amygdalina* 10000 mg/kg b.wt gave: 17800 ± 1600. The frequencies of abnormality recorded between the control and *V. amygdalina* treated rats gave a P-value of 0.748. Analysis of variance between the two types of sperm head morphology recorded gave a P-value of 0.077. These P-values indicate that *V. amygdalina* had no statistically significant effect on sperm morphology and sperm count at 5% level of significance (P > 0.05). Therefore, *V. amygdalina* is relatively safe for consumption, although it does not appear to enhance male reproductive capacity in the rats.

Keywords: Genotoxicity; sperm head abnormality assay; Bitter Leaf, *Vernonia amygdalina*.

Introduction

The African continent with its rich biodiversity endowment is a host to a wide variety of plants that are used as food, medicine, aesthetic, and as a source of raw materials for locally based industries (Simoês *et al.*, 2001). In developing countries like Nigeria, the use of medicinal plants is common, as majority still rely solely on traditional medicine as their source of primary health care. It was against this backdrop that the world health organization (WHO) in 1999 adopted among its resolutions, the support of national traditional medicine as great importance to the health of communities. Bitter leaf (*Vernonia amygdalina*) is highly cherished amongst many indigenous West African medicinal plants due to its many therapeutic uses. Documented evidence from several investigators have reported the antioxidant and hypolipidaemic properties (Igile *et al.*, 1994), hypoglycemic effect (Taiwo *et al.*, 2009; Atangwho *et al.*, 2010; Adikwu *et al.*, 2010), anti-malaria (Madureira *et al.*, 2002; Masaba, 2000; Tona *et al.*, 2004), anti-helminth and anti-parasitic properties (Hakizamungu *et al.*, 1992).

However, it has been pointed out, that while most medicinal plants may have the potential for treating a variety of diseases, many contain toxic substances that are capable of causing mutagenic effects (Vicentini *et al.*, 2001). It is therefore of paramount importance that genotoxicity tests are carried out for these plants to assess their mutagenic potential (Camparoto *et al.*, 2002). The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 1997) defined genetic toxicity or genotoxicity as a broad term that refers to any deleterious change in genetic material regardless of the mechanism by which the change is induced. The human population is exposed to a variety of drugs and chemicals which have genotoxic potentials. Therefore, appropriate sensitive and practical methods for detecting and estimating the effect of these substances is paramount (Odeigah, 1996).

Genotoxicity tests can be *in vitro* or *in vivo* tests that are designed to detect substances/compounds that induce

genetic damage directly or indirectly by various mechanisms. These tests should enable the identification of hazardous agents with respect to DNA damage. These DNA damage can be in form of gene mutation, large scale chromosomal damage, recombinational and numerical chromosomes changes (ICH, 1997). These tests can be short term- acute toxicity studies or long term- chronic toxicity studies. This present study was a two- fold investigation; first, to investigate the effect of *V. amygdalina* in albino rats using a short-term genotoxicity assay (sperm head abnormality test) and second, to explore the reproductive potential of *V. amygdalina* by checking parameters such as sperm count and motility.

Materials and Methods

Animal husbandry

Isogenic strains of adult male albino rats (*Rattus norvegicus*) weighing 130-240 g were obtained from the animal care unit of the Lagos State University Teaching Hospital (LASUTH) Ikeja, Lagos State. The animals were housed in plastic cages with mesh grid floors and maintained on food (pellets from Pfizer) and water *ad libitum*.

Plant materials

Vernonia amygdalina leaves were obtained from the local market and identified at the University of Lagos Herbarium. The leaves were properly rinsed with clean water, sun-dried to remove all water content, crushed and grounded to obtain a powdered form of the leaves.

