

Study of Metformin Effect on Antimicrobial Property

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ABSTRACT

Background: Metformin is believed that marvelous survivor for diabetes and coined as glucose eater among all oral hypoglycemic agents. Antibiotic Resistance is the major issue which compromising the treatment of bacterial, viral, fungal, parasitic infection. The present study showed that effectiveness of metformin drug on microbes.

Methods: This study conducted in the department of pharmacology in the carrier institute of medical sciences over a period of six month.

Results: Antimicrobial activity of metformin was evaluated against Gram-positive, Gram-negative bacteria and fungi using disk diffusion technique of Kierby baur methods. CIP & AK was used as standard antibiotics. Antioxidant potentiality of metformin was investigated by DPPH scavenging activity. Metformin active at 500 µg/ml, showed very good antimicrobial activity against most of the bacterial strains with an average zone of inhibition of 12-15mm. **Conclusions:** Metformin demonstrated a pronounced inhibitory action against *Pseudomonas aeruginosa*, an organism which is known to be multidrug resistant. The tested fungi are *Candida albicans* and *Aspergillus niger*. The tested drug showed very good antifungal activity with an average 13-17 mm zone of inhibition.

Keywords: Antimicrobial activity, metformin, antifungal activity, pharmacology

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INTRODUCTION

Resistance of Anti-microbial activity is a serious global strength issue which compromising the treatment of bacterial, viral, fungal and parasitic infections.^[1] New drugs or new drug combinations may be the solution in the battle against resistance development in serious infectious diseases. The concept of reversal of resistance by means of non-antibiotics may be a solution for bringing drug resistant micro-organisms back to their original sensitivity to the classical antibiotics.^[2] A variety of compounds which are employed in the management of pathological conditions of a non-infectious etiology have also been shown to modify cell permeability and to exhibit broad-spectrum antimicrobial activity *in vitro* against bacteria and other microorganisms.^[3-5] There is evidence that certain non-antibiotic compounds, alone or in combination with conventional antibiotics, may

play a useful role in the management of specific bacterial infections associated with high risk of resistance to conventional antibiotics.^[6-8] Drugs belonging to different pharmacological classes such as Antihistamines (Diphenhydramine, Bromodiphenhydramine, Promethazine), Psychotropics (Promazine , chlorpromazine , Fluphenazine, Trifluoperazine), Antihypertensive (Methyl-DOPA), Local anesthetics (procaine), Hypoglycemic possess powerful antibacterial activity. Such chemotherapeutic agents have been grouped together and are now entitled as "Non-antibiotics".^[9-14]

The present paper describes the antimicrobial and antioxidant potentiality of a hypoglycemic drug, metformin. Metformin is an oral antidiabetic drug in the biguanide class.

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It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production by the liver.^[15] It was first introduced in the 1950s in Europe, and subsequently approved in the USA. Hyperglycemia increases oxygen-reactive species generation and reduces the protective capabilities of antioxidant defense systems. In patients with type 1 or 2 diabetes mellitus (DM), the increased production of oxygen free radicals^[16,17] may be linked to the development of chronic complications of diabetes.^[18-20] In vitro studies have demonstrated that oral antidiabetic drugs have antioxidant effects that might be secondary to an inhibiting role in lipid peroxidation.

METHODS

ATCC strain of different bacteria were collected in the department of microbiology & Muller Hinton agar were prepared for the growth of organism and kept at optimum temperature and pH. The duration of the study was six months. For comparing of antibacterial activity of metformin drug with standard antibiotics, Disk diffusion test were used to evaluate zone diameter.

Preparation concentration of drug

Accurately weighed 5mg, 10mg, 15mg and 20mg of metformin (crude drug) were dissolved in required amount of DMSO and distilled water, since metformin did not dissolve in distilled water with DMSO, thus the same amounts of drugs were dissolved in 0.5ml of methanol to get concentration of 100µg/10µl, 200µg/10µl, 300µg/10µl and 400µg/10µl. The solution of 10µl was dripped onto the disc such that each disc had 100µg, 200µg, 300µg and 400µg of test drug metformin and then the disc was allowed to dry for methanol to evaporate. Since the Ciprofloxacin (5µg/disc) and Amikacin (30µg/disc) were standard antibiotic agents so the discs with respective concentrations were brought from Hi-media.

Preparation of test inoculums

Sterile plates of Muller Hinton agar were prepared and incubated at 37°C for 24 hours to access for any contaminant. ATCC strains of different bacteria were inoculated from the stock cultures onto the MHA plates and were incubated at 37°C with Sterile filter paper discs containing drug and placed at appropriate position of the flooded plate surface and the diameter of zones of inhibition (mm) was taken. Analogous procedure was followed for ciprofloxacin and amikacin and the cores providing zone diameter was compared to evaluate anti-microbial property of metformin.

RESULTS

In this study, zone diameter use as parameter to evaluate of antimicrobial property of metformin drug. The metformin dilution was prepared in the methanol. Different zone diameters observed in this study for the different bacteria. For the comparison standard drug were taken in this study. The activity of metformin drug showed dose dependent antimicrobial activity. At 500 dose, very good antibacterial activity was found average zone of inhibition 12-15 mm. The activity of metformin also showed antifungal activity which describe in Table no. 3.

