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ACUTETOXICITYTESTINGOFSYNTHESIZEDPYRAZOLINEDERIVATIVESINADULTZEBRAFISH

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ABSTRACT: The reduction products of pyrazole are pyrazolines. These 5-membered heterocyclic compounds containing nitrogen are famous for their wide range of biological actions, including antibacterial, anti-inflammatory, anticancer, analgesic, anticonvulsant, anthelmintic, antioxidant, and herbicidal effects. Intermediate chalcone has been used in the synthesis of pyrazoline derivatives. Due to their widespread use as scaffolds in the synthesis of bioactive chemicals at Vidyapeeth's College of Pharmacy, pyrazoles have recently been the focus of various techniques.responses across several platforms. In the realm of risk assessment and regulation, fish are the most often used vertebrate creature. Both adult zebrafish and zebrafish embryos possess unique traits that make them ideal model organisms for research into pharmacology and toxicology. Zebra fish, scientifically known as Daniorerio, have emerged as potent models for investigating human diseases and developmental processes. Acute toxicity (LC50), organ specific toxicity, and developmental toxicity are some of the toxicity tests that have recently been created using zebrafish assays. Using adult zebrafish and zebrafish eggs, this research examines the toxicity of newly synthesized pyrazoline derivatives in accordance with OECD recommendations for the relevant models.

Keywords: Pyrazolinederivativesare,Toxicityofsyntheticderivatives,Zebrafish,Toxicitystudy*etc.*

INTRODUCTION:

Pyrazolinearenitrogencontaining5-memberedheterocycliccompoundshaving two adjacent nitrogen atoms within the ring,andvariousmethodshavebeenworkedoutforth eirsynthesis.Numerouspyrazolinederivativeshave been found to possess considerable biologicalactivities, which stimulated the research activity inthisfield.They possess several prominent biological effects,suchasantimicrobial,anti-mycobacterial,anti-fungal, anti-amoebic, anti-inflammatory, analgesic,anti-depressant and anti-cancer activities [1,2]. It hasbeen considered that if a drug is effective, it willhave side effects or any toxic effects after certainlimitofconcentration [3].Thetoxicitystudyisimportanttodecidethemaximum tolerable dose of any drug. Fish are thewidely used vertebrate organism in risk assessmentandregulation.Outofthemadultzebrafish handzebrafish embryo has a special characteristic whichpermits its use as a model organism in toxicologicalandpharmacological studies[4].

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Zebra fish known as a *Danioreri*ohave risen aseffectiveandpowerfulmodelsforstudyingdevelopmentalprocessesandhumandisorders[5].Zebrafishsharesmanyfeatureswiththemammalians.Theydevelopmostoftheorgansfound in mammals including those of the nervous,digestive,reproductive,immune,excretory,andcardiovascularsystems,immunesystem[6].Zebrafish have various special focal points like itssmallsizeandlowcost,includefecundity,witheach female capable of laying 200 - 300 eggs perweek, external fertilizationand rapid developmentof embryos, which allows the direct observation ofdevelopinginternalorgansandtissues*in-vivo*situating them for rapid drug discovery and toxicitystudies.Theycaneasilyabsorbthecompoundswhicharesolubilizedinwater,makedrugadministrationsimpleand feasible.

Fish generate huge number of progenies, thereforeoffering them to being as appropriate as in vitroframeworks.Themaintenancecostislessexpensive than for other animal models. Zebra fishdeveloprapidly,allowingforassaysofdrugtoxicitiesonorgandevelopment[7,8].Inpresentarticle the toxicity of newly synthesized pyrazolinederivativeshas been studied.

