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Activity of drugs against bacteria, fungi and viruses

Punita Sharma ✉ and Qurratul Ain

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Abstract

We use drugs in treatment of diseases. A successful drug is one which is readily absorbed slowly excreted and having low toxicity. To evaluate the antibacterial activity of drug, add drug is in known concentration to the cultures of the test organisms. We use cup-plate agar diffusion method for this. To evaluate the Antifungal activity the compound screened in vitro against Aspergillus niger. Potato Daxtrose agar medium was used and the zone of inhibition were measured. The activity of compound was represented by (+), (++), (+++). To evaluate antiviral activity we use EMC virus and JE Virus. EMC virus has been reported from Rodents, Rhesus monkey chimpanzee and Mandrill baboon. Rats have considerable virus in their tissues, urine and faces. In Human EMC virus shows a mild febrile illness to severe encephalomyelitis. EMC virus causes fibrile CNS disease accompanied by sign of paralysis, headache, nuchal rigidity, vomiting and stiff neck etc. EMCV infection can be prevented through vaccination. JEV has a single stranded. Single stranded RNA genome which serves as m-RNA. The coat consists of several copies of 13-14 Kda capsid proteins. JEV causes acute infection of CNS producing meningo myalo encephalitis. The mortality rate is high in this disease. The virus is spread through mosquito vectors and multiplies in pigs and rats. JEV duplicates in liver, spleen, lungs and other tissues. There is urgent need to find out such agents which may act as effective antiviral.

Key words: Metabolites, Chemotherapeutic value, physiological actions, autoclave, chromic acid

Introduction

Secondary metabolites are produced by the use of any drug in treatment of disease is of two types (Bartroli *et al.,* 2008). The first type is used in treatment of specific diseases. The second type of that which has significant effect upon animal and organism but not the remedy for a particular organism but not the remedy for particular diseases (Bekhit , 2008). A successful drug is one which is readily absorbed and slowly excreted and having low toxicity (Bexon *et al.,* 2008). This relationship is shown by the ratio maximum tolerated dose (MTD) or maximum curative dose (MCD). The Langer the ratio safer would be the drug (Bhatt *et al.,* 2001). The biological reactivity of durg is due to the complete molecule. The personal reactivity of the entire atom is changed during the formation of the drug (Bhawsar *et al,* 2008). The structure-activity relationship undergo slow changes with advancement in the knowledge of chemical and physical properties of the molecules (Bhusare *et al.,* 2004). The most recent and thoroughly considered theories have not showed regularity in the relation of chemical structures of physiological actions (Biradar *et al.,* 2004). According to W.A. Sexton, physical properties and activity of a molecule after with the structural variations and will changes. In the distribution of cells and tissues in access to active sites of enzymes and receptors, the reaction rates of such sites and in excretion patterns (Bogert and Soil, 2006). This suggested a new approach in chemotherapeutic research which includes the tests of compounds related to metabolite of microorganisms (Breuer, 2008). Thus in evaluating structure-activity relationship, the total picture of steric factor, electron density, localization and the physical and chemical activities of a compound require to the considered (Chaurasia and Sharma, 1982). Chemotherapeutic value of a compound is determined in various stages.

Evaluation of Antibacterial Activity: The commonly used method is to add drug in known concentrations to the cultures of the test organisms

Author's Address
[1]Department of Chemistry, SMJN (PG) College, Haridwar
[2]Department of Engineering Chemistry, SGIT, Ghaziabad
E-mail.: *010sharmapunita @gmail.com*

(Chaurasia *et al.,* 1980). In the present work, activities of the synthesized compounds evaluated by cup-plate agar diffusion method (Chemykh *et al.,* 1979). The synthesized compounds have been covered in vitro against staphylococcus aureus. The cup-plate agar diffusion method consists of following steps.

(a) Preparation of the medium, its sterilization and tubing.
(b) Treatment of the glass apparatus and its sterilization.
(c) Pouring of the seeded medium into sterilized petri dishes and cutting of the cups.
(d) Preparation of the needed concentration of drug and their pouring into the cups.
(e) Incubation at particular temperature.
(f) Measurement of the zones of inhibitors.

