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COMPARISON OF ANTIHELMINTHIC ACTIVITY BETWEEN BISARYL BENZYL PIPERAZINE AND BENZIMIDAZOLE LINKED PIPERAZINE DERIVATIVES

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Key words:

Piperazine, Benzimidazole, *Pheretima posthuma*, Antihelminthic activity

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ABSTRACT: Helminthes infections are more common in poor countries where people suffer from poor health hygiene and sanitation, though there are lot of marketed drugs available for these disease but the interesting thing about antihelminthics is that they are either pypirazine or benzimidazole derivatives, so as medicinal chemist the attention will be drawn towards these different moieties which exert same biological action, in this present work five derivatives of piperazine were synthesized by conventional methods using two schemes, by scheme 1 three compounds were synthesized which were Bis benzyl derivatives of pierazine and by scheme 2 already prepared piperazine 2,5 di one was reacted with benzimidazole derivatives to yield finally two benzimidazole linked piperazine derivatavies and these two different classes of piperazine deiatives were screened for antihelminthic activity against Pheretima posthuma to compare their potency in context of calculating paralyzing and death time. Result showed that bis-benzyl derivatives possess to some extent better activity than that of benzimidazole linked piperazine derivatives.

INTRODUCTION: Piperazine is a saturated six member heterocyclic compound containing two nitrogen at 1 and 4 position (as called 1, 4-hexahydropyrazine**).** Piperazine was first introduced as an anthelmintic in 1953. A large number of piperazine compounds have anthelmintic action. In comparison with benzimidazole (a imidazole derivative which too possess potent antihelminthic activity and even some marketed drugs are available as antihelminthic) has always drawn the attention of many researchers to compare the potency of these two different nucleus, though both of their mechanism of action is different in contrast to antihelminthic activity [1].

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The anthelminitic activity of piperazine and related compounds is said to be, based on blockage of the response of the worm muscle to acetylcholine, at the myoneural junction. This action is mediated by its agonist effects upon the inhibitory GABA receptor, The selectivity for helminths is because vertebrates only use GABA in the CNS and the helminths' GABA receptor is a different from the vertebrates' as a result causing a flaccid paralysis in the worm, which is dislodged from the intestinal wall by normal peristaltic action and expelled in the feces [2, 3, 4].

The anthelmintic action of the piperazine compounds depends on their capacity to produce a state of narcosis in the worms. [5]. The addition product of piperazine and phosphoric acid is useful as an anthelmintic agent for poultry, canines, felines, equines, porcines, and humans [6] whereas benzimidazole derivatives like albendazole selectively bind to nematode ß-tubulin, inhibiting polymerization, thus preventing the formation of microtubules and preventing cell division. The loss

of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores thus decreasing ATP formation ultimately leads to death of worms [4, 7].

Various methods are reported for the synthesis of piperizine derivatives, though many piperazine derivatives occur naturally, piperazine can be synthesized by reacting alcoholic ammonia with 1,2- dichloroethane, by the action of sodium and ethylene glycol on ethylene diamine hydrochloride, or by reduction of pyrazine with sodium in ethanol [7] various research has been cariied out on synthesis on piperizine and pirazine and those respective deivatives were found to be useful in combating various diesase some important derivatives of piperazines which were already proved to be pharmacologically and biologically are I,e 5-methylpyrazine-2-Carbohydrazide possess antibacterial [9] and antiinflamatory activity [10] N-aryl and N-alkyl piperazine derivatives [11, 12] posses antibacterial activity and enhanced antibacterial activity is achieved by incorporation of triazine ring in the piperizine ring [13] Mannich bases derived from n-methyl piperazine is found to active against cancer [14].

Based on different researches mostly it is seen that N-alky/phenyl derivaties or bisaryl/benzyl, (benzyl substitution increase antibacterial activity in comparison to methyl phenyl substitution) [10, 11] derivatives of pipearazine are potent molecules, the potency is quantified by the nature of electron donating or electron withdrawing groups substituted on the nucleus.

As mention above that various literatures of piperazine signifies N-phenyl /alkyl or bisaryl/benzyl derivatives are found possess various pharmacological and biological activities here the work is aimed to synthesis some bis benzyl (containing N-methyl group)derivatives of piperazine in one hand and benzimidazole linked piperazine derivatives in other hand and compare their antihelminthic activities.

