STUDY THE ANTIBACTERIAL EFFECT OF NEOMYCIN SULPHATE AS SUSPENSION FORMULA FOR DIARRHOEA

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Abstract
Preparation of veterinary formulation as suspension contain Neomycin sulfate 5%and kaolin 1% (weight /volume) used orally to treat diarrhea caused by infected with positive or negative bacteria (Salmonella sp., E.coli, St. Aureus) and diarrheal conditions associated with many diseases including hemorrhage gastroentritis, inflammatory bowel disease. After preparation the formula which examined by bacteria like St.aureus and E.coli which found the antibacterial activity of suspension as same compared with Neomycin pure. Bacterial examination of the suspension composition was carried out using St. aurius and E.coli bacteria, in which the inhibitory capacity of the neomycin suspension was found to be close to that of pure neomycin. The initial examination of the suspension was carried out at zero time, ranging between (94-106)% and compared to pure neomycin, which ranged between (90-110)%. The stability study of suspension after storing in different temperatures (25 and 40) °C for a period of three months, where the effectiveness of the composition at a temperature of 25 °C ranged between (95- 94)%. While the efficiency of the suspension composition ranged at a temperature of (40) °C between (95-91)% ,the activity of formula may be due to quality of the materials used. The new formula was evaluation to treat diarrhea in small animals “dog and cat” with hemorrhagic bowel infection which showed good result obtained.

Keywords: Neomycin Sulphate; Antibacterial Activity; Suspension Formula.

Introduction

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Diarrhoea considered as a serious threat for animal all over the world and ranking second among all the causes of deaths due to infectious diseases [1]. There are different causes of diarrheal infections in the developed nations; mainly the bacterial agents such as Shigellae, Salmonellae, E. coli and Campylobacter are the most common causes of infectious diarrhea [2]. Other way; Diarrhoea present in acute or chronic condition, with primary features suggesting small or large intestinal or mixed diarrhoea usually associated with gastrointestinal tract disease or secondary to systemic disease, such as fever or hypoadrenocorticism.

Neomycin sulphate, an antibiotic belonging to the aminoglycoside has excellent activity against gram negative bacteria, and partially active against gram-positive bacteria include *Salmonella sp.*, *Proteus sp.*, *klebsiella sp.*, *E.coli*, *Entrobacter sp.*, *acid-fast bacilli and actinomycetes sp.* [3]. Neomycin inhibits the action of the bacterial cell by disrupting the protein structure within the bacteria by binding the drug to the 30s unit of the ribosome, which inhibits the synthesis of amino acids and their conversion into protein and thus inhibits the reproduction of bacteria ; considered as bactericidal, most effective and least toxic antibiotic. Neomycin treat bacterial infections of the GIT system due to slow absorption and 79% release with faeces unchanging and has respectable suppressing the growth of S. aureus strain, has a long history of resistant states with various harmful effect which cause of bacteremia and infective endocarditis as well as pleuropulmonary [4-6]

Kaolin (Kaolin), a clay mineral with a soft, yellowish-white texture, most heat-resistant and highly melting clay, not dissolves in water or acids and when wet turns dark or clay colour. Its chemical composition is (Al2O3.2SiO2.2H2O) and has a melting point above 1800°C with name derived from Kaoliang Mountain located in Jiangxi, China and under the electron microscope, consist rough hexagonal, platy crystals in size between (0.1 - 10 )µ [7,8]. The British name Light kaolin and in U.S. Kaolin and chemical name (Hydrated aluminium silicate) therefore, kaolin is used with other materials to increase its workability and to lower the temperature necessary to increase stability produce. So the kaolin in formulation, kaolin particles show shrinkage because of its relative grain structure and it has little dry strength [9]. Kaolin according to ancient research considered as an antidiarrheal agent, which act as protector to gastrointestinal mucosal layers exactly through adsorbs toxins and bacteria outside the digestive tract. In other ways, kaolin due to the electrostatic charge, helping the material to adhere to other substances and increase its release out [10-12].

In pharmaceutical technology, Kaolin like other clay minerals may used as excipients or active substances in the formulation topically or orally and in other diluting agent in pills and capsules [13-15].

