Research Article

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Study of Antimicrobial and Antioxidant potentiality of Anti-diabetic drug Metformin.

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Abstract:

Metformin, an oral hypoglycemic drug was studied in vitro for possible antibacterial and antioxidant activity. Antimicrobial activity of metformin was evaluated against 9 Gram-positive, Gram-negative bacteria and 3 fungi using disk diffusion technique. Kanamycin (30µg/disc) was used as standard. 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. Metformin demonstrated a pronounced inhibitory action against *Pseudomonas aeruginosa*, an organism which is known to be multidrug resistant. The tested fungi are *Saccharromyces cerevaceae, Candida albicans and Aspergillus niger.* The tested drug showed very good antifungal activity with an average 13 -17 mm zone of inhibition. The percentage (%) scavenging of DPPH free radical of metformin was found to be concentration dependent with IC_{50} value 56.90± 0.83µg µg/ml while IC_{50} value of standard ascorbic acid was found to be 51.89 ± 1.11µg/ml. The results of these studies indicated that metformin could be significantly protect animals from tested pathogenic bacteria as well as might be beneficial for diabetic patient more than a hypoglycemic drug.

Keywords: Metformin, Antimicrobial activity, zone of inhibition, antioxidant, free radical.

Introduction

Anti-microbial drug resistance is a serious global health issue 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 aetiology have also been shown to modify cell permeability and to exhibit broad-spectrum antimicrobial activity *in vitro* against bacteria and other microorganisms(3,4,5).There is evidence that certain nonantibiotic 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 (6,7, 8). Drugs belonging to different pharmacological classes such as Antihistamines(Diphenhydramine,Bromodiphenhydramine 9, Promethazine 10), Psychotropics (Promazine 11, chlorpromazine 12, Fluphenazine, Trifluoperazine), Antihypertensive (Methyl-DOPA 13), Local anesthetics (procaine), Hypoglycemic possess powerful antibacterial activity. Such chemotherapeutic agents have been grouped together and are now entitled as “Non- antibiotics” 14,4. The present paper describes the antimicrobial and antioxidant potentiality of a hypoglycemic drug, metformin. Metformin (Figure 1) is an oral antidiabetic drug in the biguanide class. 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 (18,19,20). In vitro studies have demonstrated that oral antidiabetic drugs have antioxidant effects that might be secondary to an inhibiting role in lipid peroxidation.

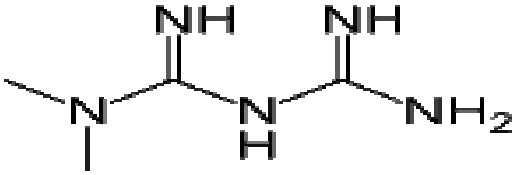

Fig 1: Metformin (N, N-Dimethylimidodicarbonimidic diamide Hydrochloride)

Experimental

Drug

The cardiovascular drug metformin (Figure 1) was obtained in pure dry powder form from Dr. Reddi, India, and was preserved at 4ºC

Preparation of various concentration of drug

Accurately weighed 50mg of metformin, was dissolved in 0.7ml of di-methyl sulphoxide (DMSO) and 50 ml of sterile water to get conc. of 50µg/ml.(stock solution). From the above stock solution 0.25ml, 0.3ml, 0.5ml solution was pipetted and was diluted and the volume was made up to 10ml with the nutrient agar which give solution of conc. 250µg/ml, 300µg/ml, 400µg/ml respectively.

Antimicrobial assay

Microorganisms:

Antimicrobial activity was tested against *B. megaterium, B. subtilis, B. cereus, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Psedomonas aeruginosa ,Salmonella paratyphi & V. parahemolyticus , Saccharromyces cerevaceae*, *Candida albicans* and *Aspergillus niger.* These microbial strains were isolated from clinical samples and obtained as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Antimicrobial screening

Media

Antibacterial activity of active metformin hydrochloride were investigated against 11gram positive and gram negative bacterial strains by the paper disk diffusion technique (21) using 100µl of suspension containing 108 CFU/ml of bacteria spread on nutrient agar medium. Sterile 6 mm diameter filter paper discs were with 500µg of each of the sterile test material and placed into nutrient agar medium. Kanamycin (30µg/disc) disc were used as positive control to ensure the activity of standard antibiotic against the test organisms. The sample discs and the standard antibiotic discs were placed gently on the previously marked zones in the agar plates pre-inoculated with the test bacteria and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 12 hours.