The medium exerts greatest effect upon the activity of a compound (Dabhi *et al.,* 2005). The other factors which influence the in-vitro tests are the conditions of the test organism, the drug concentration, incubation period, environment factors, temperature (generally 37°C), pH (generally 7.2-7.6). In present work, the nutrient agar media is employed which has the composition. Peption 10g, Beef extract 3.0 g, Yeast extract 1.5g, Sodium chloride 1.5g, Glucose 1.0g, Distilled water 1000 ml, Agar-agar 17.5 g. For the preparation (Dahm *et al.,* 2003) of medium all the above ingredients except agar-agar were weighted and mixed in water (300 ml) by gentle heat. After that 500 ml of distilled water was added. The pH was adjusted in the range of 7.5 ±0.1. Then agar-agar was added and the mixture was autoclaved for half an hour (Jones and Sanderfy, 1956) this was filtered through cotton. The medium obtained was transferred in culture tubes. The tubes plugged with cotton were sterilized by autoclave. All glass apparatus were cleaned with chromic acid and then sterilized by oven (Cotthup *et al.,* 1964). The hot medium in tubes poured into petridishes and cool upto 50°C then 0.3 ml of medium containing test organism was added to each petridish. The area of the medium was divided in three similar parts with glass marking pencil. Four holes, one in each part with a sterilized culture (Szymanski, 1969). The test solutions having concentration of 2.5 µg/ml and 50 µg/ml were obtained by mixing compounds in dimethyl formamide (DMF) which works as a control. Solutions of three different compounds having conc of 25 µg/ml were poured into three holes of petri-dish and in the second petridish, solution of the same concentration of 50 µg/ml of the same compounds were poured in the holes marked with the same numbers (Tobin, 1971). The central hole was filled with DMF (control). All petridishes were transferred to an incubator adjusted at 37°C and left for 72 hours. The zone of inhibition formed due to the test compound (Dollish *et al.,* 1974).

In the present work, the activity of compliance is represented by (+), (++), (+++) and (++++) each (+) shows a difference of 2mm in the diameter of the zones of inhibition (-) shows no zones of inhibition (Rao, 1963) .

Evaluation of Antifungal activity

All the synthe sized compounded have been screened in vitro against aspergillus niger. All the glass appends was leaned with chromic acid followed by distilled water and then sterilized by warming at 200.c in oven (Bellamy, 1963). Potato dextrose again medium was used which consisted of potatoes (infusion from) 200g/l dextrose 20 g/l, agar 30 g/l, these ingredients were weighed and dissolved in 500 ml of distilled water by gentle heat (Bellamy ,1968) then distilled water was added to make the solution to 1liter. The PH was adjusted in the range of 7.6 + 0.1. The medium was auto laved for half an hour, then transferred in 25 ml portions in sterilized conical flasks fitted with cotton plugs. Then again auto laved at 151/sq inch for one hour and then reared into sterilized petridishes. Then it was allowed to cool upto 50°C.

Fairly uniform suspension of fungus was prepared, a known compound tone tested for fungicidal activity in diethyl formamide was obtained and 25µg/ml and 50µg/ml were added to petridish. (Herzber,1945). The medium was divided into three equal parts with glass marking pencil; holes are made with a sterilized cutter. Solution of three different compounds having conc of 25µg/ml and 50µg/ml were added in three holes of two petridishes and central hole was filled with DMF (control (Bhagavartam and Venkatarayudu 1962). The zones of inhibition were measured (Wilson *et al.,* 1955). The activity of compound is represented

by (+), (++) (+++). (Dyer,1965) "Evaluation of Antiviral activity".

A. Encephalomycarditis Virus :-

The strains of this virus was isolated by Julgleblut and Sander from cotton rats which were used for the passage of the Yale – SK strain of Poliomyelitis virus (Becker and Gordy 1968), EMC virus have been reported from rodents, rhesus monkey, the chimpanzee and the mandrill baboon. Rats have considerable virus in their tissues, urine and feces (Ingold *et al.,* 1936). In human EMC virus shows a mild febrile illness to severe encep halomyelitis (Baily *et al.,* 1936). EMC virus causes fibrile CNC disease. With a lymphoctic ploocytosis accompanied by sign of paralysis, headache, nuchal rigidity, photophobia, vomiting, stiff neck and hyperactions deep reflexes, (Angus, 1936) EMC virus can also be preserved in 50% glycol or 0.05 molar glycine (Mair *et al.,* 1949). It is deactivated by a temp of 60°C for 30 minutes. Lesion in experimentally infected animals is found in the entire central nervous system and in striated and cardiac muscles (Miller, 1956). Specific antibodies which cross-react equally with all the strains seems in the sera of conalescent animals and man. EMCV infection can be prevented through vaccination. The vaccines are Arildone, Ribavirin and Enviroxime.

