

## Oral Administration of Ethanolic Leaf Extract of *Vernonia amygdalina* May Impact Negatively on Fertility in Male Wistar Rats

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### Authors' contributions

This work was carried out in collaboration with all authors. Author JAE conceptualized and designed the study. Author AIA managed the analyses of the study and also wrote the manuscript. Author VNO and Author EOO managed the literature searches. Author UO wrote the protocol and performed the statistical analysis. All authors read and approved the final manuscript.

### Article Information

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### ABSTRACT

**Background:** The use of *Vernonia amygdalina* leaves as vegetables and in folklore medicine for therapeutic activities is on the increase globally.

**Aim:** This study, therefore, sought to investigate the antifertility effect of *V. amygdalina* leaves in male Wistar rats.

**Methods:** Fresh and healthy leaves of *V. amygdalina* were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan. They were dried and extracted using ethanol as the solvent. Thirty male Wistar rats were grouped into 6 of 5 rats each. Animals in groups A, B, and C were administered normal saline for 10, 20, and 30 days respectively via the oral route. Those in groups D, E, and F were treated with 500 mg/kg body weight of leaf extract of *V. amygdalina*. At the end of treatment, the animals were sacrificed by cervical dislocation. The

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internal organs were exposed. Testes and cauda epididymis were removed and kept in the sterilized glass. Male fertility parameters were determined using standard methods.

**Results:** Ethanolic leaf extract of *V. amygdalina* decreased sperm count and sperm motility while abnormality of spermatozoa increased significantly after 10, 20, and 30 days treatment respectively. However, seminal pH and sperm mortality were only perturbed after 30 days of treatment. The changes in seminal quality of *V. amygdalina* leaf treated-animals showed antifertility effects.

**Conclusion:** Excessive consumption of high doses of *V. amygdalina* may have a negative impact on sperm count, motility and viability of sperms, thus decreasing fertility. But this does not automatically translate to such effect on humans.

**Keywords:** *Vernonia amygdalina*; sperm count; motility; mortality; abnormality; seminal pH.

## 1. INTRODUCTION

The induction of male infertility in experimental animals and humans resulting from treatment with medicinal plants and their products has drawn the attention of researchers. The antisteroidogenic and antifertility activities of extracts from plants with antimalarial properties have also been reported [1,2]. With the increased efforts in the development of more potent antimalarial agents as a result of the challenges posed by the resistant strains of the malarial parasite, the evaluation of these antimalarial agents for possible antifertility actions becomes worthwhile. This is in view of the fact that both malaria and infertility are worldwide phenomena and there is a need to avoid the risk of infertility resulting from malarial chemotherapy [3].

*Vernonia amygdalina* commonly known as bitter leaf in English, ("Oriwo" in Edo, "Ewuro" in Yoruba, "Shuwaka/Chusadoki" in Hausa, and "Olubu/Onugbu" in Igbo), is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon, and Zimbabwe. The leaves are dark green coloured with a characteristics odour and a bitter taste. It is reputed to have several health benefits. It is effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and anti-parasitic activity [4]. *V. amygdalina* is a perennial herb belonging to the Asteraceae family. The species is indigenous to tropical Africa and is found wild or cultivated all over sub Saharan Africa [5]. The leaves are eaten after crushing and washing thoroughly to remove the bitterness [6]. However, almost all parts of the plant are pharmacologically useful. Both the root and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others [7]. *V. amygdalina* has been ascertained to provide various culinary and medicinal properties. These

medicinal properties exert a bacteriostatic and bactericidal effect on some bacteria [8]. Antimalarial properties [9], as well as antitumourigenic properties [10], have also been reported for extracts from the plant. Furthermore, Ogbuagu et al. [11] have demonstrated the effect of methanolic extract of its leaves on glycemic and lipidaemic indices in Wistar rats. Its prophylactic propensity against acute ethanol-induced oxidative stress has also been reported [12]. Airaodion et al. [13] recently reported that *V. amygdalina* leaves possess haemolytic properties in Wistar rats. Many herbalists and native doctors in Africa recommend its aqueous extract for their patients for the treatment of varieties of ailment ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually-transmitted diseases among others [7].

## 2. MATERIALS AND METHODS

### 2.1 Collection and Extraction of Plant Material

Fresh and healthy plants of *V. amygdalina* free from disease were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in open laboratory space for 14 days and milled into powder using an electronic blender (Moulinex). The extraction was done using Soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [14]. About 25 g of the powder was packed into the thimble of the Soxhlet extractor and 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the

heating mantle and began to evaporate moving through the apparatus to the condenser. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35°C with a yield of 2.07 g which represented a percentage yield of 8.28%. The extract was preserved in the refrigerator for further analysis.

