***Original Research Article***

**Pessaries of Gentamicin Sulphate for the Treatment of Bacterial Vaginosis: Formulation Development**

ABSTRACT

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| --- |
| **Aim:** To develop pessaries for the treatment of bacterial vaginosis. Vaginosis is a disease resulting from abnormal bacterial or fungal proliferation of the vagina, causing discomfort to the patient. There are numerous pessaries for the treatment of fungal vaginosis, but a paucity of such pessaries exists for bacterial vaginosis. Development of pessaries for the treatment of bacterial vaginosis therefore becomes pertinent.**Study design:** *In vitro* and *In vivo* evaluation of the effectiveness of pessaries formulated for bacterial vaginosis.**Place and Duration of Study**: Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria; between May 2024 and February 2025.**Methodology:** The non-natural bacteria colonizing the vagina were isolated using standard methods and their susceptibility to gentamicin sulphate was tested to establish the effective inhibitory concentration. This concentration guided the formulation of different batches of the pessaries, which were evaluated using dissolution studies. The pessaries with the optimal inhibitory concentration were formulated to suite rat’s vagina. The effectiveness of treating vaginosis caused by bacteria using gentamicin sulphate pessaries in a rat model was investigated. **Results:** All the clinically isolated bacteria showed sensitivity to gentamicin sulphate at 0.4 mg/ml (P = .05). A 4 % formulated pessary showed good release *in vitro,* and a 5-minute dissolution sink cleared all the isolated bacteria. A suitably formulated pessary reduced *Staphylococcus aureus* load in a rat-infected model by 97.5 % and *Escherichia coli* by 55.3 %.**Conclusion:** Gentamicin sulphate pessaries could be used to treat vaginosis caused by susceptible bacteria. |

*Keywords: vaginosis, gentamicin sulphate, pessaries, vaginal bacterial inhabitants, non-natural bacterial inhabitant.*

1. INTRODUCTION

Vaginosis is a disease arising from microbial proliferation of the vagina and it’s reported that most women will encounter vaginosis at some point in their lives. This disorder results from an imbalance in the microbiome of the vaginal ecosystem. The majority of the disease's pathogens are organisms that are normally found in a healthy vagina and the vaginal microbiome is significant because it serves as a primary defense against secondary infections as well as sexually transmitted illnesses and infections (STIs) [1]. However, over population of the microbial bio-load causes destabilization of the vagina micro flora resulting to itching, exudates with foul odour, inflammation of the vagina (vaginitis) and many other symptoms characteristic of vaginosis [1, 2]. There are bacterial vaginosis as well as fungal vaginosis and most often, non-vaginal bacteria inhabitants colonizing the vagina are implicated in bacterial vaginosis [1]. Several anti-fungal pessaries exist for the treatment of fungal vaginal infections but there are very limited anti-bacterial pessaries. In most cases, oral and parenteral drug administrations are employed for the treatment of bacterial vaginosis, which are not quite sufficient.

Gentamicin sulphate is a broad-spectrum aminoglycoside. The drug has been formulated into several dosage forms, including as systemic oral and for parenteral use [3, 4]. The drug has also been presented for dermal topical applications [3, 5, 6] and as inhalations [7, 8]. It has been evaluated for rectal local activity as well as implants [9, 10]. It has also been presented for GIT localized (non-systemic) indications as a conventional oral tablet and as a pro-drug [11, 12]. Preparation of gentamicin sulphate into pessaries may help to improve the treatment of bacterial vaginosis and bridge the gap of paucity of treatment options for the disease.

In this study, gentamicin sulphate is formulated into pessaries, and the pessaries are evaluated for treatment using an infected rat model in an attempt to develop treatment options for bacterial vaginosis.

2. methodology

**2.1 Isolation of Non-natural Bacteria Inhabitants of the Vagina from Volunteers**

The non-natural bacteria specimen colonizing the vagina was collected from healthy undergraduate volunteers with informed consent supported with ethical clearance (MH/COMM/523/V.1/78) from the Anambra State Ministry of Health, Awka, Nigeria. A sterile swab was used by each of the participants to collect the vaginal fluid. The swabs were washed in sterile water, and the suspension obtained was used to inoculate the Mueller-Hinton agar. Sub-culturing was further carried out to purify the isolates before identification. This was done following a slightly modified method [13].

**2.2 Determination of Sensitivity of the Isolated Bacteria against Gentamicin Sulphate**

The sensitivity of the isolated bacteria against gentamicin sulphate was carried out using a slightly modified agar diffusion assay method [14]. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standards and were seeded onto the sterilized Mueller-Hinton agar. Holes of 8 mm diameter were bored on each quadrant of the plate. The gentamicin sulphate was reconstituted with sterile water to produce the following dilutions: 0.05, 0.1, 0.2, and 0.4 mg/ml. A drop of the dilutions was introduced into the bored holes such that in each plate, the higher concentration alternates with the lower concentration. The plates were replicated and allowed for diffusion before incubation at 37 oC for 24 hours. Thereafter, the zone of inhibition was measured and recorded.