B. Japanese Encephalitis virus (JEV) :

It belongs to the fflavivirus family. JEV has a single stranded RNA genome which serves as m-RNA. The cost consists of several copies of 13-14 kda capsid proteins (Miller and Crowford, 1946). JEV causes acute infection of central nervous system producing meningo myalo encephalitis. The mortality rate is high in this disease (Crowford and Miller, 1949). The virus is spread through mosquito vector and multiplied in pigs and vats. JE virus duplicate in liver, spleen, lung and other tissue. It spreads extra cellularly within the CNS by the infection of neurons (Tobins *et al.,* 1971). JE virus caused several epidemics in our country. JE virus epidemics also seen in Bankura district of West Bengal, Gorakhpur in Uttar Pradesh and also in Goa and Tamil Nadu. JE virus infection can be checked through vaccination. The manufacture of this vaccine has been taken up in India recently. However there is indication that limits the utility of present vaccines. Thus there is urgent need to find out such agents which may act as effective antiviral (Chaurasia and Sharma, 1982).

Material and Methods

EMCV and JEV were obtained from Dr. R.K. Maheshwari, Deptt of Pathology, University of Health Sciences, Maryland (USA) by Antiviral Screening has been conducted. Brains from mice with specific symptoms were collected septically and 10% brain homogenate was observed in a minimum essential medium (MEM). The mean Lethal dose (LD_{50}) of the virus in mice was estimated according to Reed and Muench (Pitzer and Scott, 2002). The EMCV was titrated in Swiss albino mice (15-16 g). The JE virus was spread in the brain of 1-2 days old sukling mice infected by an intracerebral injection. The brains of infected animals with specific symptoms were collected and titrated in MEM to isolate the virus. For mice the mean Lethal dose (LD_{50}) was estimated by the method of Reed and Muench. JEV was titrated in Swiss Albino mice (14-15) gm body weight (Randle and Whiffer, 2004).

Antiviral Activity Assay

In Vitro : The invitro antiviral assay was executed according to Sidwell and Huffman. The monolayers of vero cells in 96 well micro titre plate (Nunk) with 0.1 ml of the test compound in Hanks maintained salt solution with two fold serial dilution upto the lowest concentration. After that they have been incubated for 24 hours at 37°C. The plates were seen microscopically for cytotoxicity, for eg deformity, swelling and sloughing of the cells (Gray, 2006). After assessment of the cytotoxic concentration, the plates were washed with HBBS and 5 LD_{50} EMCV and JEV were challenging in each well keeping cell and virus controls separately for prophylactic studies. The plates were strained after incubation for 48 hr . with 0.1% crystal violet and studied microscopically for percent cytopathic effect inhibition. (Ingold *et al.,* 1936) After antiviral valuation, vero cells grown in 96 well microtitte plates were challenged side by side with the virus as well as two fold serial dilution of the trial compound. The plates were studied microscopically at 37°C after 48 hrs. for therapeutic administration, virus was permitted to absorb on vero cells at 37°C for 90 min and then a

serial dilution of the trial compound was added (Angus *et al.,* 1936).

In Vivo: Experiments were carried out by the method of Kant Swiss albino mice (14-16 g body weight) were injected with the trial compound intraperitoneally every 24 hrs for 3 consecutive days. All the animals have been seen for 21 days for their distinct paralytic symptoms and mortality. The mean survival time were calculated by the number of animals dying per day (Dunn and Ingogld, 1955).

Result and Discussion

Quinolinly substituted 1,2,4 triaole [3,4-b] [1,3,4} thiadiazoles have been screened for antibacterial against S.Aureus at the concentration 25µg/ml. 1,3,4 thiadiazolyl and quinolinly substituted pyrazoles were tested for antifungal activity against aspergillus niger. It has been seen that when methyl is substituted in the para position of benzene ring then activity is maximum. In case of hydrogen and chloro substitutions it was moderated. In case of bromo substitutions activity was almost nil. Compounds were evaluated in vitro for antiviral activity against cnephalomycardiaties virus (EMCV). Single compound exhibited 75% protection at 3.25µg/ml conc. Other compound did not show any activity. Some compound being tested for its in vivo efficacy shows only 40% protection against EMCV. 1,3,4 – thiadiazol substituted 1,3,4 – oridiazoles were tested fr their antiviral efficacy against Japanese Enephalitis virus.

Conclusion

Quinolinly substituted 1,2,4 triazole have been screened for antibacterial against S. Aureus, Quinolinly substituted pyrazoles were tested for antifungal activity against aspergillus niger. Single compound exhibited 75% protection against EMCV 1,3,4 – thiadiazol were shows their antiviral activity against JEV.

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