

**History:**

Received: May 01, 2021

Accepted: May 15, 2021

Published: June 9, 2021

Collection year: 2021

Status: Published

**Identifiers and Pagination:**

Year: 2021

Volume: 11

First Page: 1

Last Page: 8

Publisher ID:10.21065.1925-

7430.11.1

DOI:<http://dx.doi.org/10.21065/1925-7430.11.1>

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**Citation:**

Muzzammil M et al., Riphah

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App Sci. 2021, Vol. 11, p.1-8

**Original Research Article**

**ANESTHETIC AND ANTIBACTERIAL ACTIVITY OF KETAMINE DERIVATIVES  
SYNTHESIZED BY MANNISH REACTION**

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**ABSTRACT**

**Background:** The present study focuses on the anesthetic and antibacterial effect of ketamine which is a commonly used general anesthetic.

**Methods:** Five derivatives of ketamine i.e. Rxn-102, Rxn-103, Rxn-113, Rx-601, and Rx-801 were prepared. The Anesthetic activity of ketamine derivatives was determined using tail immersion test and the results were compared with ketamine. For biological activity of the antimicrobial complexes, Agar plate dilution test was performed in order to evaluate the Minimum Inhibitory Concentration (MIC) of ligand and newly synthesized derivatives. The antibacterial effect of ketamine was studied using six different strains of bacteria (Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogenes, Pseudomonas aeruginosa and Escherichia coli). Ciprofloxacin was used as a control to verify the methodology.

**Results:** Comparison of the tail immersion and formalin tests data indicated that derivatives had comparable anesthetic activity to that of Ketamine. The antibacterial effects of all compounds were relatively lower than ciprofloxacin. The derivatives Rxns 102, 113, 103, 601 and 801 demonstrated better effects on bacterial strains of S. aureus and S. epidermidis. However, only Rxn 113 showed better results against E. faecalis.

**Conclusions:** Ketamine not only showed profound anesthetic action but also had a strong inhibitory effect on growth of gram positive bacteria Staphylococcus epidermidis

**INTRODUCTION**

Ketamine have played an important role in veterinary medicine as well as in humans. Acting as a safe and reliable anesthetic, ketamine without depressing respiratory and circulatory functions blocks nerve paths. It can be taken<sup>(1)</sup> orally but most commonly given intramuscularly and via naso-pharyngeal route. "Ketamine has hallucinogenic, painkilling activity and is a

**Funding:**

The authors received no direct funding for this research.

**Competing Interests:**

The authors declare no competing interests

Additional information is available at the end of the article.

short acting general anesthetic. Ketamine modulates neuronal responses and abnormal behavior i.e. exhibits "State Dependency".<sup>(2)</sup>

Ketamine suppresses the spinal cord activity by blocking afferent impulses which are associated by emotional <sup>(3)</sup>component of pain perception inside the medial medullary reticular formation and interact with various neurotransmitters.<sup>24</sup> Ketamine inhibits neuronal uptake of neurotransmitters especially inhibits the uptake of serotonin, glutamate and dopamine and thus depress the communication between cerebral cortex and thalamus. It results in altering neuronal activity associated with motor functions, memory, sensory and emotional experiences <sup>(4)</sup>. As ketamine alters catecholenergic neurotransmission it has effects on peripheral organs

Ketamine blocks the closed channels from both aqueous and membrane phase thus blocks the NMDA<sup>(5)</sup> receptor, which are important for consciousness, cognition and sensory perception. 14, 26

Ketamine results in analgesia by non-competitive antagonism of NMDA receptor thus preventing central sensitization in dorsal horn neurons at spinal cord <sup>(6)</sup>thus interfere with pain transmission. It also blocks the synthesis of nitric oxide by inhibiting the enzyme synthase which is involved in pain perception. With a lower affinity ketamine binds with sigma and opiod receptors but it doesn't contributes to analgesia.<sup>(7)</sup>

Ketamine metal complexes have been synthesized and their characterizations have been done by many techniques. However, there still is room to study its bases and derivatives for various biological activities<sup>(8)</sup>. To date literature analysis reveals that many ketamine derivatives are known to possess good biological activities like anesthetic and antibacterial activities. In view of these findings and in continuation of our research work on ketamine, we studied anesthetic and antibacterial activities of various derivatives of ketamine<sup>(9)</sup>. These derivatives (Rxn 102, 113, 103, 601and 801) were prepared by treating ketamine with their respective metal halides and their characterizations were done by TLC and NMR techniques.

