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Synthesis of Some New Chlorosubstituted Pyrazolines and Their Curative Effect on Induced Hepatotoxicity in Albino Rat

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Chlorosubstituted 4-aroyl-Δ^2-pyrazolines (**2a–d**) were synthesized by condensing chlorosubstituted 3-aroylflavanones (**1a–d**) with phenylhydrazine hydrochloride in DMSO containing a little piperidine and assayed these compounds (**2a–d**) on cythion induced activities in albino rats with special reference to blood serum (VLDL, LDL and HDL) hepatotoxicity. The effects of intraperitonial administration of 4-aroyl-Δ^2-pyrazolines (100mg/kg body wt.) were studied on cythion induced blood serum hepatotoxicity. The blood serum VLDL, LDL and HDL were estimated in order to assess the liver functions by established procedures. Biochemical observations were supplemented with histological examination of liver section. It is evident from the results that the levels of serum lipoproteins were more altered in cythion treated animals than controls. There was small but significant decrease in the concentration of total serum lipids which may be largely due to the reduction of the total lipid concentration in the VLDL, LDL and HDL. However, the altered lipoprotein concentration levels were restored to almost normalcy in 4-aroyl-Δ^2-pyrazolines treated animals.

Key Words: Chlorosubstituted pyrazolines; Hepatotoxicity, Albino rat.

INTRODUCTION

A widespread use of pesticides in every field of life has become alarmingly hazardous to human health. Most of the pesticides cause metabolic transformations in the body of higher animals and the main site of metabolism is the liver[1, 2]. Any metabolic disturbance in the liver produces characteristic hepatic diseases[3–6], and thus the identification of the various characteristic metabolic disturbances constitutes the physiological and chemical basis for tests of liver functions.

In fact, the use of pesticides and their interactions with liver and its most important bioconstituent lipoprotein is the root cause of many diseases and fatty liver. The major form in which triglycerides released by the liver are very low density lipoproteins (VLDL)[7]. In the circulation low density lipoprotein (LDL) is found in considerable amounts and in rats the high density lipoprotein (HDL) is the major lipoprotein density class[8].

Kheshimov[9] reported that the intraperitoneal administration of phosphamide and HCCH insecticides on rats and rabbits reduces total serum protein. The action of low and high doses of cyclohexamide both cause a rapid and almost complete inhibition of protein[10].

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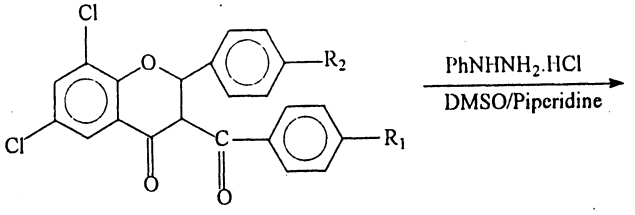
Serum protein analysis is regarded as a valid procedure for estimating the degree of damage to the parenchyma of the liver. It is also used to detect slight derangement of liver functions. There are certain Ayurvedic preparations such as Liv-52 and resinous substances produced by honey bees, *i.e.*, propolis which have shown good hepatoprotective effect[11, 12].

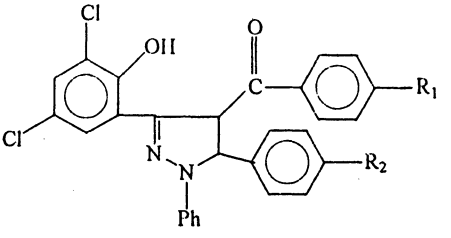
It has been reported that the heterocyclic compounds containing pyrazoline ring present a broad spectrum of biological activity[13]. It has been revealed that substitution in the phenyl ring enhances their antibacterial activity. Heterocyclic compound niridazole[14] undergoes an extensive first pass hepatic metabolism. The paucity of data in the hepatoprotective effects of 4-aroyl-Δ^2-pyrazolines, the two main constituents of liver protective drugs fascinated us to undertake the study of these drugs on cythion induced activities in albino rats. We report here the synthesis of some heterocyclic chlorosubstituted 4-aroyl-Δ^2-pyrazolines from 3-aroyl flavanones on reaction with phenylhydrazine hydrochloride in DMSO containing a little piperidine. The effects of these compounds have been studied on cythion induced activities in albino rats.

