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Antioxidant and Antimycobacterial Activity of Pyrrole and Imidazole Derivatives.

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ABSTRACT: The purpose of this study was to screen antioxidant and anti mycobacterial activity of pyrrole and imidazole derivatives against Mycobacterium tuberculosis, Mycobacterium smegmatis and Mycobacterium phlei. In these studies,27 pyrrole and imidazole derivatives were used to check antimycobacterial potential. Antioxidant activity of pyrrole and imidazole derivatives were employed to determined DPPH Radical Scavenging Activity, OH Radical Scavenging Activity and SOR Radical Scavenging Activity assay. Antimycobacterial activity was also performed by using agar well diffusion method. Among the all 27 pyrrole and imidazole derivatives 26 are show DPPH scavenging activity at the range of 41.27-70%, 24 are show OH radical scavenging activity at the range of 13-98.8%. all 27 pyrrole and imidazole derivatives show SOR scavenging activity at the range of 18.75-86%. Among the all 27 pyrrole and imidazole derivatives "6" are show good anti mycobacterial activity against three mycobacterium species.

KEYWORDS: Pyrrole and imidazole derivatives, Antioxidant activity, Antimycobacterial activity.

I. INTRODUCTION:

Tuberculosis is a highly infectious disease that is widely distributed throughout the world.The disease is influenced by economic and nutritional factors,immunity and hormonal status have been associated with prevalence. The most popular causative agent is "Mycobacterium tuberculosis" and other Mycobacterium species. It is commonly a disease of lungs(pulmonary tuberculosis) where it forms a localized infection after inhalation.It can affect extra pulmonary regions like lymph nodes,bone and joints, subcutaneous, eyes. Meninges, The Kidneys and also the Gastro-intestinal tract, where it causes insidious disease that develops without any striking clinical evidence.It can also cause Congenital tuberculosis transmissible from infected mother to fetus following ingestion of the amniotic fluid containing M.tuberculosis.[1]

It spread through the air when people who have an active T.B infection cough,sneeze or otherwise transmit their saliva through the air.Most infections are asymptomatic and latent but about one in ten latent infections eventually progress to active disease and ills more than 50% of those infected.The classic symptoms of active T.B infection are "Chronic cough" with blood-tinged sputum,fever, night sweats and weight loss.Infection of other organs causes a wide range of symptoms. Diagnosis of active T.B relies on radiology as well as microscopic examination and microbial culture of body fluids. Diagnosis of latent T.B relies on the Tuberculosis Skin test or blood test. Treatment is difficult and requires administration of multiple antibiotics over a long period of time.

One third of the world's population is thought to have been infected with M.tuberculos is with new infectious occurring at a rate of about one per second. In 2007 there were an estimated 13.7 million chronic active cases globally while in 2010 there were an estimated 8.8 million new cases and 1.5 million associated deaths, mostly occurring in developing countries. About 80% of the population in many Asian and African countries test positive in Tuberculosis test,while only5-10% of the United States population tests positive.

I. Free Radical Formation In Tuberculosis:

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism.A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom.Free radicals are highly reactive due to the presence of unpaired electrons. Any free radical involving oxygen can be referred to as Reactive oxygen species[ROS].Oxygen

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centered free radicals contain two unpaired electrons in the outer shell.When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules thus the chain reaction continues and can be" thousands of events long.The electron transport chain which is found in the inner mitochondrial membrane utilizes oxygen to generate energy in the form of ATP. Oxygen acts as the terminal electron acceptor with in electron transport chain.From 2-5% of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide free radical via electron escape.Excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism ,leads to a variety of pathophysiological process such as inflammation, diabetes,geno toxicity and cancer[2].

In tuberculosis, oxidative stress is a result of tissue inflammation poor dietary intake of micro nutrients due to illness. Free radical burst from activated macrophages and anti-tuberculosis drugs.These free radicals may in turn contribute towards pulmonary inflammation if not neutralized by antioxidants. The formation of free radicals is a normal consequence of a variety of essential bio chemical reactions and can occur at elevated rates under pathophysiological conditions. Inflammation related oxidative stress has been implicated in the pathogenesis of lung fibrosis and dysfunction in patients with pulmonary tuberculosis. This fibrosis is thought to be mediated by activated macrophages and results in a granulomatous response with central necrosis. Activated macrophages are capable of releasing a variety of chemicals including oxygen free radicals.