MATERIALANDMETHOD:

PyrazolineDerivatives: Pyrazolineisreductionproduct of pyrazole. Literature survey shows thatpyrazoline derivatives show promising antitumor,analgesicandanti-inflammatoryactivity.Thepyrazolinederivativesaresynthesizedfromthechalcone.Thechalconeissynthesizedfromaromatic ketone and aromatic aldehyde in ethanolicNaOH[9]. Further this chalcone is used to synthesizeN-acetamide and benzamidepyrazoline derivatives.The pyrazoline derivative obtained from the sameinstituteand thecompoundare selectedonthe basisof their results of cell line study .The compoundwhich shows High to moderate activities in cell linestudywere selected for toxicity study. Acute toxicitytesting of following four pyrazoline derivatives wasconductedinadultzebrafishandinzebrafishembryo.

Compound1:N-(2-(1-carbamothioyl–5-(4-chlorophenyl) – 4 - 5 dihydro-1-H- pyrazol - 3-yl)phenyl) acetamide:

Molecularweight:372,Meltingpoint:229◦C,Molecular formula: $C_{18}H_{27}ON_4SCl$ Nature: Whitishpowder,$R_f$value:0.55[chloroform:methanol(10:1)]UVλ_{max}: 315.

Compound 2: N-(3-(4-chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1-H-pyrazole - 1 - carbonothioyl)acetamide

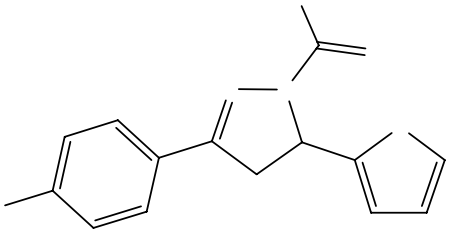
Molecularweight:363,Meltingpoint:174°C,Molecular formula: $C_{16}H_{14}ON_3S_2Cl$ Nature: Darkyellow colored crystals, R_f value: 0.67[n-Hexane:Ethylacetate(3:1)] UVλ_{max}: 301 nm

Compound3:N-(2-(5-(4-bromophenyl)-1-carbamothioyl - 4 - 5- dihydro - 1- H -pyrazol-3-yl)phenyl) acetamide

Molecularweight:417,Molecularformula:$C_{18}H_{17}ON_4SBr$,Nature:whitepowder,Meltingpoint: 231°C, R_f value: 0.55 [chloroform: methanol(10:1)] UVλ_{max}: 316 nm

Compound4:N-[5-(4-bromophenyl)-3-phenyl-4,5 - dihydro - 1H – pyrazole - 1-carbonothioyl)benzamide

Molecular weight: 464, Melting point:161-163 °C,Molecular formula: $C_{23}H_{18}N_3OSBr$ Nature: Browncolored crystals, Rf value: 0.60 [n-Hexane: Ethylacetate(2:1)], UVλ_{max}: 296 nm



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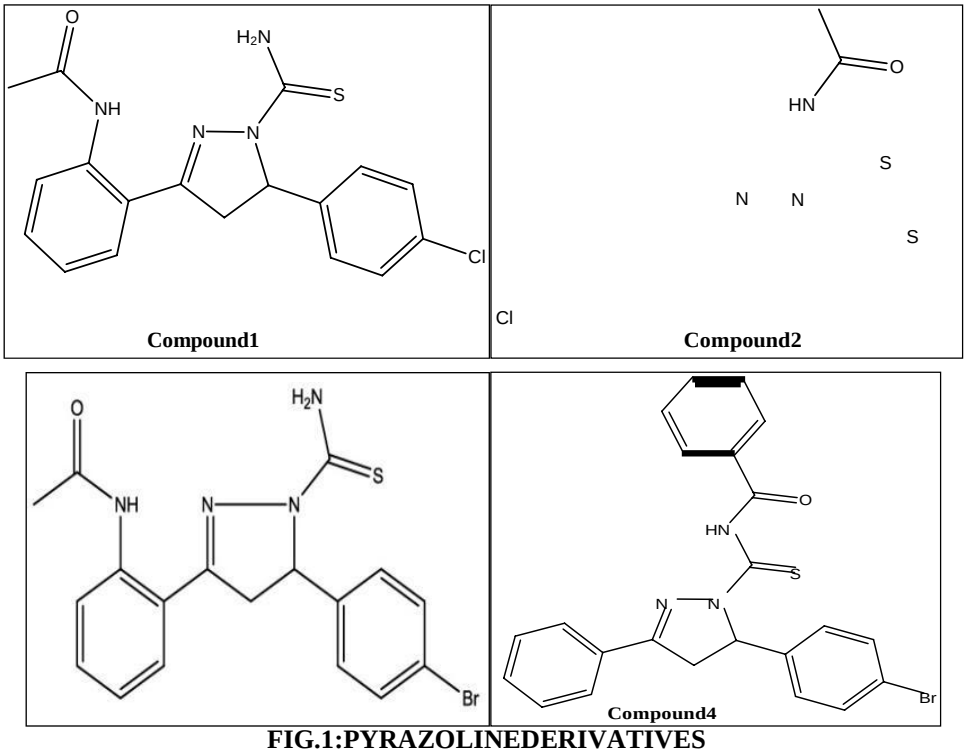