## 2.2 Experimental Design and Animal Treatment

Thirty male Wistar rats weighing between 185 and 200 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were acclimatized for 7 days during which they were fed *ad libitum* with standard feed and drinking water. Animals were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into 6 groups of 5 rats each. Animals in groups A, B and C were administered normal saline for 10, 20, and 30 days respectively via oral route. Those in groups D, E, and F were treated with 500 mg/kg body weight of leaf extract of *V. amygdalina* for 10, 20, and 30 days respectively via the oral route. At the end of treatment, the animals were sacrificed by cervical dislocation. The internal organs were exposed. Testes and cauda epididymis were removed and kept in the sterilized watched glass.

## 2.3 Determination of Male Fertility Parameters

The cauda epididymis was separated from both of the testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. Sperm counts were done with the aid of hemocytometer according to the method of Eliasson [15]. The motility of spermatozoa was determined according to the methods of Tijee and Oentoeng [16]. For the study of abnormality of spermatozoa, a film of semen was prepared on slide. These films on slide were fixed in methanol. The slides were stained in eosin for 40 minutes. The films were washed in tap water and after drying, the slides were examined under the microscope to observe abnormality of

spermatozoa. Seminal pH was measured using a pH meter.

## 2.4 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean  $\pm$  standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey's (HSD) multiple comparison tests. Differences between means were considered to be significant at  $p < 0.05$ .

## 3. RESULTS

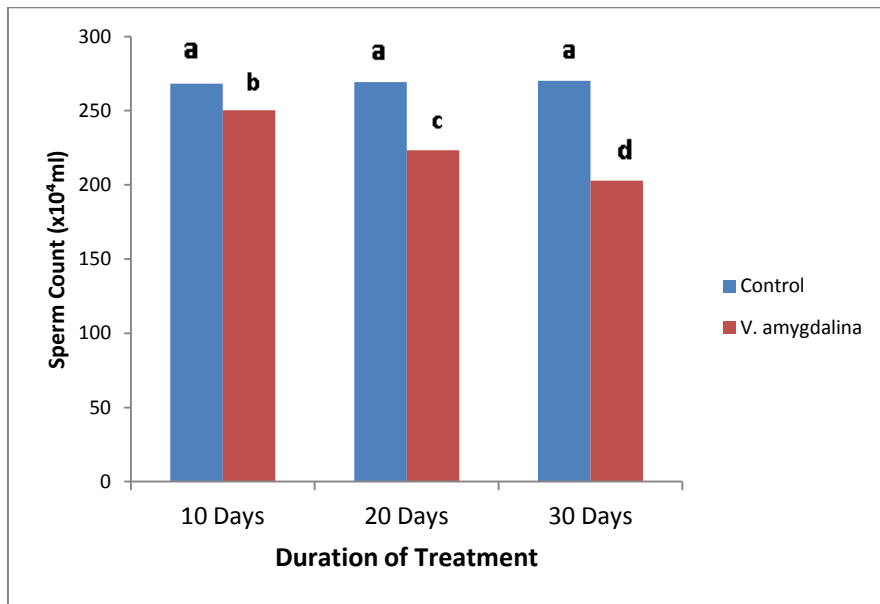
The results of the effect of *V. amygdalina* leaf extract on male fertility parameters of animals are presented in Figs.1-5.