EXPERIMENTAL

A mixture of 3-aroyl flavanone (0.01 mol) and phenylhydrazine hydrochloride (0.02 mol) in DMSO (20 mL) containing a few drops of piperidine was refluxed for 2.5 h. After cooling the reaction mixture was acidified with dil. HCl (1 : 1). The solid product thus obtained was recrystallised from ethanol-acetic acid mixture to get 4-aroyl isoxazole. It gives colouration with neutral $FeCl_3$ solution and dissolved in NaOH indicating thereby the presence of free phenolic —OH group.



(1a-d)



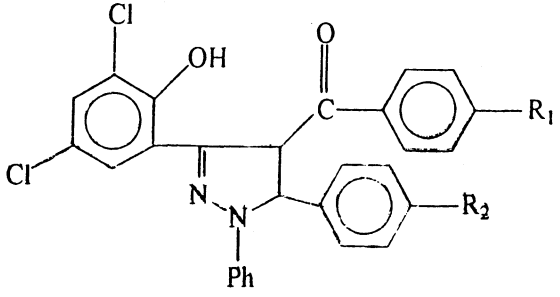
(2a-d)

The spectral analysis of the compound **(2d)** is as under: IR (nujol) shows absorption bands at 3100–3000 cm^{-1} ν(OH), 1615 cm^{-1} ν(C=N), 1265cm^{-1}

ν(=C—N) stretching in aromatic tert. amines), 1045 cm^{-1} ν(C—O), 680cm^{-1} ν(C—Cl); UV-Vis ($CHCl_3$) showed λ_{max} 395 nm corresponding to n → π* transition; PMR ($CDCl_3$) showed 3.70 (s, 3H, Ar—OCH_3), 5.20 (d, 1H, CH—C), 5.65 (d, 1H, CH—C), 6.80 to 7.60 (m, 16H, Ar-H), 10.70 (s, Ar—OH).

Melting points recorded are uncorrected and their purity was tested by TLC on microscopic slides with silica gel-G layers in benzene.

From elemental analysis, chemical properties and spectral data, the compound **(2d)** assigned the structure 3-(2-hydroxy-3,5-dichlorophenyl)-4-benzoyl-5-(4-methoxy phenyl)-1-phenyl-Δ^2-pyrazolines.



(2 a-d)

Similarly the other compounds **(2a–c)** were prepared and their elemental analysis and physical data are listed below (Table-1).

TABLE-I
ANALYTICAL AND PHYSICAL DATA OF 3,5-DIARYL-4-AROYL-1-PHENYL-Δ^2-PYRAZOLINES **(2a–d)**

S. No.	m.f.	Yield (%)	m.p. (°C)	Nitrogen (%)	
				Found	Calculated
2a	$C_{28}H_{20}N_2O_2Cl_2$	70	174	5.70	5.74
2b	$C_{30}H_{24}N_2O_4Cl_2$	80	170	5.00	5.11
2c	$C_{29}H_{22}N_2O_3Cl_2$	80	169	5.30	5.41
2d	$C_{29}H_{22}N_2O_3Cl_2$	60	160	5.30	5.41

The healthy albino rats of 6–8 weeks of age and 100–200 g body weight were obtained from Dr. Punjabrao Deshmukh Medical College, Amravati and maintained in suitable environment. They were supplied commercial pelleted diet and water *ad libitum*. The animals were divided into four groups (A, B, C, D). The animals of group A were fed on stock diet and used as control species. Animals of group B were given cythion pesticide intraperitonially (40 SD/kg body wt/day) for one week. Animals of groups C were given newly synthesised chloro-substituted 4-aroyl pyrazolines **(2a–d)** separately. Animals of group D were given 4-aroyl pyrazolines in conjugation with cythion. The total period of observation was ten weeks.

The blood samples were collected from animals of groups A, B, C, D and left to clot at room temperature for at least 30 min to remove the clot and cell debris to perform the following analysis.