Kaolin exhibits exceptional physical, mechanical, and structural properties that reason makes it very useful for many pharmaceutical applications [16]. Kaolin adsorbs molecular substances such as lecithin, quinoline, proteins, bacteria, and viruses beside increase the viscosity of the stomach helping to release toxic substances and acts as protective cover for the lining of the intestine in cases of severe diarrhoea. In that way, combined of kaolin with different antibiotic lead to increase the antibacterial activity in the bodies to treat diarrhoea [17]. The actual work of most of the antibiotics used for treatment of gastrointestinal injuries have a direct effect on the microorganisms present leading to block the balance of the intestinal medium and increasing the permeability of the intestinal wall and thus activating harmful bacteria causing various symptoms. So in present study decided to use antibacterial neomycin with kaolin as additive to treatment diarrhoea.

**Materials and methods**
Table (1). Composition of Neomycin sulphate suspension formula.

<table>
<thead>
<tr>
<th>Item no.</th>
<th>Ingredient</th>
<th>Reference</th>
<th>Description</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neomycin sulfate</td>
<td>(18-19)</td>
<td>White or slightly yellow powder, hygroscopic.</td>
<td>soluble in water</td>
</tr>
<tr>
<td>2</td>
<td>kaolin</td>
<td>(18-19)</td>
<td>White or slightly</td>
<td>soluble in water</td>
</tr>
<tr>
<td>3</td>
<td>Methyl parabene</td>
<td>(20-21)</td>
<td>White</td>
<td>slightly soluble in water</td>
</tr>
<tr>
<td>4</td>
<td>Propyle parabene</td>
<td>(20-21)</td>
<td>White</td>
<td>slightly soluble in water</td>
</tr>
</tbody>
</table>

Preparation of suspension formula

All material used in formula were weighted separately by sensitive balance (IKA) according to different formulation and sieved with 200Micron size to facilitate the process of dissolving in water. Materials placed in glass beaker with distilled water and mixing process continues for 1-5 hours to obtain a homogeneous suspension. Then the suspension is packaged in impervious bottles. Samples of the formula suspension sent to the microbiological part for the bacterial examination and the stability [22 ].

Table (2). Composition of neomycin sulfate and active ingredient (gm) in different formula

<table>
<thead>
<tr>
<th>No.</th>
<th>material</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neomycin sulphate</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>kaolin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Additive</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Suspending agent</td>
<td>0.1</td>
<td>0.5</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>D.W.</td>
<td>qs</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Antibacterial activity of formula

Agar diffusion method used to detect the antibacterial effect of formula against Gram negative and positive bacteria by using Muller-Hinton Agar medium. After preparation the plate with bacteria and made 4 pits placed it with 50 μl of the neomycin formula and incubated at 37 °C for 18-24 hours. The inhibitory activity was determined by measuring the diameter of the non-growth area surrounding the pit containing neomycin which represents the inhibitory zone of bacteria [23].

Stability of formulations

The stability of formulation was evaluated at different temperature 25°C, 30°C and 40°C for 3 months to determine the effect of different temperature degree on antibacterial activities of formula [24]. The data were analyzed using SPSS software (version 11.5, USA). A p<0.05 was considered statistically significant for Kruskal-Wallis and corrected for Mann-Whitney.

Results and Discussion
Table (3). Represent the antibacterial activity of different formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Inhibition Zone for S. aureus (mm)</th>
<th>Inhibition Zone for E. coli (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>20.</td>
<td>20.9</td>
</tr>
<tr>
<td>F-2</td>
<td>24.5</td>
<td>24.4</td>
</tr>
<tr>
<td>F-3</td>
<td>24.1</td>
<td>24.0</td>
</tr>
<tr>
<td>F-4</td>
<td>28</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure (1). The antibacterial activity of neomycin sulphate suspension at concentration 1% and 5% in Staph. aurius bacteria.

