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Biological & toxological studies of 4,6-dimethyl-2-pyrimidinol, 5-bromo-2, 4-dihydroxy pyrimidine, 2,4-dihydroxy pyrimidine-5-carboxylic acid, 4-hydroxy-2-mercapto pyrimidine, 2-hydroxy-4-mercapto pyrimidine

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ABSTRACT : In this paper we have compiled the biological pharmacokinetic, toxic study, human health effect, therapeutic & other study on 4,6-dimethyl-2-pyrimidinol, 5-bromo-2,4-dihydroxy pyrimidine, 2,4-dihydroxy pyrimidine-5-carboxylic acid, 4-hydroxy-2-mercapto pyrimidine, 2-hydroxy-4-mercapto pyrimidine.

Keywords : human health effect, therapeutic activity, toxic study, pyrimidine compounds.

INTRODUCTION

It is well known that the n-heterocyclic molecules like pyridine, pyrimidine, adenine, uracil, anisols, purine, pyradizine, are very important because of bactericidal, fungicidal, germicidal and pharmacological activities [1-5]. In these compounds pyrimidine and its derivatives are of great importance due to their pharmacogonisticol and biological effect. It is similar to benzene and pyridine containing two nitrogen atoms at one and six position in six member ring. It is isomeric with two other forms of diazine. Three nucleobases found in nucleic acids namely cytosine, thymine and uracil are pyrimidine derivatives. In DNA, RNA these bases form hydrogen bonds with their complementary purines. Thus the purines, adenine and guanine pair up with the pyrimidines, thymine and cytosine respectively. In R.N.A. The complement of pairs are adenine: uracil and guanine : cytosine. [6-26].

Various workers have studied the pharmacopiol & spectral studies [26-30]. Quintero *et. al.*, [31] have been studied the antitumoral activity of pyrimidine derivatives. Balzarini *et. al.* [32] have studied the antiretrovirus activity. of pyrimidine nucleosides. Barbons *et. al.*, [33] have studied the spectroscopic determination of nuclosides against cerebral vascular effect.

Jacob *et. al.*, [34] have studied the effect of 5-bromo-2-deoxyuridine & other bromonated uracils and pyrimidine derivatives in the cultivation media of bacteria [*E. Coli, P. mirabili* etc.) results in the distinct increase of U.V. sensitivity with protective effect.

Engelbrecht [35] have studied and showed .absorption-distribution and excretion of orodin chemical related to pyrimidine class by injection resulted rapidly distributed in blood.

Singh [36] have studied the biological and toxic studies of nitro anisoles administered on albino rats, showed the gene toxin, mutagenic liver kidney, urinary effect (positive and negative).

J.S. Mason *et. al.*, [37] have studied the sex - linked recessive lethal mutations and chromosomal reciprocal translocations in germ cells of male *Drosophila melanogaster* to test the twenty uridine, pyrimidine derivatives chemical produce effect on body system and also these chemical were tested for reciprocal translocations test.

These above studies show the high significance of bioactivity of bimolecule *i.e.*, pyrimidine and its substituted derivatives.

The present biomolecules under study are very significant for medicine purposes, industrial and toxicology purposes.

To review the above studies and literature [29, 30, 38] supported by data banks [39-46] of various national and international journals, the present study has been under taken for the purposes :

(*i*) To study the biological activity of 4-hydroxy-2-mercapto pyrimidine, 2-hydroxy-4-mercapto pyrimidine, 4,6 - dimethyl -2- pyrimidinol, 5-qromo-2, 4- dihydroxy pyrimidine, 2,4-dihydroxy pyrimidine-5--carboxylic acid on different laboratory rates (male & female).

(*ii*) To study the significance of said chemicals for pharmacological and therapeutic purpose.

(*iii*) To study the effect of said chemicals on general body system E.N.T. system, cardiac, hemaopoietic, respiratory, urinary,. endocrine, alimentary system of testing sets of rats.

(*iv*) To compile the treatment and safety data for said molecules.

(*v*) To study the toxicity, physical chemical property, pharmacokinetic, physiological study of said biomolecules.