The Anesthetic activity of ketamine derivatives have been determined using tail immersion test and formalin Test and the results were compared with ketamine. 33

Anesthetic agents have well documented antibacterial activity, they not only effect on excitable cells but on all cells. Lidocain a local anesthetic has good antibacterial<sup>(10)</sup> activity. 17 The antibacterial activity of ketamine was studied by disc diffusion method and ciprofloxacin was used as control. Ketamine exhibited good antibacterial activity on higher doses.

## MATERIAL AND METHODS

All the chemicals and solvents used for the synthesis were of reagent grade and were obtained commercially from Merck Company with the exception of the cadmium nitrate, which was obtained from Aldrich. The solvents were purified by standard methods<sup>(11)</sup> [Perrin, D.D.; Armarego, W.L.F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: Oxford, **1980**; pp. 68, 174-217.].

The present research was carried out in the Multi-Disciplinary Laboratory Department of Pharmaceutical chemistry, Riphah Institute of Pharmaceutical Sciences Islamabad.

For biological activity of the antimicrobial complexes, Agar plate dilution test was used to

determine Minimum Inhibitory Concentration (MIC).

### **Biological Activity of Ketamine derivatives**

#### **Anesthetic Activity**

The Anesthetic activity of ketamine derivatives have been determined using tail immersion test and the results were compared with ketamine-<sup>33</sup>

#### **Mean tail immersion latency**

The tail immersion test was performed on mice and mean tail immersion latency was measured; the samples were prepared in normal saline which is also used as control. The tail immersion latency was measured after 5min of interval time for 40 min after drug injection. Each point on the graph represents the mean of tail immersion after injection of ketamine, Rxn-102, Rxn-103, Rxn-113, Rx-601, and Rx-801 in saline in 7-9 animals.

#### **Formalin Test**

Formalin test was conducted to measure the acute and chronic pain in animal groups (mice). The animals were climated first 12 mice were injected intraperitoneally with the drug (Rxn-102, Rxn-103, Rxn-113, Rx-601, and Rx-801) and the control respectively, 30min prior to formaldehyde injection (50 µl, 2.5%) which is injected subcutaneously in the hind paw. The animals were placed in glass chamber to observe the pain response of drug formaldehyde for up to 1hr.

The first acute phase is of 15min after the injection of formaldehyde. The chronic phase is divided into initial and late phase (15-60min) respectively.

#### **Antibacterial Activity**

##### **Minimum Inhibitory Concentration Determination**

Agar plate dilution test was performed in order to evaluate the MIC of ligand and newly synthesized derivatives.<sup>(12)</sup>

The ketamine and newly synthesized derivatives were diluted with sterile distilled water and then added to molten agar, according to NCCLS guidelines 1997.

Mueller-Hinton was autoclaved (100ml) in each conical flask, then ketamine, and newly synthesized derivatives at 10X conc. were added and mixed and this media is poured on the plates. (Washington and wood 1995)

The microbial test organisms were obtained from overnight agar culture of the test organisms. Inocula were prepared by taking 3-5 isolated colonies from agar plate culture. And the growth was transferred<sup>(13)</sup> to tube which contain normal saline and broth culture was incubated to achieve turbidity of 0.5% McFarland standards. ( $2 \times 10^8$  CFU/ml).

NCCLS, 1997 describes the preparation of 0.5% McFarland solution.

The agar plates containing ketamine and other newly synthesized products and control plate were inoculated with 2 µl suspension of microbial suspension, the inoculated plates were allowed to stand until the spot is completely absorbed and incubated for 12hrs at 35C.