**2.3 Formulation of Gentamicin Sulphate Pessaries in Macrogol Bases**

2.3.1 Determination of composition of macrogol base

The macrogol pessaries base was composed of mixtures of Polyethylene glycols 1500, 4000, and 6000 in the ratio of 20: 33: 47 respectively. These ratios were arrived at via preliminary assessment using physical stability and melting point as guides.

2.3.2 Determination of displacement value (DV) of gentamicin sulphate in macrogol base

A suppository mould whose cavity holds 2.2 g of macrogol base was employed for the purpose of calculating DV, based on a modified knowledge [15]. The quantities of the respective polyethylene glycol required to make up seven (7) suppositories, each weighing 2.2 g, were measured and melted on an evaporating dish over a hot plate. The molten base was used to fill six (6) suppository cavities and allowed to cool and solidify. The solidified suppositories were removed from the mould, weighed, and termed ‘un-medicated suppositories’. Similarly, the quantity of the base for seven (7) suppositories was measured, and 30 % quantity was removed and replaced with an equal quantity of gentamicin sulphate powder. The mixture was melted and used to fill the cavity. On solidification, the weights were taken and termed ‘medicated suppositories’. The quantity of the pure gentamicin sulphate and base in the medicated suppository was thereafter calculated and input in the formulae below to obtain the Displacement Value (DV):

$$DV=\frac{Weight of drug displacing}{Weight of base displaced}………….(1)$$

2.3.3 Formulation of gentamicin sulphate pessaries

Three (3) batches: 1 %, 2 % and 4 % of gentamicin sulphate suppository for insertion into the vagina (pessaries) were formed using the 2.2 g mould and the calculated DV of 0.5. The respective quantities of the drug and the polyethylene glycols sufficient to make 2.2 g per pessary were employed to make six (6) pessaries with adjustment for overage. The mixture was then melted and poured into the mould cavities and allowed to solidify [15].

**2.4 Bio-guided Dissolution Studies of the Gentamicin Sulphate Pessaries**

Dissolution was done using the modified beaker method, by employing 300 ml of distilled water maintained at a temperature of 27 oC and 50 rpm [16]. The dissolution was allowed to run and sink collected at 0-, 5-, 15-, 30-, 45-, and 60- min with replacement using distilled water. A drop of the sink was introduced into holes bored in agar plates seeded with each of the organisms isolated in subsection 2.1. Incubation was done at 37 oC for 24 hours. Thereafter, zones of inhibition were measured.

**2.5 Anti-bacterial Evaluation of Formulated Pessaries in a Rat Model**

2.5.1 Formulation of gentamicin sulphate pessaries suitable for rats

Series of trials were carried out using pessaries of different sizes and shapes to arrive at a shape and size that will be suitable for rat’s vagina. To this end, pessaries of the size of the circumference of a 1-ml syringe were found to be suitable. Thus, rats’ pessaries were obtained by pouring the molten base containing 4 % of gentamicin sulphate into the suppository mould cavities. A 1 ml syringe (whose end holding the needle has been cut off) was inserted into the molten base and allowed to solidify. The formed pessaries were removed by gently pushing with the plunger from the other end. The pictures/plates below **(Figure 1)** emphasize more on the adapted improvise.

 

 A B

**Figure I. Improvise for producing rats’ pessaries (A - mould with column of 1ml syringe; B - 1ml syringe column and formed rats' size pessaries)**

2.5.2 *In vivo* anti-bacterial evaluation of formulated pessaries in rat model

The approval for the use of animals in this study was obtained from the Ethics Committee of the Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Female rats weighing between 150 to 200 g were employed in the study. The rat’s vagina were swabbed and cultured to ascertain the base line bacteria load and type. The non-natural bacteria inhabitant obtained (*Staphylococcus aureus* and *Escherichia coli*) were multiplied and used to infect the rat’s vagina. To this end, a turbid 48-hour culture for each of *Staphylococcus aureus* and *Escherichia coli* was prepared in 10 ml sterile water and adjusted to 0.5 McFarland turbidity standards. Thereafter, a sterile swab stick was dipped into the bacterial suspension and carefully applied on the vagina of the rats. The infected rats were allowed two (2) weeks for full development of vaginosis. Disease development was confirmed by vagina swabbing and examination by culturing on their respective selective agar to obtain baseline bacterial load.