Preparation and determination of the plant extract concentration

An unknown quantity of dried *V. amygdalina* powder was dissolved in 1000 ml of hot distilled water. The solution was left to stand for fifteen minutes and filtered to obtain a fine solution. 1ml of the solution was pipetted into three evaporating dishes A, B and C with known weight in gram. The evaporating dish containing the extract was then placed on a Bunsen burner, stirred with magnetic stirrer

until all the water evaporated. The concentration of the extract was calculated using the following formula;

$$\text{Concentration (mg/ml)} = \frac{\text{weight of extract after evaporation (g)}}{\text{ml}}$$

Experimental design and administration of extract

The experimental animals were randomly assigned to one of three groups of eight rats each. Group 1 served as the control, which were given 5 ml distilled water for forty-two days. Group 2 and Group 3 were the treatment groups. Group 2 were given 1000 mg/kg body weight of extract for 42 days while Group 3 received 10,000 mg/kg body weight of extract for the same duration. The administration of materials was by oral gavage.

Determination of dosage of extract;

$$\text{Administration volume} = \frac{\text{weight of animal (g)} \times \text{Dosage (mg/kg)}}{\text{concentration (mg/ml)} \times 1000}$$

Sperm motility test

The rats were sacrificed by exposure to chloroform fumes in an anaesthetic chamber forty-two days of extract administration. The two caudal epididymis of each rat were surgically removed and weighed. One of the two epididymis of each rat was minced with sharp dissecting pins in a petridish containing 0.5 ml eosin Y solution (to enhance contrast) and 5ml of saline solution. A drop of the homogenous suspension was introduced with a Pasteur pipette unto a clean glass slide, covered with a cover slip and examined under the microscope at 400X for motile cells (Topham, 1980; Odeigah, 1997).

Sperm count

The remaining solution from sperm motility test solution was serially diluted in physiological saline and a drop was introduced unto a haemocytometer chamber (Odeigah, 1997). The set up was transferred to a microscope and observation was made at 100X. Using the L or 7 methods (only sperm heads within the count area and within the L or 7 lines were considered in order to avoid double counting), the number of sperm cell per mm³ for all experimental animals groups was determined using;

$$\text{Number of cells per 1mm}^3 = \frac{N}{4} \times 10 \times 20$$

Sperm head abnormality assay

The second epididymis was minced with sharp dissecting pins in a petridish containing 0.5 ml of 1% eosin Y and 5 ml of saline solution to release sperm cells into the solution. The suspension was left for 30 min for proper spermatozoa staining (Topham, 1980; Odeigah, 1997). Air dried smear were prepared on glass slides and examined for head abnormalities.

Statistical analysis

The difference between the control and experimental group were analyzed using Minitab 16 statistical software to derive the analysis of variance. The test was considered negative because the frequency of abnormality was very low (not significant) both in control and *V. amygdalina* treated groups with $P < 0.05$ as the criterion of significance. The other statistical tools employed were mean, standard error of mean and standard deviation.

Results and Discussion

Preliminary studies carried out to monitor the body weight changes in the rats showed that the rats increased in weight steadily during the course of treatment. The mean body

weight (g) pre-treatment and post-treatment for the control group were 192.5 g and 214.4 g, while the corresponding values for the *V. amygdalina* treated with 1000 mg/kg extract and 10000 mg/kg extract were 189.9 g and 214.0 g; and 199.5 g and 220.2 g, respectively. This increase in weight gain further strengthens the research findings of Agbede *et al.* (2007). The observed weight gain in infant animals by these researchers was attributed to the synergistic effect of *V. amygdalina* leaf crude protein content: 17 to 33 g/100g DW; fat content: 2 to 15 g/100g DW (24.54% saturated and 65.45% polyunsaturated) in combination with the soybean meal fed to the infants (Agbede *et al.*, 2007).

It was found that the sperm motility in the *V. amygdalina* treated group did not show a dose dependent relationship. Most of the sperm cells observed were moderately active to very active in both *V. amygdalina* treated rats and control (distilled water) rats. This observation (sperm motility) appear to be different from earlier findings of Oyeyemi *et al.* (2008), as this study shows that *V. amygdalina* does not have adverse effect on sperm motility because the cells of the treated groups were as motile as those of the control group. The sperm count result (cell/mm³) on the other hand, revealed a positive linear relationship with post treatment body weight as shown in Fig. 1 below. The mean Sperm Count (cell/mm³) for 8 rats in the control group gave: 23500 ± 4200. The rats treated with 1000 mg/kg *V. amygdalina* extract values were 16500 ± 2300. The corresponding value for 10000 mg/kg *V. amygdalina* extract treated rats were 17800 ± 1600. The mean sperm count did not show a dose dependent effect; however, Fig. 2 portrays a somewhat significant picture. The difference in mean sperm counts between the control group and the 1000 mg/kg *V. amygdalina* treated rats; And the 1000 mg/kg and 10000 mg/kg *V. amygdalina* treated rats observed cannot be attributed to the treatments but probably to individual rat's innate capacity to produce sperm cells as sperm counts in individual rat showed similar patterns of highs and lows across board.