MATERIALS AND METHOD

The detail spectral analysis have been carried out of adopting experimental & other techniques & procedures [18-26] in previous chapters. The procedure & method of testing of present chemicals 4,6 -dimethyl -2- pyrimidinol, 4-hydroxy

- 2- mercapto pyrimidine, 2-hydroxy -4-mercapto pyrimidine, 5-bromo - 2,4 - dihydroxy pyrimidine and 2,4 -dihydroxy pyrimidine - 5- carboxylic acid [here after referred as 4,6,2- - DMP, 4, 2 - HMP, 2,4 - HMP, 5,2,4-BDHP, 2,4,5 - DHPCA respectively] are as follows as depicted in literature of W.H.O. geneva [39, 40, 47] and AOAC (U.S.A.) [41] as proved a standared methods and some are taken from compendium of analytical methods [41, 42]. Official methods by Canada board [43, 44], American Heart association [45]. Mannual for therapeutics by Alpers. *et. al.*, [46] & some others [47-62]. While the toxicity (human and environmental and water) were determined due to methods and procedures [16-26] by using different instruments such as - electrostatic precipitators, digital pH meter, spectrophotometers, multivariate analysis etc.

The procedure and methods used for plant analysis, physicochemical analysis, pharmaco chemical analysis as follows as due to Pravin Jain [63], Rajiv Sharma [30].

These activities and programmes were constituted at bio chemistry deptt. medical college, toxicology deptt. c.c.s. university and different labs of NGO's with their great courtesy. The literature used for methods are taken from lDR, S.M. Medical Univ. Library. NGO's Library, .OSH net library and pusa institute and NISCAIR.

Analysis and Interpretation

The analysis of study administered for all chemicals are given in assay form with all complete details here the little efforts are made to study the bioactivity, mutagenesis, human and non human toxicity, metabolism, major uses, presence, therapeutic uses, clinical effect, human health effect, treatment overview, range of toxicity, pharmacokinetics, absorption, distribution, excretion, pharmacology, physical, chemical data of all present chemicals respectively.

4,2 HMP

This compound has a great importance. It is found not in chemical form but also in the seeds of Brassica [following methods [29, 30, 63]. Which is the member of Cruciferae. The procedure of detecting the presence of this chemical is follows as described by Praveen Jain [63]. *i.e.*, paper chromatography and others [30]. It is a pharmacobotanical quality of 4,2 -HMP.

This compounds having mole. Wt. 128.15, M.P. > 260 ^{o}C (dec). decomposition temp >300 ^{o}C founds in powder form. It is appear in off white form. The odour of it is found to be slightly musty and bitter teste.

Hazardous decomposition products : This compound is founds to be toxic and harmful by applying methods. [20-35]

Not Only this it forms hazardous byproducts in the form of oxides of sulfur, Carbon monoxides, carbon dioxides, irritating and toxic fumes and gases. when comes in the contact of fire flame.

It is found to be insoluble in water, but it is soluble in alkaline solution. Health effect - This compound is harmful & toxic when comes in contact with human skin & eye. Some irritation have found due to this in eye & skin.

A small course (seven days) study have administered to know health -effect on animals. By feeding smaller dose of it to the two male and two female rats containing 32, 98, 178, 284, 380, 450 mg of chemical while one extra group was taken as control set which are well expressed in vitro and in vivo studies. After due time of completing dose, it is found that it damages liver and thyroid organ.

Therapeutic pharmacokinetic uses the this compounds is very effective and inhibits the thyroid activity. It is used as an antimetabolic and antithyroid agent. Coronary vasodilator and in congestive heart failure although its use has been largely supplanted by other drugs. It is known to cause blood dyscrasias and suspected of terato and carcinogenesis.

The *E. coli* & *Salmonella* Test

Testing of chemicals for mutagenicity in *Salmonella typhimurium* is based on the knowledge that a substance that is mutagenic in the bacterium is more likely than not to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans. Although about three-fourths of chemicals that are positive in the Ames test are found to be rodent carcinogens, not all substances that cause cancer in laboratory animals are mutagenic in this assay. However, the ease, rapidity (results in 3-4 weeks) and low cost of the test make it an important tool for screening substances for potential carcinogenicity.