Chemistry: Synthesis comprises of two schemes, scheme1 is two step reactions in fist step ethylene diamine reacts with substituted benzoic acid in presence of ammonium purpurate and methanol to give N, N'-Bis-(4-substituted benzyl) -ethane-1,2-diamine derivatives which is then reacted in second step with oxalic acid to give 1,4-Bis-(4-substituted benzyl)-piperazine-2,3-dione. [I] (PZ1, PZ2 PZ3) in second scheme o-phenylene diamine reacts with substituted benzoic acid to give substituted 2-phenyl -1H-benzo[d] imidazole. Separately glycine is reacted with ethylene glycol to form 2, 5-diketopiperazine. Then substituted 2-phenyl -1H-benzo[d] imidazole and 2, 5 diketopiperazine is refluxed to give substituted 2-phenyl-1-((piperazine-1-yl) methyl)-1H-benzo[d] imidazole (PZ-4, PZ-5).

MATERIALS AND METHODS:

The chemicals used for the experimental work were commercially purchased from local source of supplier Rankem (New Delhi), and thionyl chloride was used of Merck (Mumbai), These solvents and reagent were of AR and LR grade and purified before use, The commercially available grades of solvents were distilled. All the compounds were synthesized by conventional methods; melting point was determined by open capillary tube method and synthesized compounds were purified by re-crystallizing using suitable solvents and yield value and percentage purity were determined respectively.

Experimental portion:

A. General procedure for synthesis of 1,4-Bis-(4-dimethyl amino benzyl)-piperazine-2,3-dione:

In a beaker, the solution of ethylene diamine (2ml) and 4-dimethyl amino benzaldehyde (40Mm, 5 gm) in 50 ml of methanol was added with 40mM of ammonium purpurate was added portion wise with continuous stirring for 3 hours at ice cold temperature and allowed to stand for few minutes. The crystalline deposit was settled down which was re-crystallized from methanol to produce the intermediate compound N, N'-Bis-(4-dimethyl amino benzyl)-ethane-1, 2-diamine. Again 7.5 Mm (2.44gm) of intermediate compound and 7.5 Mm (0.675 gm) of oxalic acid was stirred in ether at ice cold temperature for 1 hour.

The reaction mixture was left over night and the separated solid product was obtained by filtration and dried. The crude drug was re-crystallized from

ethanol affording 1, 4-Bis-(4-dimethyl amino benzyl)-piperazine-2,3-dione.