An increased circulating level of free radical activity has been found in patients with active pulmonary tuberculosis.Such findings contribute to the belief that a range of free radicals are produced in inflammatory process with fibro genic potential and that tuberculosis may be measure of different stages of the disease process[3].

As a result, potential damage to cells and tissues.There are enzymes,small molecular weight molecules and micro nutrients with antioxidant capabilities that can protect against the adverse effects of free radical reactions.There is therefore a critical balance between free radical generation and antioxidant refences.An imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage is termed as"Oxidative stress"

Mycobacteria can induce reactive oxygen species(ROS) production by activating phagocytes and although these are an important part of the defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation. This may further contribute to immune suppression. Particularly in those with impaired antioxidant capacity, such as HIV infected patients. More over the malnutrition which is commonly present in patients with tuberculosis can add to the impaired antioxidant capacity in the patients.

Examination of antioxidants in patients with tuberculosis may identify deficiencies that predispose to serve oxidants injury and immune deficiency. Antioxidant profile and its relation to lipid peroxidation in tuberculosis is very limited. Thus, to further study the interaction between tuberculosis and antioxidants. It affects the antioxidant status because of lipid peroxidation products in T.B patients.

II. METHODS AND MATERIALS:

I. Selection And Culturing Of Bacteria For Antimycobacterial:

Bacterial cultures were obtained from Life Science Department Laboratory of S.R.T.M.U. Nanded (M. S., India). From these cultures, master cultures were prepared.

Mycobacterium tuberculosis.
Mycobacterium smegmatis.
Mycobacterium phlei.

Derivatives of pyrrole and imidazole were selected for anti-mycobacterial activity.The list of Pyrrole and imidazole derivativesis given here,(Hydralazine, Levosimendan, Bemoradan, Minaprine, Pipofezine, Emorfazone, Imazodan, prizidilol, Amipizone)

II. Antioxidant Activity:

DPPH RADICAL SCAVENGING ACTIVITY:

The scavenging activity of DPPH free radicals by pyrrole and imidazole compounds were determined according to the reported method [11].(0.1mM) DPPH was prepared in methanol solution. The reaction mixture contained (0.1mM) 30 µl of sample(pyrrole and imidazole compounds),30 µl DPPH,120 µl of methanol were added. The reaction was started by adding DPPH,incubation for 20 minutes at room temperature and absorbance determined at

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517nm.Ascorbic acid(0.1mM) used as reference compound[12].

OH RADICAL SCAVENGING ACTIVITY:

The OH radical scavenging activity was demonstrated with Fenton reaction[12].The reaction mixture contained 10µl $FeCl_3$,15µl 1,10 phenanthroline,50µl phosphate buffer (7.8pH),50µl sample and 25µl H_2O_2.The reaction was started by adding H_2O_2.After 5 minutes incubated at room temperature the absorbance was recorded at 560nm.Ascorbic acid(0.1mM) used as reference compound[13].

SOR RADICAL SCAVENGING ACTIVITY:

The SORscavenging assay performed by the reported method[13]. Super oxide anion radicals were generated in a nonenzymatic Phenazine methosulphate Nicotinamide adenine Dinucleotide(PMS –NADH) system.It was assayed by the reduction of Nitro blue tetrazolium(NBT).In this experiment SOR radical was generated in 100ul Tris HCL buffer(100mM, pH 7.4) contained 25µl of NBT(300 mM),25 µl of NADH(936 mM) and 10µl of sample(0.1mM).The reaction was initiated by adding 25µl of PMS(120mM).After 5 minutes incubation at room temperature the absorbance was measured at 560nm.Ascorbic acid(0.1mM) used as reference compound[14].