Various Strains of the *S. typhimurium* bacterium may be used for testing. Each is genetically different, so using several strains in a test increases the opportunity of detecting a mutagenic chemical. *Salmonella* tester strains has recently begun to routinely employ. *E. coli* strain as- a bacterial tester strain in the Ames test. This *E. coli* strain is similar in mutagen detection to *S. typhimurium* strain. All the bacterial strains used in the Ames test carry a defective (mutant) gene that prevents them from synthesizing the essential amino acid histidine from the ingredients in standard bacterial' culture medium. Therefore, these "tester" strains can only survive and grow on medium that contains excess histidine. However, in the presence of a mutagenic chemical, the defective histidine gene may be mutated back to the functional state, allowing the bacterium to grow on standard medium that does not contain supplemental histidine. These mutations, which lead to a regaining of normal activity or function, are called "back" or "reverse" mutations and the process is referred to as "reversion." The mutant colonies, which can make histidine, are called "revertants".

Many chemicals are not mutagenic in their native forms, but they are converted into mutagenic substances by metabolism in the liver. Since bacteria do not have the same metabolic capabilities as mammals, some test protocols utilize extracts of rat or hamster liver enzymes to promote metabolic

conversion of the test chemical. This permits the investigator to determine if a chemical must be metabolized to express mutagenic activity. Some mutagenic chemicals are active with and without metabolism, while others are active only under one condition or the other. Occasionally, other sorts of activation enzymes may be employed.

A test tube containing a suspension of one strain of Salmonella typhimurium (or *E. coli*) is incubated for 20 minutes at 37°C with the test chemical. Control cultures, with all the same ingredients except the test chemical, are also incubated. In addition; positive control cultures are prepared; these contain the particular bacterial tester strain under investigation, the various culture ingredients, and a known potent mutagen. After 20 minutes, agar is added to the cultures and the contents of the tubes are thoroughly mixed and poured onto the surface of Petri dishes containing standard bacterial culture· medium. The plates are. incubated, and bacterial colonies that do not require an excess of Supplemental histidine appear and grow. These colonies are comprised of bacteria that have undergone reverse mutation to restore function of the histidine-manufacturing gene. The number of colonies is usually counted after 2 days.

Spontaneous mutations (those that occur by chance, not by chemical treatment) will appear as colonies on the control Petri dishes. If the test chemical was mutagenic to any particular strain of bacterium, the number of histidine-independent colonies arising on those plates will be significantly greater than the corresponding control plates for that strain of bacteria. The positive control plates are also counted, and the number of mutant colonies appearing on them must be significantly increased over the spontaneous control number for the test to be considered valid. Failure of the positive control chemical to induce mutation is reason to discard the experiment.

Several doses (usually at least 5) of each test chemical and multiple strains of bacteria are used in each experiment. In addition, cultures· are set up with and without added liver enzymes at varying concentrations. Therefore, a variety of culture conditions are employed to maximize the opportunity to detect a mutagenic chemical. In analyzing the data, the pattern and the strength of the mutant response are taken into account in determining the mutagenicity of a chemical. All observed responses are verified in repeat- tests. If no increase in mutant colonies is seen after testing several strains under several different culture conditions, the test chemical is considered to be nonmutagenic.

The Mouse Lymphoma Test. This mammalian cell mutagenicity assay is used to determine whether a chemical is capable of inducing a change in cultured mammalian cells. Cells are treated with the test chemical and then placed into suspension cultures with selective medium for replication and fixation of induced mutations. Cells are then plated for colony growth, and after several days, colony numbers and colony size are recorded. The number of mutant colonies is a measure of the ability of the test chemical to induce a genetic change at the thymidine kinase locus in these transformed cells. The mouse lymphoma can detect both point mutations and chromosomal alterations.

The highest dose of test chemical was determined by solubility or toxicity, and did not exceed 5 mg in the absence of dose-limging toxicity. Mouse lymphoma cells were maintained at 39°C.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6 × 106 cells in 10 ml of medium. This volume included the 89 fraction in those experiments or of metabolic activation. Incubation with the test chemical contiru time the medium plus chemical was removed and the cells were resuspended in of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3 × 106 cells were plated in medium and soft agar supplemented with TFT Pates were incubated at 37°C in 5% CO_2 for 10 to 12 days. At the end of incubation, colonies were counted with an automated counter. The test was initially performed from the livers of either Aroclor induced or non-induced male Fisher rats.