## 4. DISCUSSION

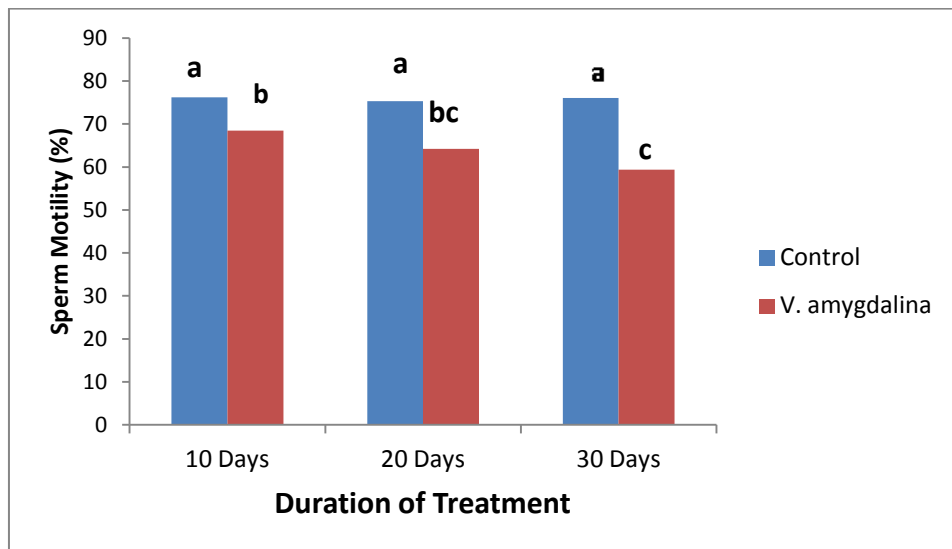
Herbs have been used for centuries in the treatment of different ailments [17,18]. Some of them have unexploited effects which can result in serious unplanned consequences. These consequences could be teratogenic, and as such babies born of such pregnant mothers can be malformed with ignorance of its cause [19]. This study, therefore, sought to investigate the effect of ethanolic leaf extract of *V. amygdalina* on some male reproductive parameters.

In this study, it could be clearly be demonstrated that sperm count declined significantly ( $p < 0.05$ ) when animals treated with leaf extract of *V. amygdalina* were compared with the control groups throughout the administration as presented in Fig. 1. This result is similar to the findings of Airaodion et al. [20] who treated animals with *C. papaya* leaf extract. It also corresponds with the report of Kumari et al. [21] who treated animals with *C. papaya* seed extract. This might be suggestive that leaf extract of *V. amygdalina* interfered with steroid hormone biosynthesis, which results in impaired spermatogenesis [22]. Disturbance in steroid hormone biosynthesis, as well as spermatogenesis, may affect the seminal quality of animals. The decrease in sperm count observed in this study is dependent on the number of days treated. This is indicative of the fact that continuous consumption of *V. amygdalina* leaves as vegetables will sustain a reduction in sperm count.

In this study, a significant ( $p < 0.05$ ) decrease was observed in sperm motility of animals treated with leaf extract of *V. amygdalina* when compared with the control groups throughout the



**Fig. 1. Effect of *V. amygdalina* on Sperm Count of Animals after 10, 20, and 30 days of Treatment** Results are presented as mean values of 5 rats. Bars with different letters represent means that are significantly different at  $p < 0.05$



**Fig. 2. Effect of *V. amygdalina* on sperm motility of animals after 10, 20, and 30 days of treatment**

Results are presented as mean values of 5 rats. Bars with different letters represent means that are significantly different at  $p < 0.05$

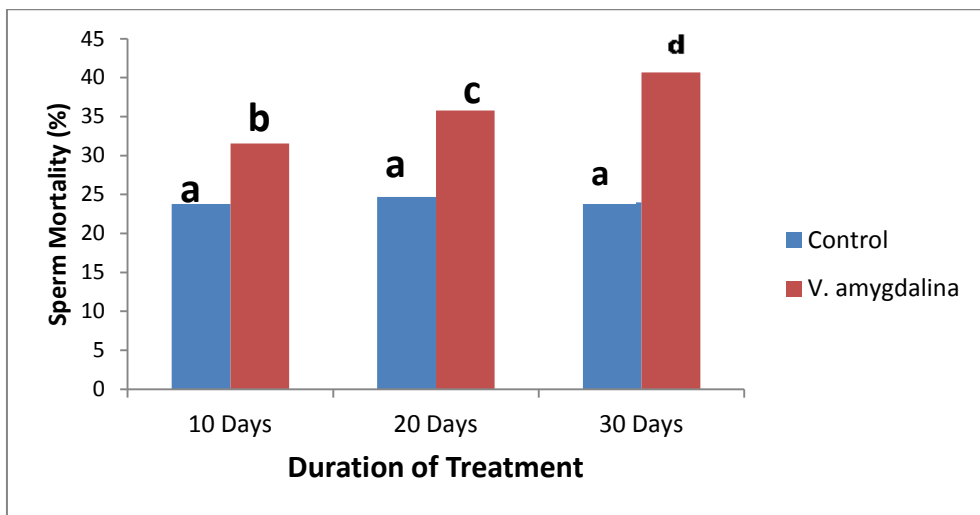
period of administration. This corresponds with the findings of Airaodion et al. [20] who treated animals with *C. papaya* leaf extract. It also agrees with the report of Kumari et al. [21] who treated animals with *C. papaya* seed extract. The reduced sperm motility observed in this study might be an indicator that *V. amygdalina* leaf extract can reduce the ATPase activity in all tissue of the animals [23]. This causes the