ANTI-MYCOBACTERIAL ACTIVITY:

The antimycobacterial activity was carried out by using agar well diffusion method.L-J agarmedium and borosil glass petri plates wereautoclaved at 121^0c at 15 lbs for 15 minutes. 30ml of nutrient agar was poured in each plate and allowed tosolidify. The suspension of above culture (Mycobacterium tuberculosis, Mycobacterium smegmatis, Mycobacterium phlei.) was prepared and 100 µlof suspension wereuniformlyspread onL-J agar medium containing plateswith the help of sterile glass spreader.Wells were made on the plates with sterile cork borer having 9 mm diameter. The concentration of samples was taken 0.03mg/ml and 50µl of sample was dropped into the prepared wells. Rifampicin an antibiotic 50µg/ml was introduced into the well of plate as a control. After introduction of pyrrole and imidazole compounds and antibiotics, the plates were kept in refrigerator for 30 minutes and then plates were incubated at 37 ^{0}c for 24 to 48 hrs. The antimycobacterial activity of pyrrole and imidazole compounds were determined by measuring diameter of zone of inhibition.

III. Rifampicin A Good Antibiotic For Tuberculosis:

Rifampicin or a Rifampin is a bactericidal antibiotic drug of the "rifampicin group".It is a semi synthetic compound derived from "Amycolatopsisrifamycinia".Rifampicin is used in the treatment of a number of bacteria but known for activity against "mycobacterium" strains such as cause tuberculosis and "Hansen's disease".This drug is always used against active infections in combination with other antibiotics.

MECHANISM:

Rifampicin inhibits DNA dependent RNA polymerase in bacterial cells by binding its beta-subunit,thus preventing transcription to RNA and subsequent translation to proteins.Its lipophilic nature makes it a better to treat the tuberculosis.

IV. Activity On Standard Free Radicals:

DPPH: It is the violet-colored free stable radical(2,2 –diphenyl-1-picryl hydroxyl)which now is used as standard and as calorimetric reagent for redox process.It is also useful in a variety of investigations such as polymerization inhibition or radical chemistry and determination of antioxidant properties of amines,phenols, or natural compounds(vitamins,plant extracts,medicinal drugs).DPPH is insoluble in water[11]

OH: The hydroxyl radical OH is the neutral form of the hydroxide ion(OH^-).Hydroxyl radicals are highly reactive and consequently short lived;however,they form an important part of radical chemistry.Most notably hydroxyl radicals are produced from the decomposition of hydroperoxides or in atmospheric chemistry, by the reaction of excited atomic oxygen with water.It is also an important radical formed in radiation chemistry.Since it leads to the formation of hydrogen peroxide and oxygen.The hydroxyl radical can damage all types of macromolecules carbohydrates,nucleic acids,lipids and amino acids.The hydroxyl radical has a very short invivo half-life of approximately 10^{-9} seconds and a high reactivity.This makes it a very dangerous compound to the organism.

SOR: Superoxide also known by other name "Hyperoxide" is a compound that contains the superoxide anion with the chemical formula O_2^-. Superoxide anion is particularly important as the product of the one electron reduction of dioxygen O_2,which occurs widely in nature with one unpaired electron.Superoxide is produced in large quantities by the enzyme NADPH oxide for use in oxygen

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dependent killing mechanisms of invading pathogens.Superoxide may contribute to the pathogenesis of many diseases the evidence is particularly strong for radiation poisoning and hyperoxic injury.

V. Anti-Mycobacterial Activity On Following Mycobacterium Species:

Mycobacterium tuberculosis: It is a slow growing bacterium causing a respiratory transmitted disease tuberculosis.It is asmall aerobic,nonmotile bacillus.It divides every 16 to 20hrs,which is an extremely slow rate compared with other bacteria.Mycobacteria have an outer membrane lipid bilayer.They contain high lipid and mycolic acid content of its cell wall.Mycobacterium tuberculosis can with stand weak disinfectants and survive in dry state for weeks.In nature the bacterium can grow only with in the cells of host organism but Mycobacterium tuberculosis can be culturedin the laboratory.The most common acid-fast staining technique is the Ziehl-Neelsen stain and the auramine - rhodamine stains are used.