Table-1: Metformin dissolution for the preparation of disc

Solvent	Metformin (weight)	Dissolution
Water	5-50mg	Not seen
Di-methyl sulphoxide (1 ml)	50 mg	Not seen
Methanol	5mg	100(µg /10 µl)
	10 mg	200(µg /10 µl)
	15 mg	300(µg /10 µl)
	20 mg	400(µg /10 µl)

Table-2: Zone diameter of different drug by Kirby Baur Method

Bacterial Strains	Metformin Concentration		Standard drug with Concentration	
	(250 µg /µl)	(500 µg /µl)	Ciprofloxacin (5 µg)	Amikacin (30 µg)
Pseudomonas aeruginosa	11mm	13 mm	16 mm	14 mm
Escherichia coli	13 mm	15 mm	20 mm	16 mm
Staphylococcus aureus	9 mm	12 mm	15 mm	18 mm
Salmonella paratyphi	10 mm	13 mm	13 mm	18 mm
Vibrio parahemolyticus	11 mm	14 mm	12 mm	15 mm

Table-3: Different zone diameter of metformin for antifungal activity

Fungus	Concentration (250 µg /µl)	Concentration (500 µg /µl)
Candida albicans	12 mm	14 mm
Aspergillus niger	14 mm	17 mm

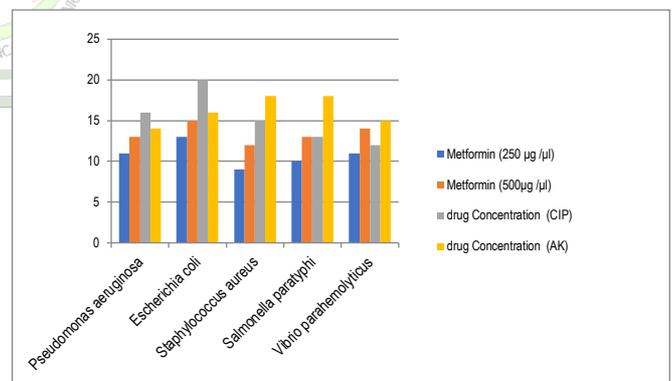


CHART:-1 Zone diameter of different drug by Kirby Baur Method

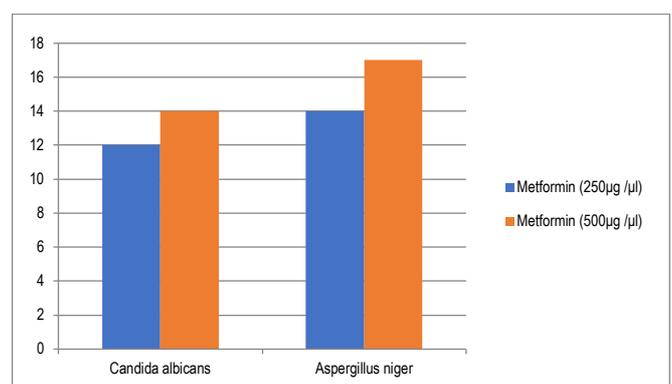


CHART:-2 Different zone diameter of metformin for antifungal activity

DISCUSSION

Metformin was found to have commanding antibacterial activity for gram positive as well as gram negative bacteria and also had antifungal activity. It may be defined here that metformin established a noticeable inhibitory action against different organisms which is known to be multidrug resistant. The proposed mechanism by which non-antibiotics exert their in-vitro antimicrobial activity is thought to be via effects on the inner membrane of bacteria.^[21,22]

DPPH radical scavenging activity of Metformin

The percentage searching of DPPH free radical was found to be concentration dependent i.e. concentration of the extract between 25-200 µg/ml greatly increasing the inhibitory activity with the IC50 value 56.90± 0.83µg/ml active metformin. While IC50 value of standard ascorbic acid was found to be 51.89 ± 1.11µg/ml. Safer Antioxidants are vital to avoid the development of free radical mediated disorders. They can either scavenge ROS/RNS to stop radical chain reactions (primary antioxidants or free radical scavengers) or inhibit the reactive oxidants from being formed into ROS/RNS (secondary or preventive antioxidants).^[23] The percentage scavenging of DPPH free radical revealed by metformin was found to be concentration dependent i.e. concentration of the extract between 25-200 µg/ml greatly increasing the inhibitory activity. This Potential DPPH radical scavenging activity might confirm its hydrogen donating capacity and also its proposed ability to protect the consumers' health from various free-radical related diseases. Previous studies had demonstrated efficiency of metformin in managing oxidative stress.^[24]

CONCLUSION

The present study concludes that, the potential of metformin as a noteworthy antimicrobial agent, because such properties are likely to improve its usage in humans. Furthermore, the antimicrobial efficiency of metformin may be enhanced by structural modifications or be augmented by suitably combining metformin with conventional antimicrobial agents to produce synergism. DPPH radical scavenging efficacy of metformin would be very much useful for diabetic patient.

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