The Minimum inhibition concentration indicates that the Ketamine and newly synthesized

derivatives had inhibited <sup>(14)</sup>completely the growth of microorganisms. The results were interpreted according to CLSSI 2009 guidelines.

To check the authenticity of the method, ciprofloxacin 5ug has been used as control.

Ketamine 250 µg caused inhibition zones with diameters of 8–25 mm. These inhibition zones were different for each derivative.

## RESULTS

### 3.1 Biological activity of ketamine derivatives

#### 3.1.1 Anesthetic activity of ketamine derivatives

The results indicated a marked anti-nociception 2-25 min after ketamine and its derivatives Rxn 102, 113, 103, 601and 801 injections.

##### 3.1.1.1 Mean tail immersion latency

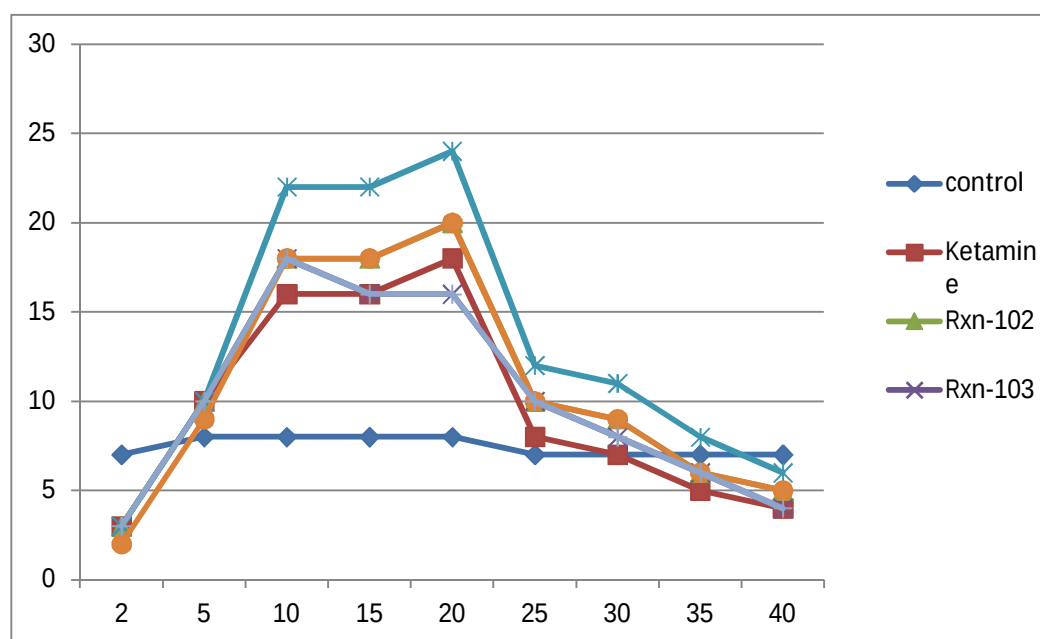
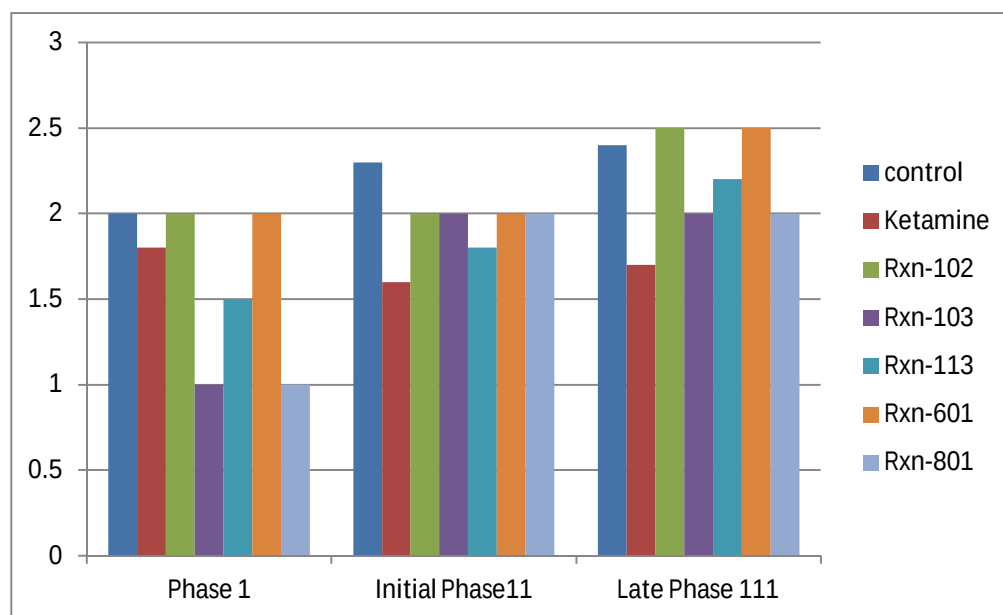