(I) Isolation of LDL and VLDL by precipitation with heparin and $MnCl_2$: To 5 mL serum were added 0.2 mL of 5% heparin solution and 0.25 mL of $MnCl_2$ solution. A precipitate appeared immediately and the reaction mixture was centrifuged for 10 min at 600 rpm. The precipitated lipoprotein sedimented at the bottom. The supernatant liquid was decanted and precipitate was dissolved in 10% sodium bicarbonate solution. The manganese associated with lipoproteins as bicarbonate salt was removed by concentration. 5 mL of tris-HCl buffer was added to the clear yellow suparnatant and lipoproteins were completely precipitated by the addition of 2 mL $MgCl_2$. The precipitate was separated by centrifugation and redissolved in 5% NaCl. In order to remove contaminating serum protein, the lipoproteins were precipitated by adding 5 mL tris-HCl buffer and 2 mL $MgCl_2$. The solution was then dialyzed for 24 h against the tris-HCl buffer to remove heparin. The dialysis bag was transferred to another flask containing 5% $BaCl_2$ solution. After 24 h the insoluble heparin-barium salt was removed by centrifugation.

The supernatant was dialyzed against tris-HCl buffer in order to remove $BaCl_2$. This resulted in a clear yellow solution of concentrated lipoprotein. The lipoprotein isolated in this way was the mixture of LDL and VLDL which were separated by ultra-centrifugation. After 24 h at 10,000 rpm the VLDL formed an opalescent band at the top of the tube and the clear yellow LDL sedimented at the bottom.

(II) Isolation of HDL by precipitation with sodium phosphotungstate and $MgCl_2$: To 5 mL of serum were added 5 mL of 4% sodium phosphotungstate. (NaPhT) and 2 mL $MgCl_2$. The precipitates of LDL and VLDL were removed by centrifugation. 4.5 mL of NaPhT was added to the clear supernatant (I). The precipitate which appeared immediately was free of lipids and contained mostly V-globulins which were removed by centrifugation. To supernatant (II) was added 0.0875 mL from 2 mL $MgCl_2$. The precipitation was completed after 2 h and the mixture was centrifuged for 30 min at 20,000 rpm. The clear supernatant (III) (pH 7.1) was decanted and the precipitate was dissolved in 2.5 mL of solution of the following composition (1% NaCl + 0.4%, NaPhT + 0.1 M $MgCl_2$). After washing, the precipitate was recovered by centrifugation and was suspended in 1% NaCl, and 10% Na_2CO_3 solution was added dropwise with stirring until redissolution was achieved. This concentrated neutral solution of HDL, contaminated by small amount of serum protein, was further purified by ultra-centrifugation.

The serum concentrations of the VLDL, LDL and HDL were determined by measuring their proteins and total lipid concentrations.

(III) Protein: The protein contents were estimated by Lowry *et al.*[15] method and values were expressed as mg/100 mL of serum,

(IV) Total lipids: They were estimated gravimetrically by Folch *et al.*[16] method.

(V) Lipoproteins: A suitable aliquot of the isolated fraction was estimated according to Folch *et al.*[16] method by using chloroform-methanol (2 : 1) mixture. These extracts were evaporoted and taken in a known volume of chloroform and stored in sealed stopper tubes at 20°C. until required for further estimations.

RESULTS AND DISCUSSION

Serum VLDL, LDL and HDL

It is evident from Tables 1 to 9 that the level of serum lipoproteins was more altered in animals treated with cythion than controls. There was small but significant decrease in the concentration of serum total lipids which was largely or solely due to a reduction of the total lipid concentration in VLDL, LDL and HDL.

Tables 1–3 show total lipid protein and lipoprotein changes in serum VLDL.

TABLE-1
SERUM TOTAL LIPID CHANGES IN VERY LOW DENSITY LIPOPROTEIN (VLDL) ON EXPOSURE TO CYTHION AND CHLOROSUBSTITUTED 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	61.05 ±6.35	59.70 ±5.50	60.80 ±5.49	60.30 ±5.55	60.70 ±5.44	60.65 ±5.39
4	61.00 ±6.30	59.01 ±5.82	60.30 ±5.85	60.10 ±5.45	60.35 ±5.39	60.45 ±5.37
6	60.28 ±6.18	58.18 ±5.23	60.10 ±5.29	59.97 ±5.30	59.79 ±5.30	59.90 ±5.10
8	60.78 ±6.38	57.10 ±5.34	59.86 ±5.33	59.60 ±5.25	59.10 ±5.25	59.20 ±5.39
10	61.08 ±6.36	55.81 ±5.49	58.99 ±5.10	58.26 ±5.05	58.55 ±5.11	58.63 ±5.21