Typically, 4 solvent control cultures and 3 positive control cultures were included in each experiment. Two or three cell cultures were used for each concentration of test chemical that was studied in a single experiment. Five to six concentration levels were tested per experiment. All experiments were replicated at least once.

It is found that atesting chemical has a negative call.

2,4,5 - DHPCA. It is an important pyrimidine derivative ($C_5H_4N_2O_4$) having mol. wt. 15.10, MP - 350°C, dissociation constant pKa = 2.07, Octanol partition Coefficient log kow = –0.83, Henry's low constant = 8.1 × 10-15 atm-cum mol at 25°C, Hydroxyl radial reaction rate constant = 8.9 × 10-12 cu cm/mol. sec at 25°C may exist in ionized form in water.

The potential of this is for bio concentration in aquatic organisms is low. It have high mobility in soil.

Hazardness. It emits toxic fumes of nitrogen oxides when heated.

Major uses. It is mainly use in the biosynthesis of nuclic acids.

Human health effect. To know the effect on human health by following the literature [26-35] a small survey was administered on physician desk. Some major results are found. When this compound comes through inhalation and dermal contact and ingestion, it may produce mild to moderate oral and esophageal burns and stomach burns. On dermal contact sever burns may occur. Inhalation may result in dyspea, pleuritic chest· pain, pulmonary edema, hypoxemia, bronchospasm, pneumonitis, tracheobronchitis. In eye contact irritation, pain, swelling, corneal, erosions

and blindness may occur. It may be poisonous, shortness of breath may develop following inhalation of acid vapours, mists. Inhalation may produce dyspea, pleuritic chest pain, upper airway edema & hypoxemia. Ingestion of acids may result in burns, gastrointestinal bleeding, gastritis, perforations, dialation, adema, necrosis, vomiting, stenosis, fistula, and duodenal/jejunal injury, Systemic toxicity may result in acute hepatic injury, Hepatic injury been reported following chronic Exposure to chromic acid. Renal failure is a rare complication of severe poisonings. Hemoglobinuria may develop secondary to Hemolysis. Nephritis may develop after hydrochloric acid ingestion. Massive fluid and electrolyte shifts may occur with extensive dermal or gastrointestinal burns. Hyperkalemia may occur with hemolysis. Hyperphosphatemia, hypocalcemia and hyperchloremia have been reported.

Hemolysis may occur following significant acid ingestion. Disseminated intravascular coagulation has been reported.

Chemical burns to the skin are often associated with concurrent thermal burns and trauma, complications seen with thermal burns including cellulites, sepsis, contractures, osteomyelitis, may occur as well as systemic toxicity from absorbed acid. Deep or extensive burns may require grafting. Alopecia was reported following application of an acidic formulation of a hair-relaxing product.

Treatment Saftety

In Muscosal Decontamination. If no respiratory compromise is present, dilute immediately with water or milk; no more than 8 ounces In adults and 4 ounces in children.

In Gastric Decontamination. Ipecac contraindicated. Consider insertion of a small, flexible nosagastric or orogastric tube to suction gastric contents after recent large ingestions; the risk of further mucosal injury must be weighted against potential benefits.

Endoscopy. Because acid ingestions may cause severe gastric burns with relatively few initial signs and symptoms, endoscopic evaluation few initial signs and symptoms, endoscopic evaluation is recommended with 2 hours in any patient with a definite history of ingesting a strong acid, even if asymptomatic. If burns are found, follow 10 to 20 days later with a barium swallow.

Pharmacologic Treatment. Corticosteroids are controversial. Consider use in second degree burns within 48 hours of ingestions in patients without gastrointestinal bleeding or evidence of perforation. Antibiotics are indicated for suspected performation or infection and in patients receiving corticosteroids.

Surgical Options. Initially, if severe esophageal burns are found a string may be placed in the stomach to facilitate later dilation. Insertion of a specialized nasogastric tube after confirmation of a cirumferential burn may prevent strictures. Dilation is idicated after 2 to 4 weeks if strictures are confirmed; if unsuccessful, either colonic intrapositiori or gastric tube· placement may be performed. Consider early laparotomy in patients with severe esophageal and/or gastric burns.