Figure 1. Mean tail immersion latency (s), Time (min).

##### 3.1.1.2 Formalin Test

Pain Score 0-3



**Figure 2. Comparison of the tail immersion and formalin tests data indicated that derivatives from Rxn 102, 113, 103, 601and 801had comparable anesthetic activity to that of Ketamine.**

Chronic formalin pain (phase II) is mediated by inflammatory mediators and CNS activation.

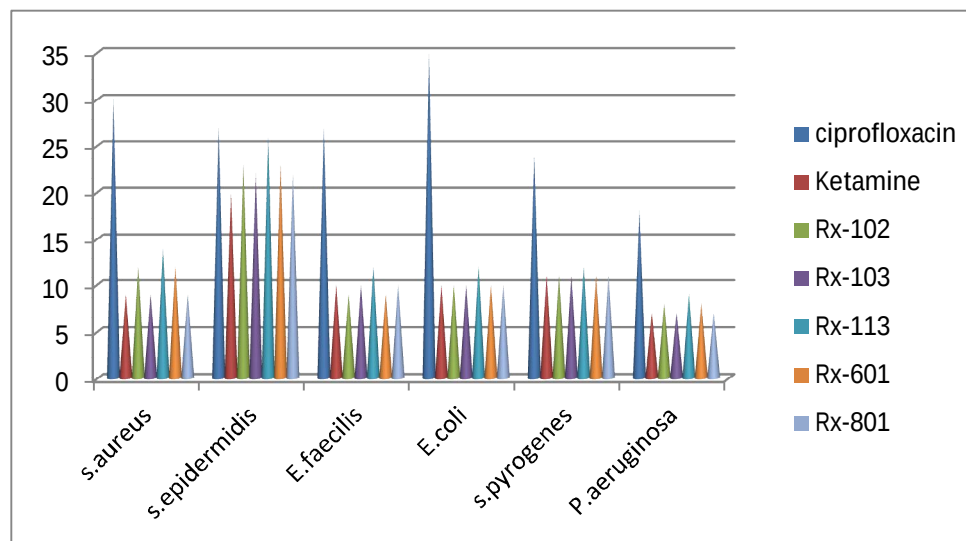
### 3.1.2. Antibacterial Activity

**Table 1. Antibacterial Activity of Ketamine Derivatives Rx-102, Rx-103, Rx-113, Rx-601 and Rx-801**

| Bacteria                  | ciprofloxacin | (L) Ketamine | Rx-102 | Rx-103 | Rx-113 | Rx-601 | Rx-801 |
|---------------------------|---------------|--------------|--------|--------|--------|--------|--------|
| S. aureus ATCC 29213      | 30            | 9            | 12     | 9      | 14     | 12     | 9      |
|                           | 31            | 9            | 12     | 8      | 12     | 12     | 8      |
|                           | 30            | 10           | 12     | 9      | 14     | 12     | 9      |
| S. epidermidis ATCC 12228 | 27            | 21           | 24     | 20     | 26     | 24     | 20     |
|                           | 27            | 20           | 24     | 22     | 26     | 24     | 22     |
|                           | 28            | 20           | 22     | 24     | 24     | 22     | 24     |
| E. faecalis ATCC 29212    | 27            | 10           | 9      | 10     | 12     | 9      | 10     |
|                           | 28            | 10           | 9      | 10     | 12     | 9      | 10     |
|                           | 27            | 10           | 9      | 10     | 14     | 9      | 10     |
| E. coli ATCC 25922        | 35            | 10           | 12     | 10     | 14     | 12     | 10     |
|                           | 35            | 11           | 10     | 12     | 12     | 10     | 12     |
|                           | 35            | 9            | 10     | 10     | 10     | 10     | 10     |
| S. pyogenes ATCC 19615    | 24            | 11           | 10     | 11     | 12     | 10     | 11     |
|                           | 24            | 10           | 12     | 10     | 13     | 12     | 10     |
|                           | 24            | 11           | 10     | 11     | 11     | 10     | 11     |

|                      |    |   |   |   |    |   |   |
|----------------------|----|---|---|---|----|---|---|
| <i>P. aeruginosa</i> | 18 | 7 | 8 | 7 | 9  | 8 | 7 |
| ATCC 27853           | 19 | 7 | 8 | 7 | 9  | 8 | 7 |
|                      | 18 | 8 | 8 | 6 | 10 | 8 | 6 |

Antibacterial activity of ketamine derivatives was compared with ketamine and ciprofloxacin as a controlled antibacterial drug. The antibacterial effects of all compounds were relatively lower than ciprofloxacin.