TABLE-2
SERUM PROTEIN CHANGES IN VERY LOW DENSITY LIPOPROTEIN (VLDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	13.07 ±2.11	12.93 ±2.63	12.90 ±2.33	12.40 ±2.40	12.60 ±2.55	12.55 ±2.40
4	12.98 ±2.68	11.27 ±1.44	12.71 ±2.10	12.10 ±2.10	12.30 ±2.30	12.20 ±2.35
6	12.87 ±2.57	10.19 ±1.50	12.49 ±2.05	11.91 ±1.70	12.00 ±2.45	11.99 ±2.05
8	12.97 ±2.68	9.76 ± 1.34	12.00 ±1.55	11.60 ± 1.65	11.70 ±2.10	11.40 ±1.99
10	13.01 ±2.09	9.20 ±1.48	11.70 ±1.65	11.15 ±1.39	11.55 ±1.90	11.30 ±1.81

TABLE-3
SERUM LIPOPROTEIN CHANGES IN VERY LOW DENSITY LIPOPROTEIN (VLDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	73.13 ±10.45	70.80 ±10.46	72.90 ±10.53	72.60 ±10.40	72.88 ±10.51	72.75 ±10,50
4	73.02 ±10.35	69.58 ±10.25	72.93 ±10.45	72.46 ±10.37	72.85 ±10.72	72.65 ±10.35
6	72.90 ±10.48	68.28 ±10.32	72.50 ±10.39	71.55 ±10.45	71.99 ±10.11	71.36 ± 3.70
8	72.93 ±10.52	67.40 ±10.34	71.63 ±10.45	71.25 ±10.30	71.42 ±10.25	71.30 ±10.30
10	72.98 ±10.54	64.95 ±10.36	71.54 ±10.30	71.45 ±10.20	71.34 ±10.19	71.15 ±10.29

Tables 4–6 show the trend of VLDL total lipids, proteins and lipoproteins in albino rat treated with cythion and 4-aroyl-Δ^2-pyrazolines.

TABLE-4
SERUM TOTAL LIPID CHANGES IN LOW DENSITY LIPOPROTEIN (LDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	70.88 ±10.69	69.20 ±10.35	70.60 ±10.45	70.57 ±10.38	70.40 ±10.49	70.55 ±10.56
4	71.05 ±10.09	69.10 ±10.08	70.91 ±10.39	70.89 ±10.39	70.88 ±10.39	70.44 ±10.38
6	70,90 ±10.90	68.90 ±10.02	70.35 ±10.15	70.25 ±10.25	70.40 ±10.25	70.30 ±10.22
8	70.95 ±10.73	68.82 ±10.01	70.23 ±10.09	70.10 ±10.05	70.50 ±10.10	69.98 ±10.01
10	71.10 ±10.21	67.70 ±10.02	70.76 ±10.01	70.05 ±10.21	69.70 ±10.02	69.60 ±10.00

TABLE-5
SERUM TOTAL LIPID CHANGES IN LOW DENSITY LIPOPROTEIN (LDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	35.13 ±2.68	34.88 ±2.56	35.05 ±2.60	34.90 ±2.30	35.01 ±2.65	35.03 ±2.67
4	34.96 ±2.67	33.30 ±2.19	34.65 ±2.55	34.55 ±2.44	34.57 ±2.33	34.59 ±2.39
6	34.98 ±2.84	32.20 ±2.80	34.40 ±2.60	34.36 ±2.56	34.33 ±2.40	34.43 ±2.35
8	35.03 ±2.53	32.01 ±2.74	34.55 ±2.70	34.61 ±2.67	34.60 ±2.34	34.59 ±2.30
10	35.06 ±2.56	31.08 ±2.03	34.66 ±2.50	34.44 ±2.45	34.39 ±2.50	34.50 ±2.25