Mycobacterium smegmatis: Mycobacteriumsmegmatis lives in aggregate layers of cells attached to each other in acommunity called biofilm.It is mostly found in the soil,water and plants.They tend mostly to exist near large bodies of water.It is 3.0 to 5.0um long with a bacillus shape and can be stained by Ziehl-Neelsen method and the auramine-rhodamine fluorescent method.The bacteria will be finely wrinkled and creamy white while it is growing on accessible nutrients.Mycobacterium smegmatis is grow with in 48hrs of time and is abundant.It will also be waxy because of the high amount of unique gram-positive cell-wall coated with mycolic acids.

Mycobacterium phlei: Mycobacteriumphlei is an acid-fast bacterium of the genus mycobacterium.They are named this way because they contain mycolic acids.It is one of the fast-growing bacteria.On egg media and sheep's blood agar,the colonies are flat with a distinctive pigmentation ranging from deep yellow to orange.Mycobacteriumphlei has only occasionally been isolated in human infections,and patients infected with Mycobacterium phlei generally respond well to anti mycobacterial therapy.

III. RESULT AND DISCUSSION:

Antioxidant activity of pyrrole and imidazole derivatives determined by following three assays

I. DPPH Radical Scavenging Activity:

Among the all 27 pyrrole and imidazole derivatives 26 are show DPPH scavengingactivity at the range of 41.27-70%. In which (4-chloro-2-(2-chloro phenyl)-5-(piperidin-1-yl)pyrrole and imidazole) shows lowest activity as 41.27% and (tert-butyl-4-(5-chloro-1-(3-chloro-4-flurophenyl)-1,6,dihydro-6-oxopyridazin-4-yl) piperazine-1-carboxylate) shows highest activity.

II. OH Radical Scavenging Activity:

Among the all 27 pyrrole and imidazole derivatives 24 are show OH radical scavengingactivity at the range of 13-98.8%. The (5-(2-(dimethyl amino)ethylamine)-4-chloro-2-(3-chloro-4-flurophenyl)pyrrole and imidazole) shows lowest activity as 13% and (4-chloro-2-(2-chlorophenyl)-5-(cyclophenyl amino)pyrrole and imidazole) shows highest activity.

III. SOR Radical Scavenging Activity:

All 27 pyrrole and imidazole derivatives show SOR scavenging activity at the range of 18.75-86%. The (5-(2-dimethyl amino)ethyl amino)-4-chloro-2-(3-chloro-4flurophenyl)pyrrole and imidazole)shows lowest activity as 18.75% and (4-chloro-2-(3-chloro-4-flurophenyl)-5-(cyclopentyl amino)pyrrole and imidazole) shows highest activity.

IV. ANTI MYCOBACTERIAL ACTIVITY:

Among the all 27 pyrrole and imidazole derivatives "6"are show good antimycobacterial activity against three mycobacterium species. Three derivatives show activity against M. tuberculosis, two derivatives show activityagainst M.smegmatis, one show activity against M.phlei.

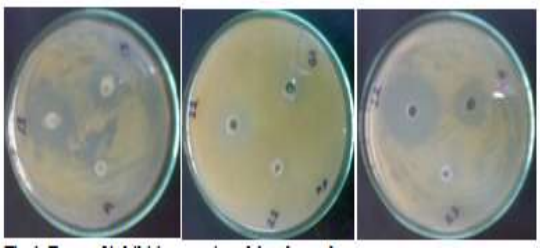

Fig:1 Zone of inhibition against M. tuberculosis

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Fig: Zone of inhibition against M. smegmatis,

Fig: Zone of inhibition against M. phlei.

IV. CONCLUSION

The pyrrole and imidazole derivatives have pharmacological properties and they show anti-inflammatory applications in asthma, chronic obstructive pulmonary disease and also show anti mycobacterial activity. According to the presence study all 27 pyrrole and imidazole derivatives show good antioxidant activity and also anti mycobacterial activity. Some compounds show good antioxidant activity but not show the anti-mycobacterial activity, this is because changing the position of "N" group, methoxy group and other functional groups. So, from present study we can concluded that pyrrole and imidazole derivatives can used as good antioxidants and also anti T.B agents in tuberculosis disease.

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