FIG.1:PYRAZOLINEDERIVATIVES

Acute Toxicity Testing of Synthetic PyrazolineDerivatives: Toxicitystudywasperformedaccordingto OECDtestguideline-203(Fish,AcuteToxicityTest)inordertodecidelethalconcentration(LC_{50})oftestcompoundinadultzebrafishandaccordingtoOECDguideline236(FishEmbryoToxicityTest)todetermineacutetoxicityof chemicalsonembryonicstages of fish.

Procedure for Acute Toxicity in Adult Zebra-fish: Thewildtypeadultzebrafishweighingbetween 0.5-1.5gmwereprocuredfromlocalsupplier. They were kept into water tank and properaeration was provided. 12 hrs light and 12 hrs darklight cycles was maintained. Fish were fed threetimesdailywithmicropellets.Theywerekeptfor 15daysforacclimatization[10].Total119fisheswere divided in toseventeen differentgroups with7 fishes per group. The four different concentrationofeachcompoundwerepreparedingeometricserieswith an increasingfactor2.2.

Group I served as a control group (1ml DMSO).GroupIItogroupVreceived0.20mg,0.44mg, 0.96mgand2.12mgdoseofcompound1respectively. Group VI to group IX received 0.20mg,0.44mg,0.96mgand2.12mgdoseofcompound 2 respectively. Group X to group XIIIreceived 0.30 mg, 0.66 mg, 1.45 mg and 3.19 mgdose of compound 3 respectively. While group XIVto group XVII received 0.20 mg, 0.44 mg, 0.96 mgand 2.12 mg dose of compound 4. The drug wasdissolvedinDMSOpriortoadditionintorespective fish tank.The fishes wereexposedtotestcompoundsforaperiodof96hours.Themortalitieswererecordedat24,48,72and96hours and the concentration which killed 50 % ofthefish (LC_{50}) was determined[11].

ProcedureforAcuteToxicityinZebrafishEmbryos: Zebrafish embryos were procured fromlocalsupplier.Zebrafishembryosarekeptinembryonic medium [12]. Embryos were divided in totwenty different groups with 12 embryos per group.96 well microtitre plates were used for this study.Eachembryowastransferredinindividualwellplate.Thefivedifferentconcentrationoftestcompound were prepared by diluting the solvent ina geometric series with an increasing factor of 2.Group I served as a control. Group II to V, GroupVI to IX, Group X to XIII and Group XIV to groupXVIIreceived10µg,8µgand6µgand4µgdose of compound 1, 2, 3 and 4 respectively. The drugwasdissolvedinDMSOpriortoadditionintore

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specti ve groups. The embryos were exposed totestcompoundsforaperiodof96hours.Coagulation of embryos, lack of somite formation,andnon-detachmentofthe tail,lackofheartbeatindicates mortality. The mortalities were recorded at24,48,72and96hrsandthetoxicconcentration wasdetermined [13].