Determination of zone of inhibition

After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (22). The experiment was carried out in triplicate and the mean value was taken.

In vitro antioxidant activity screening by DPPH Free radical scavenging activity

The free radical scavenging activity of active metformin hydrochloride, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical was determined by the method described by Braca (23) . The 0.1 mmol/L solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 mL of metformin solution at different concentrations (5, 10, 25 and 50 µg/mL). After 30 min, absorbance was measured at 517 nm. Vitamin C (ascorbic acid) was used as a reference drug. The percentage inhibition activity was calculated from the following equation.

Percentage of inhibition = $[(A_0–A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition.

Results & Discussion

In vitro Antimicrobial activity of Metformin

The antimicrobial efficacy of active metformin hydrochloride against nine pathogenic bacteria and three fungi were shown in Table 1 & table 2.

The samples of active metformin hydrochloride showed dose dependant antimicrobial activity. At 500µg/ml dose, very good antibacterial activity was found with average zone of inhibition 12-15 mm.

Result of Antifungal screening of active metformin showed very good (13-17 mm, zone of inhibition) antifungal activity in a dose dependant manner.
Metformin was found to possess powerful antibacterial activity both for gram +ve & gram –ve bacteria. It may be pointed out here that metformin demonstrated a pronounced inhibitory action against *Pseudomonas aeruginosa*, an organism 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 (24,25).

DPPH radical scavenging activity of Metformin
The percentage (%) scavenging 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 (Figure 1) with the IC_{50} value 56.90± 0.83µg/ml active metformin hydrochloride. while IC_{50} value of standard ascorbic acid was found to be 51.89 ± 1.11µg/ml.

Safer Antioxidants are essential to prevent the progression 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) (26). 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 (Figure 2) .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 (27).

Conclusion

The present study indicated 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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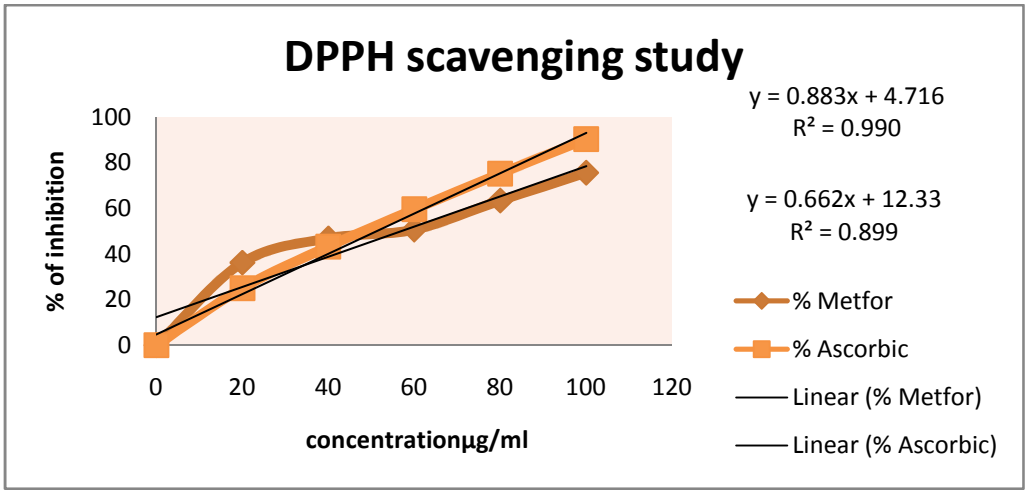

Fig 2: Determination of LC_{50} values for standard and metformin from linear correlation between logarithms of concentration versus percentage of mortality.

Table 1: In vitro antibacterial activity of Metformin active and kanamycin discs .

Name of Bacteria	Prepared sample of Metformin		Kanamycin 30µg/disc
	Concentration 250µg/ml	Concentration 500µg/ml	
Gram positive Bacteria			
Staphylococus aereus	9	12	30
Bacillus megaterium	11	13	32
Bacillus subtilis	10	12	30
Bacillus cereus	11	14	25
Sarcina lutea	12	13	33
Gram negative Bacteria			
Salmonella paratyphi	10	13	28
Escherichia coli	13	15	32
Vibro parahemolyticus	11	14	33
Psedomonas aeruginosa	11	13	28
Fungus			
Candida Albicans	12	14	
Asperagillus niger	14	17	
Sacharomyces cereveceae	11	13	