suppression of energy metabolism. If ATPase activity is decreased, it could suppress the motility rate of sperm, as ATP is the main energy source of sperm and it is directly related to sperm motility. Lohiya et al. [24] observed total inhibition of motility in human sperm after treatment with *C. papaya* seed extract. *C. papaya* seed extract has also shown inhibitory action on sperm motility in rats [25,26]. The inhibitory motility observed in

the sperm of rats treated with leaf extract of *V. amygdalina* in this study might follow the same mechanism as that reported for *C. papaya*. The decrease in sperm motility observed in this study is dependent on the number of days treated. This is indicative that continuous consumption of *V. amygdalina* leaves as vegetables will sustain reduction in sperm motility.

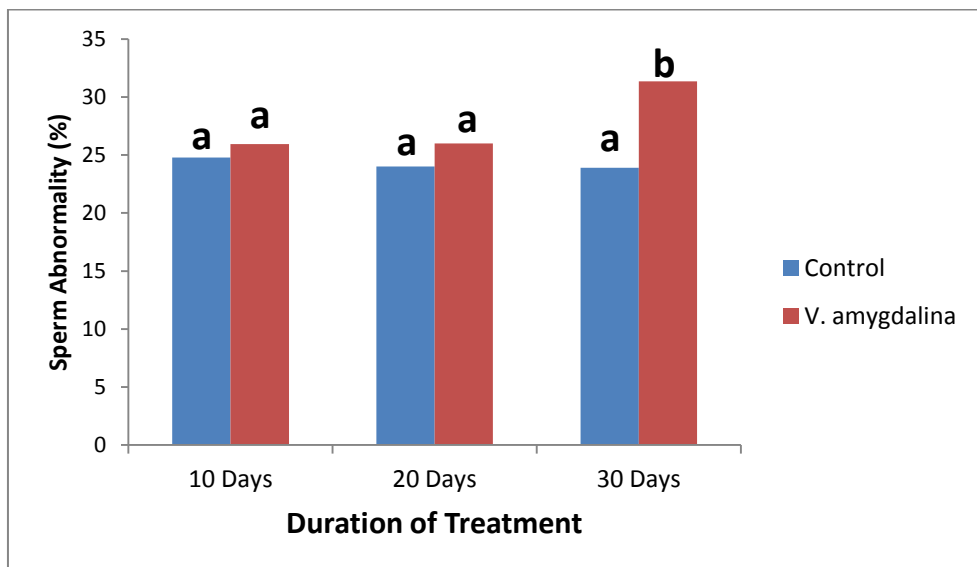
In this study, the leaf extract of *V. amygdalina* was observed to increase the number

of abnormal spermatozoa when compared with the control animals after 10 days of treatment. Increased abnormality of spermatozoa in *V. amygdalina* treated animals might be as a result of damage to Sertoli cell [27]. For normal testicular function Sertoli cell plays a vital role in maintaining a conducive environment for spermatogenesis. Damage in Sertoli cell may affect the maturation process of spermatozoa, which might result in an increased abnormality of sperms observed in this study.



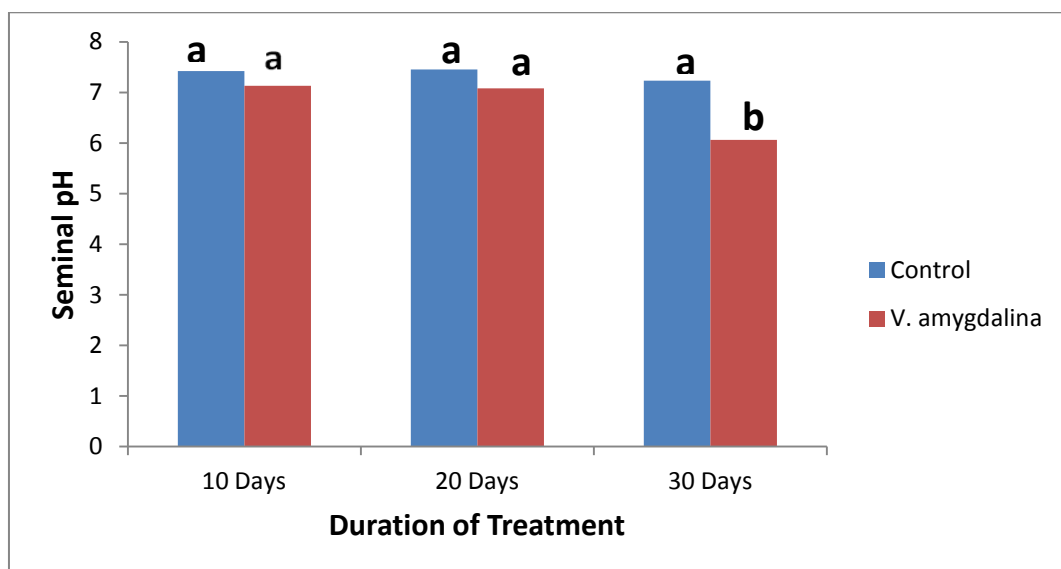
**Fig. 3. Effect of *V. amygdalina* on sperm mortality of animals after 10, 20, and 30 days of treatment**