RESULT: The maximum concentration at whichthe 50 % of the fish was killed was recorded. Theabnormal changes and mortality of fish for everytest compound was recorded after 24, 48, 72 and 96hoursgiven infollowing **Tables**.

TABLE1:TABLESHOWINGTHERESULTSOFACUTETOXICITYTESTIN ADULTZEBRAFISH

Compounds	Group	Dose	Abnormalchanges		Mortality			
			Swimming	Pigmentation	24 hrs	48 hrs	72 hrs	96 hrs
Control	GroupI	-	-	-	-	-	-	-
	GroupII	0.2mg	-	-	-	-	1	1
Compound1	GroupIII	0.44mg	-	-	1	1	2	3
	GroupIV	0.96mg	□	□	1	2	3	3
	GroupV	2.12mg	□	□	2	4	7	7
Compound2	GroupVI	0.2mg	-	-	-	-	1	1
	GroupVII	0.44mg		□	-	2	3	4
	GroupVIII	0.96mg	□	□	-	2	5	7
	GroupIX	2.12mg	□	□	1	3	6	7
Compound3	GroupX	0.3mg	-	□	-	-	1	2
	GroupXI	0.66mg	□	□	-	2	3	6
	GroupXII	1.45mg	□	□	-	3	6	7
	GroupXIII	3.19mg	□	□	4	6	7	7
Compound4	GroupXIV	0.2mg	-	-	-	1	2	2
	GroupXV	0.44mg	-	□	-	1	4	5
	GroupXVI	0.96mg	□	□	1	2	5	7
	GroupXVII	2.12mg	□	□	2	7	7	7

TABLE2:TABLESHOWINGTHERESULTSOFACUTETOXICITYTESTIN ZEBRAFISHEMBRYO

Compounds	Group	Dose (µg)	Mortality			
			24 hrs	48hrs	72hrs	96hrs
Control	Group I	-	-	-	-	-
Compound1	GroupII	10	8	10	12	12
	GroupIII	8	6	8	10	12
	GroupIV	6	4	7	8	8
	GroupV	4	4	6	7	7
Compound2	GroupVI	10	8	12	12	12
	GroupVII	8	7	10	12	12
	GroupVIII	6	6	8	10	10
	GroupIX	4	6	7	8	10
Compound3	GroupX	10	11	9	7	5
	GroupXI	8	11	9	7	4
	GroupXII	6	5	7	9	10
	GroupXIII	4	4	5	8	9
Compound4	GroupXIV	10	9	12	12	12
	GroupXV	8	6	8	8	10
	GroupXVI	6	5	6	6	7
	GroupXVII	4	3	4	4	5

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DISC

USSION: We set out to find the lethal dosage of each of the substances in our investigation. To determine the fatal concentration (LC50) of the test substance in adult zebrafish, the OECD test guideline 203 (Fish, Acute Toxicity Test) was used. The fish in the control group did not exhibit any unusual alterations in their swimming behavior, coloration, or survival rate. The zebrafish that were exposed to the test chemical showed alterations in their coloring and swimming patterns. The LD50 values for compounds 1, 2, 3, and 4 were 2.5 mg, 1.9 mg, 1.25 mg, and 1.47 mg, respectively, according to the results. The OECD guideline 236 (Fish Embryo Toxicity Test) was used to determine the fatal concentration of the test compounds for acute toxicity of chemicals. The LD50 values for compounds 1, 2, 3, and 4 were 4.31 mg, 3.18 mg, 4.86 mg, and 5.85 mg, respectively.

CONCLUSION: According to the results of this research, zebrafish exposed to test chemicals for longer periods of time at lower doses exhibit toxicity. Even at low concentrations, the pyrazoline derivatives cause serious harm. This research proved that zebrafish may be used as a reliable model to test the toxicity of various chemicals. Adult zebrafish were utilized in the regenerational and developmental angiogenesis test based on the results of this toxicity investigation.

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