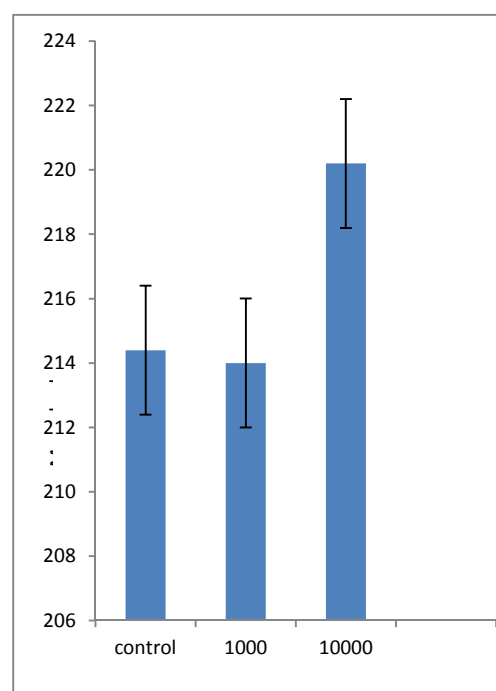


Fig. 1: Effect of *V. amygdalina* extract on body weight (g) in albino rats.

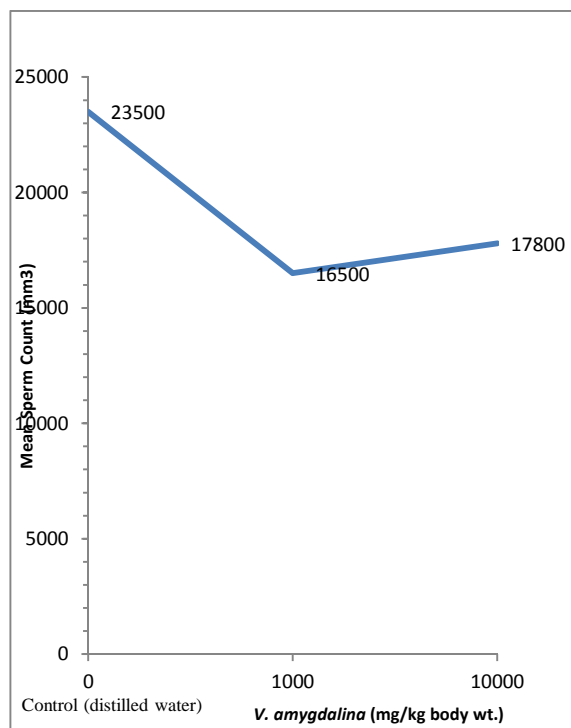
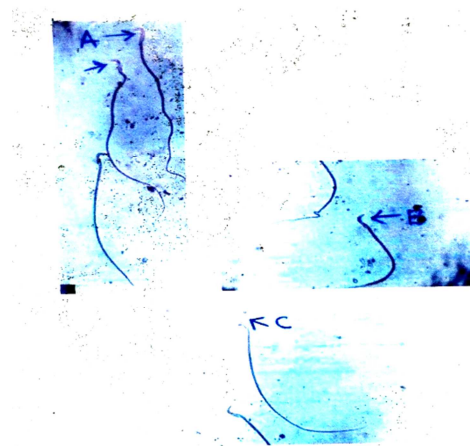


Fig. 2: The effect of different doses of *V. amygdalina* on sperm count in rats. Each point represents the mean haemocytometer readings per treatment group.