The diseased rats were divided into two main groups (IT: infected and treated, IUT: infected and untreated). Each main group was further divided into two sub-groups for each of the test bacteria, with each subgroup containing four rats. Treatment consists of daily insertion of one pessaries (measuring 1 cm) carefully into the vagina of each of the treated animal for 5 days.

Thereafter, the assessment of the activity of the pessaries *in vivo* was evaluated. Suspensions of vagina swabs taken (post-treatment) were cultured on both selective agars. The plates were incubated at 37℃ for 24 hour. The *in vivo* activities of the pessaries were determined by the reduction in bacterial load post-treatment when compared to the pre-treatment bio-load [17].

3. results and discussion

**3.1 Results**

The following bacteria: *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhi* wererecurring non-natural bacteria isolated from female volunteers. The bacteria were thereafter subjected to a susceptibility test using different concentrations of gentamicin sulphate (**Table 1)**. As can be seen from Table 1, the inhibition of the bacteria is concentration-dependent. The gentamicin sulphate had an inhibitory effect at concentrations as low as 0.05 mg/ml for *S. aureus* with an IZD of 9.5 mm and 4.5 mm for *S. typhi* at the same concentration. *E. coli,* on the other hand, is more resistant to the drug with an IZD of 1.5mm at a concentration of 0.4 mg/ml (atP*=* .05).

**Table 1. Sensitivity of isolated non-natural bacteria inhibiting rat’s vagina**

|  |  |  |  |
| --- | --- | --- | --- |
| **Conc (mg/ml)** | ***S.aureus*****IZD** | ***E.coli*****(mm)** | ***S.typhi*** |
| 0.4 | 19.5 ± 0.7 | 1.5 ± 0.70 | 13.5 ± 0.00 |
| 0.2 | 14.5 ± 0.7 | 0.00 | 12.5 ± 0.00 |
| 0.1 | 11.5 ± 0.7 | 0.00 | 9.5 ± 0.70 |
| 0.05 | 9.5 ± 0.7 | 0.00 | 4.5 ± 0.70 |

The gentamicin sulphate, having shown activity against the isolated bacteria, was formulated into batches of pessaries with concentrations **(Table 2)**. Upon dissolution and inoculation of the sink into the bacteria-seeded agar, the IZDs as shown in Table 2 were obtained. A batch containing 4 % of gentamicin sulphate was able to inhibit all the isolated bacteria from dissolution time of 5 min onward.

**Table 2. Bio-guided dissolution studies of 1 %, 2 %, and 4 % gentamicin sulphate pessaries batches**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Time**  | **(minutes)** |  |  |
| **Batch : 1 %** | **5** | **15** | **30** | **45** | **60** |
| *S,aureus* | -- | -- | -- | -- | -- |
| 1. *coli*
 | -- | -- | -- | -- | -- |
| *S. typhi* | -- | -- | 12.0 ± 0.0 | 14.0 ± 0.0 | 17.0 ± 0.0 |
| **Batch: 2 %** |  |  |  |  |  |
| *S,aureus* | 10.0 ± 1.0 | 10.0 ± 0.5 | 10.0 ± 0.5 | 10.0 ± 1.0 | 11.0 ± 0.0 |
| *E.coli* | -- | -- | 11 ±1.0 | 11 ± 0.0 | 12 ± 0.5 |
| *S. typhi* | -- | 12.0 ± 0.5 | 11.0 ±1.0 | -- | -- |
| **Batch: 4 %** |  |  |  |  |  |
| *S,aureus* | 11.0 ±1.0 | 12.0 ± 0.5 | 13.0 ±1.0 | 14.0 ± 0.5 | 13.0 ± 0.5 |
| *E. coli* | 11.0 ±1.0 | 13.0 ±1.0 | 12.0 ±0.5 | 14.0 ±1.0 | 12.0 ± 1.0 |
| *S. typhi* | 12.0 ± 0.5 | 12.0 ± 0.5 | 12.0 ±1.0 | 15.0 ± 0.5 | 13.0 ± 1.0 |

The results of the *in vivo* studies conducted with the 4 % pessaries suitable for rat’s vagina is shown **(Table 3)**. The untreated animal’s bacterial load increased from 699.33 to 998.12 CFU/ml, which represents a 42.71 % increase for *S aureus* and 33.89 % for *E. coli,* whose increase was from 702.33 to 940.33 CFU/ml. For the treatment group, there was a reduction in the bacterial load. The *S aureus* load was reduced from 723.0 to 18.20 CFU/ml representing a 97 % reduction, while that of *E. coli* was reduced from 772.40 to 345.60 CFU/ml, representing a 55.26 % reduction.