Results are presented as mean values of 5 rats. Bars with different letters represent means that are significantly different at  $p < 0.05$



**Fig. 4. Effect of *V. amygdalina* on sperm abnormality of animals after 10, 20, and 30 days of treatment**

Results are presented as mean values of 5 rats. Bars with different letters represent means that are significantly different at  $p < 0.05$



**Fig. 5. Effect of *V. amygdalina* on seminal pH of animals after 10, 20, and 30 days of treatment**  
Results are presented as mean values of 5 rats. Bars with different letters represent means that are significantly different at  $p < 0.05$

No significant difference was observed in the seminal pH when animals treated with leaf extract of *V. amygdalina* were compared with the control animals after 10 and 20 days of treatment respectively. However, the difference became significant after 30 days of treatment. This is contrary to the findings of Airaodion et al. [20] who reported a significant decrease in seminal pH when animals were treated with *Carica papaya* leaf extract for 30 days. This result indicates that *V. amygdalina* leaf extract perturbed the normal pH range after about 30 days treatment. If the pH is decreased, the medium of the seminal plasma becomes acidic which in turn makes sperms highly fragile, thus leading to a higher rate of mortality.

In this study, no significant difference was observed in sperm mortality when animals treated with leaf extract of *V. amygdalina* were compared with the control animals after 10 and 20 days of treatment. However, the difference became significant after 30 days of treatment. This also contradicts the findings of Airaodion et al. [20] who reported a significant increase in sperm mortality when animals were treated with *Carica papaya* leaf extract for 30 days. There is a correlation between seminal pH and sperm mortality. The significant increase observed in the sperm mortality of animals treated with leaf extract of *V. amygdalina* after 30 days might be attributed to the significant ( $p < 0.05$ ) decrease in seminal pH at the same period of treatment. A low pH of epididymal fluid of bovine has been

reported to result in an increased rate of mortality of spermatozoa [28].

Generally, antimalarial remedies have been reported to have antifertility effects. Some of these remedies reported include chloroquine [29], *Azadirachta indica* [30], *Alstonia boonei* [31], dihydroartemisinin [32] and *Carica papaya* leaves [20]. Airaodion et al. [9] have previously reported that *V. amygdalina* leaves possess antimalarial properties. Thus, there is a possible relationship between its antimalarial properties and antifertility activities. Studies have reported the presence of proteins in the sperm as well as the semen. Edwards et al. [33] reported the presence of serum proteins (albumin, lactoferrin), glycoproteins, a kinase and prostatic in sperm cells. These proteins nourish the sperm cell. The reduction in sperm count observed in animals treated with leaf extract of *V. amygdalina*, might be attributed to the proteolytic action of the proteases in *V. amygdalina*. These enzymes may have hydrolyzed the semen proteins, making them unavailable for use by the sperm cells, thereby leading to malnutrition and defects seen in the cells.

## 5. CONCLUSION

The results obtained from this study revealed that ethanolic leaf extract of *V. amygdalina* causes a decreased sperm count and sperm motility while abnormality of spermatozoa increased significantly after 30 days of treatment.

Seminal pH and sperm mortality was perturbed after 30 days of treatment. The normal range of sperm count, sperm motility, seminal pH, and abnormality of spermatozoa are essential factors for fertility. Any disturbance of such a normal range of the seminal quality may affect the fertility of animals. These changes in seminal quality of *V. amygdalina* leaf treated-animals are indicative of antifertility effects. This may not automatically translate to such adverse effects on humans. Therefore, its effect on the fertility of man needs to be further confirmed.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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