The different kinds of observed sperm head are shown in Fig. 3 below. Most of the sperm heads had a definite head shape that is accented by a marked hook. However, some abnormalities were observed in both control and treated groups. Some sperm head had a short hook (hook is shorter than normal) and long hook (hook is longer than normal). The frequencies of abnormality recorded were very low and shows no statistical significance based on the analysis of variance result. At 5% (The level of significance being $P > 0.05$), there is no significant difference between the control (distilled water) and treatment (1000 mg/kg *V. amygdalina* and the 1000 mg/kg *V. amygdalina*) because the P-value (0.748) is greater than 5%. Also for sperm head abnormality result at 5% level of significance, shows no statistical difference between the short hook and long hook sperm heads because the P-value (0.077) is greater than 5%. Previous studies by Camparoto *et al.* (2002) have shown that *V. amygdalina* like other medicinal plants contain some components such as alkaloids, flavonoids, saponins, tannis and glycosides. According to these researchers, these substances are sometimes produced as part of plants protective mechanisms and may therefore be toxic when isolated in pure forms. It is likely that these substances do not cause sperm head morphology changes in rats. This agrees with the view that *V. amygdalina* is relatively non-toxic.



(A) Normal sperm head with a definite head shape; (B) Short hook; the hook on the head is shorter than normal; (C) Long hook; head is elongated than normal.

Fig 3: Types of sperm head abnormalities found in control and *V. amygdalina* treated rats.

Okabe *et al.* (1998) carried out an extensive study on the genetics of the mammalian gametes; they showed that the variations in DNA content of spermatozoa with some attendant morphological defects are often genetically controlled. The shape of the sperm head is already predetermined at the genetic level, this shape remain stable afterwards. The abnormalities recorded in this study presumably occurred during spermatogenesis as mistakes are known to happen during differentiation of spermatozoon in the spermatogenetic process (Padmalatha and Vijayalaxmi, 2001). Some scientists have attributed these abnormalities to chromosomal aberrations that occur during the packaging of the genetic material in sperm head (Padmalatha and Vijayalaxmi, 2001). On the other hand, some have argued that abnormal sperm heads are due to point mutations during spermatogenesis. Plant extracts may add to these mistakes during the process.

The antimutagenic and anticancer activities of flavonoids in *in vitro* and *in vivo* systems have been reported in *Chrysanthemum morifolium* and green tea (Miyazawa and Hisama, 2003 and Zeng *et al.*, 2015) in Asia. These research findings further corroborates earlier reports that lower mortality rates due to cancer exist in tea producing regions than areas where tea is not cultivated. The combination of two or more of these complexes (flavonoids, saponins, tannis and glycosides) as found in some medicinal plants such as *V. amygdalina*, cumulatively work together to suppress mutagenesis and possibly genotoxicity. Horikawa *et al.* (1994) probed the activity of six Chinese medicinal herbs on *Samonella* and found that tannin and catechin compounds were responsible for the inhibition of mutagenicity caused by benzo [a] pirene.

Flavonoids compounds isolated from *V. amygdalina* exhibit growth inhibitory effects on some drug resistant microorganisms (Akinpelu, 1999). The use of *V. amygdalina* as chewing stick can be attributed to this antimicrobial activity, it is alleged to have a beneficial effect on dental caries (Yeap *et al.*, 2010). The cytotoxic effects of *V. amygdalina* chloroform extract on human carcinoma narsopharynx cells has also been reported by Yeap *et al.* (2010) as well as the antioxidant activities of the flavonoids compound (Igile *et al.*, 1994). The aqueous extract of *V. amygdalina* has also been found to significantly reduce triglyceride levels and normalize

cholesterol concentrations in drug induced diabetic rats (Taiwo *et al.*, 2009; Atangwho *et al.*, 2010; Adikwu *et al.*, 2010).

Conclusion

V. amygdalina is generally not genotoxic on rats sperm cells, it does not have any effects on sperm quality and therefore cannot be recommended as tonic to enhance the male reproductive system. Its consumption at relevant concentrations can be continued by the general population, caution should be exercised to avoid indiscriminate use because the safety of any substance cannot be guaranteed on prolonged exposure at very high concentrations.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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