TABLE-6
SERUM LIPOPROTEIN CHANGES IN LOW DENSITY LIPOPROTEIN (LDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES (**2a–d**) IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	106.03 ±2..97	105.72 ±3.38	105.99 ±3.40	105.25 ±3.34	105.89 ±3.50	105.90 ±3.35
4	105.97 ±3.22	104.40 ±3.62	105.50 ±3.20	105.10 ±3.27	105.29 ±3.23	105.35 ±3.46
6	105.98 ±3.27	103.37 ±2.52	105.35 ±3.10	105.19 ±3.33	105.21 ±3.37	105.15 ±3.16
8	105.98 ±3.21	102.48 ±2.42	105.10 ±3.26	105.00 ±3.25	105.02 ±3.40	105.03 ±3.19
10	106.06 ±2.96	101.49 ±2.44	105.69 ±3.12	105.25 ±3.10	105.87 ±3.15	105.89 ±3.39

Tables 7–9 show the declining trend of HDL total lipid, protein and lipoprotein in albino rat treated with cythion, 4-aroyl-Δ^2-pyrazoline **(2a–d)**.

TABLE-7
SERUM TOTAL LIPID CHANGES IN HIGH DENSITY LIPOPROTEIN (HDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	113.01 ±5.06	112.16 ±5.01	112.99 ±5.09	112.79 ±5.00	112.87 ±5.25	112.89 ±5.07
4	113.10 ±3.01	111.81 ±2.53	112.96 ±5.06	112.60 ±5.02	112.81 ±5.22	112.82 ±5.21
6	113.07 ±3.05	110.19 ±5.11	112.60 ±5.25	112.46 ±5.12	112.75 ±5.26	112.67 ±5.08
8	112.97 ±5.08	105.05 ±3.22	112.46 ±5.01	112.28 ±5.10	112.41 ±5.30	112.50 ±5.12
10	113.04 ±5.03	13.08 ±2.22	112.30 ±5.21	112.10 ±5.09	112.25 ±5.11	112.27 ±5.10

TABLE-8
SERUM PROTEIN CHANGES IN HIGH DENSITY LIPOPROTEIN (HDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	121.05 ±4.96	119.70 ±4.50	120.79 ±4.31	120.59 ±4.21	120.66 ±4.33	120.69 ±4.26
4	121.02 ±4.90	118.32 ±4.52	120.55 ±4.33	120.41 ±4.31	120.44 ±4.12	120.46 ±4.25
6	121.07 ±4.96	116.40 ±4.15	120.47 ±4.35	120.30 ±4.22	120.33 ±4.19	120.39 ±4.31
8	121.04 ±4.93	113.49 ±3.01	120.35 ±4.22	120.29 ±4.20	120.25 ±4.29	120.21 ±4.22
10	121.00 ±4.92	110.51 ±3.23	120.25 ±4.11	120.10 ±4.11	120.17 ±4.30	120.19 ±4.12

TABLE-9
SERUM LIPOPROTEIN CHANGES IN HIGH DENSITY LIPOPROTEIN (HDL) ON EXPOSURE TO CYTHION AND EXOGENOUS 4-AROYL-Δ^2-PYRAZOLINES IN ALBINO RAT

Weeks	Control	Induced	4-aroyl-Δ^2-pyrazolines			
			a	b	c	d
2	235.08 ±7.32	234.17 ±7.48	235.00 ±7.50	234.70 ±7.44	234.82 ±7.20	234.83 ±7.55
4	235.02 ±7.96	230.08 ±7.17	234.98 ±7.11	234.69 ±7.14	234.80 ±7.11	234.81 ±7.29
6	235.06 ±7.33	228.00 ±7.09	234.86 ±7.19	234.51 ±7.33	234.45 ±7.31	234.70 ±7.22
8	235.01 ±7.31	222.70 ±7.22	234.70 ±7.40	234.35 ±7.25	234.45 ±7.29	234.56 ±7.40
10	235.00 ±7.30	213.18 ±8.56	234.40 ±7.32	234.21 ±7.21	234.30 ±7.22	234.31 ±7.30

Conclusion

The results of serum estimations in VLDL, LDL and HDL containing total lipids, proteins and lipoproteins reveal that there is a declining trend in the values of these constituents in cythion treated albino rats; and the administration of 4-aroyl-Δ^2-pyrazolines shows remarkable curative effect.

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