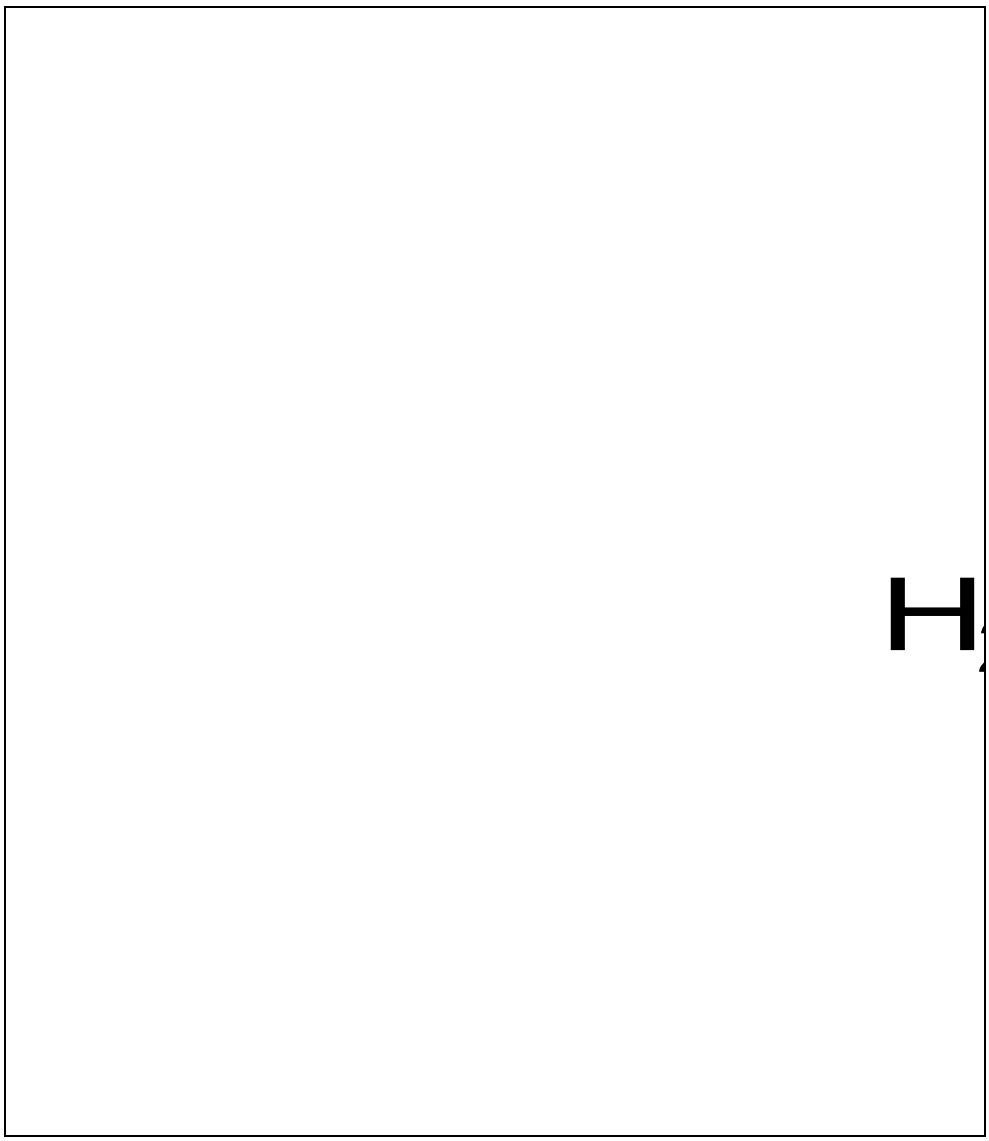
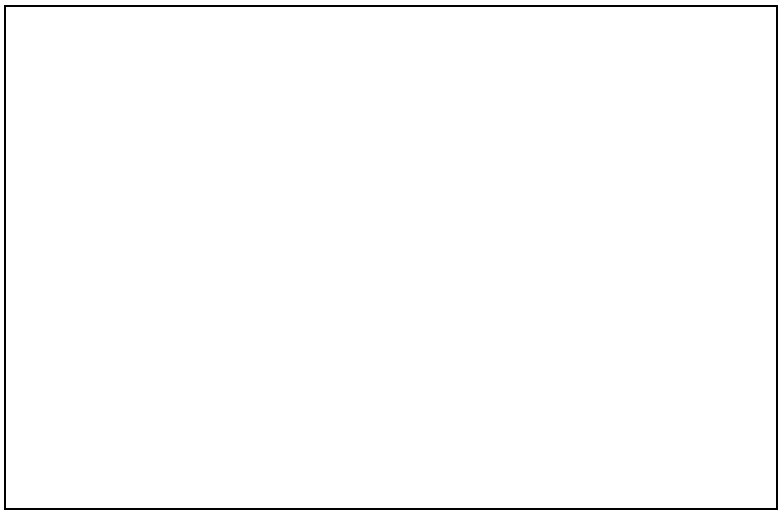


FIG.1: SYNTHESIS OF 1, 4-BIS-(4-DIMETHYL AMINO BENZYL)-PIPERAZINE-2, 3-DIONE.