**Figure 3. Graphical representation Antibacterial Activity of Ketamine Derivatives**

All Compounds obtained from rxns 102, 113, 103, 601 and 801 demonstrated better effects on bacterial strains of *S. aureus* and *S. epidermidis*. However, only rxn 113 showed better results against *E. faecalis* as compared to all derivatives of ketamine.

Although the antibacterial effect on *S. epidermidis* are comparable, ketamine and its derivatives were less effective against all bacteria as compared with ciprofloxacin.

## DISCUSSION

<sup>(15)</sup> reported different pharmacological and clinical effects of ketamine. At higher doses causes standard unconscious like any other anesthetic. It may not selectively enhance the post synaptic action of electrophoretically administered Gaba. Ketamine has been used as general clinical purpose for long ago different researches has been made to check its effectiveness.

<sup>(16)</sup> ketamine is also preferred as an anesthetic agent in experimental animal studies. The doses used in animal studies are calculated as 40–100 mg kg<sup>-1</sup> and these doses are higher than the doses used in human clinical practice.

<sup>(17)</sup> reported ketamine and its optical isomers are sedative analgesic and anesthetic drug. Its main purpose is to checked its effectiveness as an antibacterial agent. It reduces the response of central nervous system to NMA. All the derivatives comparable activity to that of ketamine

<sup>(18)</sup> this mean that they can serve as an alternate to ketamine for profound anesthetic action

To check the effectiveness of ketamine against bacteria *E.coli* and *Staphylococcus pyogenes* were tested etc. Antibacterial activity of Ketamine<sup>(19)</sup> derivatives was compared with Ketamine and Ciprofloxacin as controlled antibacterial drug. The antibacterial effects of all compounds were relatively lower than Ciprofloxacin<sup>(20)</sup>

## CONCLUSION

All the derivatives showed comparable anesthetic activity to that of ketamine. This means that they can serve as an alternate to ketamine for profound anesthetic action. The antimicrobial activity of ketamine and its derivatives was not as effective as commonly used antibiotic Ciprofloxacin however they do have the tendency to inhibit growth of some gram negative as well as gram positive bacteria which can turn out to be a plus point of using ketamine and its derivatives in surgery as these drugs will produce profound anesthesia and will also decrease the chances of post-operative infection due to microbial inhibitory effect found in this research

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