**Table 3. Bacteria load following vaginal administration of the formulated gentamicin sulphate pessaries**

|  |  |  |  |
| --- | --- | --- | --- |
| ***Staphylococcus aureus*** | **Pre-treatment bio-load** | **Post-treatment bio-load** | **Reduction/Increase (%)****100 (Post trt - Pre trt) / Pre trt** |
| IUT | 699.33 ± 58.53 | 998 ± 97.12 | 42.71 |
| IT | 723.00 ± 157.93 | 18.20 ± 15.26 | -97.48 |
|  |  |  |  |
| ***Escherichia coli*** |  |  |  |
| IUT | 702.33 ± 120.50 | 940.33 ± 29.02 | 33.89 |
| IT | 772.40 ± 153.60 | 345.60 ± 91.58 | -55.26 |

*Key: IUT = infected and untreated; IT = Infected and treated*

**3.2 Discussion**

Several bacteria can invade the vagina and destabilize the natural microflora thereby causing vaginosis. A literature report revealed that bacterial vaginosis is most common among sexually active individuals and is unrelated to sociodemographic variables [18]; this research didn’t investigate this claim. However, vaginosis (in the broad sense) is well caused by over proliferation of the natural inhabitants such as *Lactobacillus spp* to cause bacteria vaginosis or *Candida albican* to cause fungal vaginosis, if the vagina environment is destabilized. It could be seen from Table 1 that all the isolated bacteria were sensitive to gentamicin sulphate at varying concentrations, and the sensitivity follows the order: S. aureus*> S. typhi> E. coli* (atP*=* .05). This observation is consistent with earlier observations where *S aureus* displayed the highest sensitivity among similar isolated bacteria for gentamicin sulphate [11]. The sensitivity is also observed to be concentration dependent implying that gentamicin sulphate could be used to treat bacterial infections of the vagina.

To realize this goal, macrogol base was preliminarily composed and optimized for suitability as a suppository base. The suitable composition consists of the following: Polyethylene (PEG) 1500, 4000, and 6000 in the ratio of 20: 33: 47 respectively. This composite was found to be physically stable and melted at a temperature of 59.3 ± 3.1 oC, which is well above body temperature. The macrogol base was used to formulate gentamicin sulphate pessaries having concentrations of 1-, 2-, and 4% using a determined displacement value of 0.5. A bio-guided dissolution study was used to evaluate these batches to ascertain the most suitable batch to formulate. To this end, the sinks from the dissolution of the respective batches were introduced into holes bored in the agar media containing the isolated organisms. The results of the assay are shown in Table 2.

It is apparent from the result as contained in Table 2 that the 4 % pessaries have the highest activity against a wider range of tested bacteria. Thus, a 4 % gentamicin sulphate pessaries suitable for insertion into the rat’s vagina was formulated and used to treat disease induced rats.

The rats were originally swabbed, and two bacteria isolated (*Staphylococcus aureus* and *Escherichia coli*) were multiplied and used to induce disease in the rats. The formulated rats’ pessaries were used to treat the diseased rats by daily insertion for five days. Thereafter, the treated rat’s vaginas were swabbed and the suspension of the swab was used to check the presence of the bacteria, after treatment. The result of such evaluation is presented in Table 3.

It can be seen from Table 3 that the *S.* infected and untreated (IUT) rats' *S aureus* load increased by 42.71 % after 5 days, while there was a 97.48 % reduction in the infected and treated (IT) rats. Similarly, the infected and untreated (IT) rat’s *E. coli* load rose by 33.89 % after 5 days, while the infected and treated (IT) rat’s *E. coli* load was reduced by 55.26 %. Invariably, from the foregoing evidence, gentamicin sulphate pessaries could be used to treat vaginosis caused by susceptible bacteria and could add to the treatment arsenal available to the patients.

4. Conclusion

The effectiveness of using gentamicin sulphate to treat vaginosis caused by bacteria was investigated. The clinically - isolated bacteria from vagina showed sensitivity to the drug. Gentamicin sulphate pessaries were formulated using macrogol base as the delivery matrix, and the formulated pessaries showed good release *in vitro*. The sink of the 4 % formulation cleared the bacterial load in a rat-infected model, suggesting that gentamicin sulphate pessaries could be used to treat vaginosis caused by susceptible bacteria.

More clinical studies with the formulated pessaries (in humans) are recommended to further confirm the findings. Stability studies are also recommended.

Consent

All authors declare that written informed consent was obtained from the human volunteers, supported with ethical clearance and approval (MH/COMM/523/V.1/78) from the Anambra State Ministry of Health, Awka, Nigeria.

Ethical approval

All authors also declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws of Nigeria. All experiments have been examined and approved by the appropriate ethics committee.

All authors also declare that all the human experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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