B. General procedure for synthesis of 1, 4-Bis-(4-Chloro benzyl)-piperazine-2, 3-Dione:



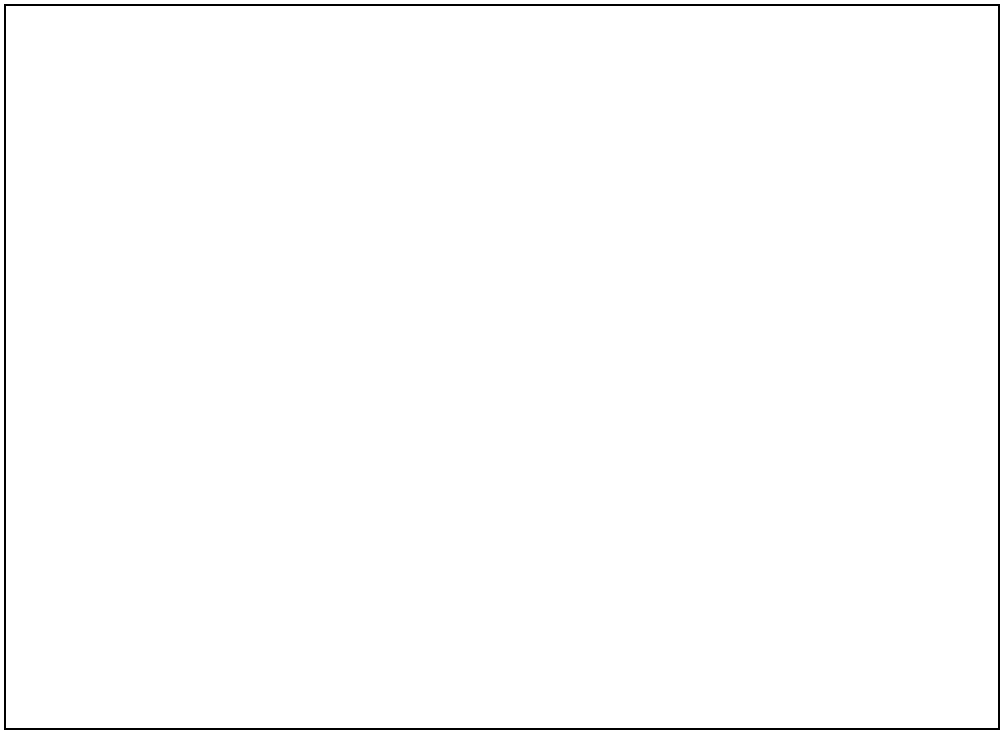


FIG.2: SYNTHESIS OF 1,4-BIS-(4-CHLORO BENZYL)-PIPERAZINE-2,3-DIONE.