Figure (2). The antibacterial activity of neomycin sulphate suspension at concentration 5% in comparable to Standard neomycin in Staph. aurius and E. coli bacteria.
Table (4). Stability of neomycin Suspension formulation storage in different temperature for 3 months

<table>
<thead>
<tr>
<th>Time of storage</th>
<th>(% of antibacterial activity after storage at 25°C for 3 months)</th>
<th>(% of antibacterial activity after storage at 30°C for 3 months)</th>
<th>(% of antibacterial activity after storage at 40°C for 3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One month</td>
<td>95.4±0.12</td>
<td>95.4±0.19</td>
<td>95.5±0.13</td>
</tr>
<tr>
<td>Two month</td>
<td>95.3±0.22</td>
<td>95.2±0.17</td>
<td>92.4±0.21</td>
</tr>
<tr>
<td>Three month</td>
<td>95.2±0.23</td>
<td>94.4±0.21</td>
<td>91.5±0.22</td>
</tr>
</tbody>
</table>

Figure (3). Activity of neomycin sulphate formula at different temperature

Diarrhea is one of the animal’s regular incidences in summer and there are different reasons for diarrhea and more complicated. The causes of diarrhoea divided into
infectious and non-infectious like disease, feed factor and the climatic factor’s etc. the infection factors such as antibacterial or viruses and parasitic. Diarrhea cause weakened of the abilities of digestive and absorption of food in intestinal tract, causes malnutrition, dehydration, loose of vitamin, electrolyte shortage and delay of growth and reduce the fertility performance of animals.

In the present research tried to prepare veterinary suspension formula neomycin sulfate combined with kaolin to treat diarrhea; other study found neomycin sulfate gives a good antibacterial effect against gram-positive and negative bacteria. Tight-junction proteins regulate paracellular permeability and Gut permeability which may be modulated by antibiotics like neomycin combined with kaolin to be useful for the treatment of diarrhea induced by drugs [25]. Neomycin sulphate suspension analysed by gram positive and negative bacteria to estimate the antibacterial activity for accepted as a medicinal formula.

The research observed acceptable result of all formulations and the antibacterial activity was parallel according to antibiotic dose according to other research displayed the greatest potency of neomycin through used alone or conjugated with others, and the antibacterial activity was currently depending on the dose as antimicrobial use. in the formula we chose to focus effort on characterizing the effects of combinations of neomycin and kaolin, with the anticipation that they may have the greatest likelihood of having a clinical impact and ease of advancement of the prepared formulations used to treat diarrhea. The study showed that neomycin has the effect to increase the antimicrobial properties when present in combination with other active materials in pharmaceutical formulation. Beside that ; there was much research acceded the idea supporting that there are agent’s off-target may effect account for neomycin ability to potentiate the antimicrobial activity of formula and that agent-specific to each antibiotic than others. the obvious research improved that the combination of neomycin (5%) with kaolin 1% in the new formula has excellent antimicrobial activity which shows in the zone of inhibition assays designed to measure the combination’s performance in the table above, compared to another concentration was tested. For that reason, in this research, we prefer to test different concentrations of neomycin sulfate to find the potent effect to prepare the formula [26] as shown in table 3.

The research shows the formulation of neomycin sulfate suspension at dose 5% was effective in zero time ranged from (95.5-106)% compared to the pure material neomycin (90-110)% and within the constitutional limits of drug which ranging from (90-135)%.

The stability of the F-4 formula studied through storage at different temperatures (30 and 40) °C for three months, the percentage antibacterial activity of F-4 formula at 30 °C ranged from (95-94)% while at 40 °C ranged from (95-91)%, as shown in Table 3.

The research indeed invent that Neomycin has the ability to inhibit S. aureus in vitro activity and according to ancient hypothesized shows, the inhibitor’s function of any antibiotic will improve when combined with others. In that regard, recent research Indeed, neomycin showed a high effect when bound to the specific component in the presence of another active agent leads to inhibition Escherichia coli, Streptococcus sp. and others.

So the presence study approving that formula of neomycin sulphate 5% according to the antibacterial activity of initial and after stability period was physically and chemically stable for at least 3 months with different temperature (30-40) °C. The research showed that the neomycin sulphate and kaolin 5% has a high susceptibility to the treat diarrhea associated with hemorrhagic enteritis.

**Conclusions**

The newly suspension formula neomycin sulphate and kaolin show good antibacterial activity at concentration 5%(w/v).

**Reference**