In Inhalation. Move patient to fresh air. Monitor for respiratory distress. If cough or difficulty breathing develops, evaluate for respiratory tract irritation bronchitis, or pheumonitis. Administer oxygen and assist ventilation as required. Treat bronchospasm with inhaled beta2 against and oral or parenteral corticosteroids. If respiratory symptoms develop obtain chest x-ray, monitor pulse oximetry and/or blood gases. Treat bronchospasm with inhaled beta against. If acute lung injury develops, consider PEEP. Evaluate for esophageal, dermal and eye burns as indicated.

In Eye Contract. Irrigate exposed eyes with copious amounts of room temperature water for at least 15 minutes. If irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

The Pharmacological Action & Therapeutic Uses. The therapeutic use of this chemical in association with mgo show the heart vitalizing activity. It reduces heart damages. To check the pharma action a small study was administered on male fisher rats initiated with 1, 2 - dimethylhydrazine 2 hcl (100 mg /kg) given 18 hour after partial hepatectomy & exposed to a diet containing one percent of present chemical for 13 months developed a 100% incidence of hepatocellular carcinoma. The creation of nucleotide pool imbalances by dietary present chemical an increase in uridine nucleotides and a decrease in adenine nucleotides was considered as a possible mechanism for promotional effect of present molecule on liver. The significance of this hypothesis is that altered nucleotide pool affect both genomic as well as membrane organization. The finding of this study is ·that liver damages are shown in these fisher rats. Hence it is harmful for lives action.

5,2,4-BDHP. This compound ($C_4H_3BrN_2O_2$) having mp > 300^oC, mol. Wt- 190.98 is found to be a toxic chemical. It is appear in powder form with unhealthy smell and hazardness to human health.

In the metabolites it is found that this potent oxidant may play a role in host defenses against invading parasites and cosinophil - mediated tissue damage. Thymidine phosphorylase, a pyrimidine salvage enzyme, transforms this compound to 5-bromodexoyridine a mutagenic analogive of thymidine. These result came in the result of small study done in bio chemistry deptt by following the methods made & direction given by Henderson [69]. Not only this the non- human toxicity shows the effect of dietary pyrimidine analogs on growth and survival of drosphila melanogaster.

Pyrimidine analogs on growth and survival of Drosophila melanogaster was determined. One pair of male and female, 8-hours-old, newly hatched, wild type Drosophila, melanogaster adults were transferred to a vial containing food supplemented with thymine, 5,2,4 - BDHP, orotic acid, cytosine, or uridine monosphosphate at 0.13% of diet.Files

grown without supplement served as controls. Flies were allowed to mate and lay eggs. One week later, the parental flies were removed, eggs were allowed to hatch and after metamorphosis through larval, and pupal stages at 25 ^{o}C. Newly hatched adults to hatch and after metamorphosis through larval and pupal stages at 25 ^{o}C. Newly hatched adults were counted. The number of adult files that hatched on each supplemented diet compared with the number that hatched on the control diet was used to calculate toxicity. The pyrimidines used in this experiment were ranked as follows: thymine greater than 5,2,4, BDHP greater than uracil greater than orotic acid greater than cytosine greater than uridine monophosphate in decreasing order of toxicity. Correlating pyrimidine structure with toxicity to wild type *Drosphila melanogaster*, the data suggest than the toxicity was associated with the presence of a methyl or bromide group attached to carbon 5 of the pyrimidine ring. The presence of a ribose-phosphate group at nitrogen 1 on the pyrimidine ring alleviated pyrimidine toxicity.

The presence of this compound as well as 5- bromo - 2 - deoxyuridine In the cultivation media of bacteria results in the distinct increase of UV sensitivity.

The therapeutic uses of this compound shows that it is effective antitumor agent as studied in vivo with the support of U.P. Singh [70].

2,4-HMP. This is the another pyrimidine mercapton derivative but it is different in qualities from previous compound. This compound having mol. wt. 128.15, MP - 295^{o}C, decomposition temp. > 301°C founds in powder form. It is appear in slightly white form. It is also found in bitter taste & Smelly odour. But there is no activity related to bioassay, human health have found for this chemical. It is found to be insoluble in water but soluble in alkali solution.

4,6,2-DMP. This compounds is found to be very toxic no other study are found for it.

These studies are well supported by literature cited. [58-68].

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