C. General procedure for synthesis of 1, 4-Bis-(2-hydroxy benzyl)-piperazine-2, 3-Dione:

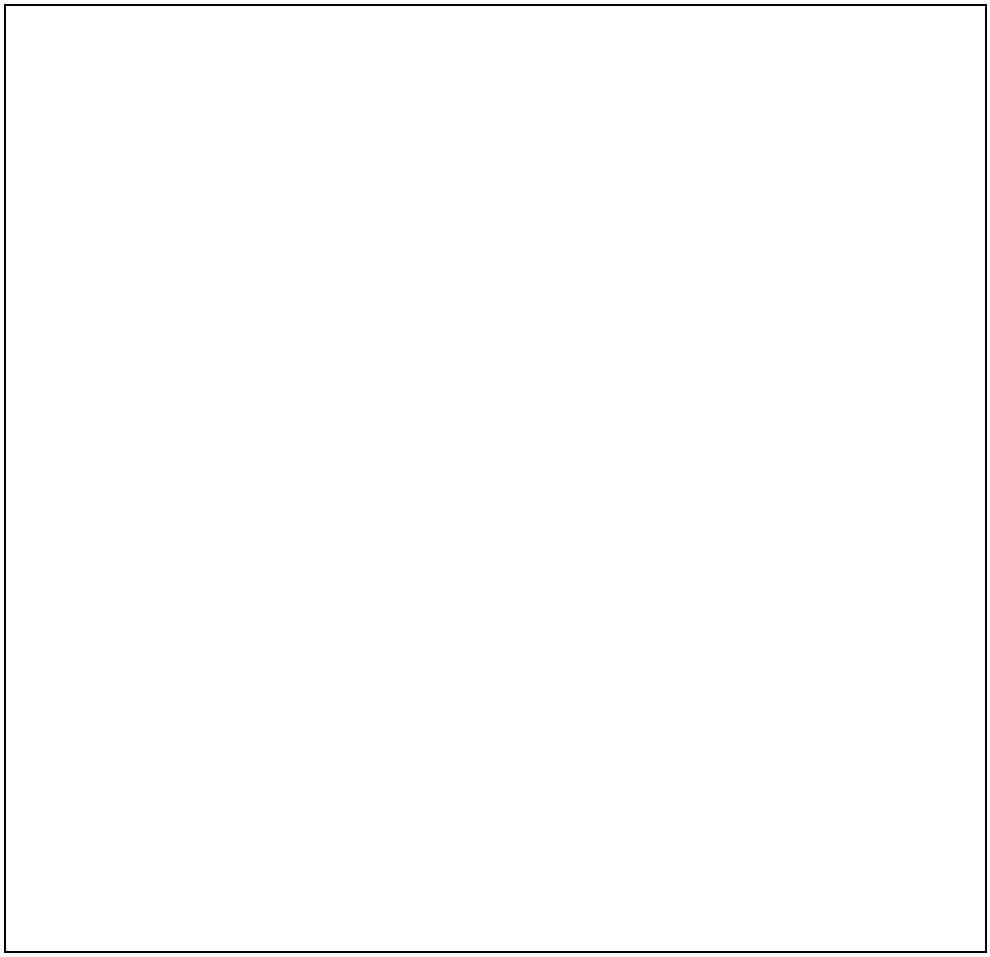


FIG.3: SYNTHESIS OF 1,4-BIS-(2-HYDROXY BENZYL)-PIPERAZINE-2,3-DIONE.

D. General procedure for synthesis of 1-((2-(Substituted phenyl)-1-H-benzo[d]imidazole-1-yl) methyl) piperazine -2,5-dione. (PZ4, PZ5):

Step 1: Preparation of diketopiperazine:

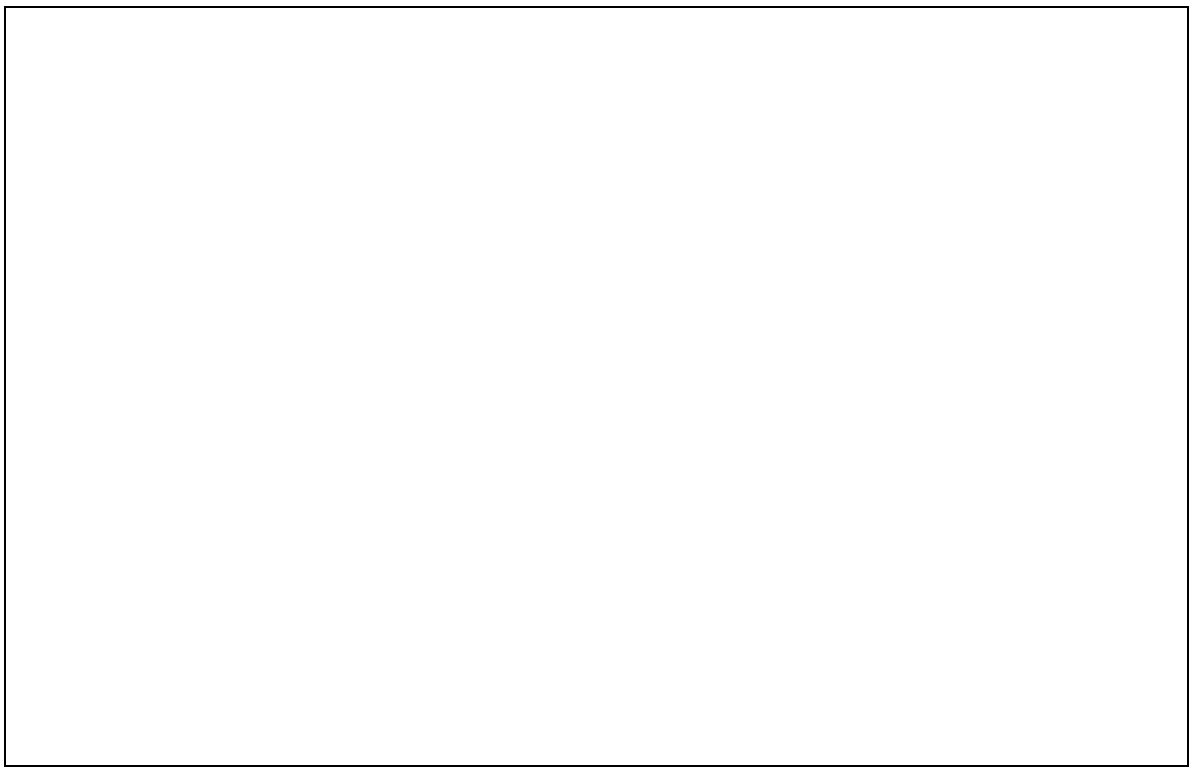
At first, 15 gm of glycine and 75 ml of ethylene glycol was mixed in a three naked flask fitted with condenser and mechanical stirrer. The mixture was heated in sand bath to 175^0C, maintaining the temperature, it was continuously stirred for 1hour. The dark brown reaction product was cooled to room temperature and kept at refrigerator for overnight. The next day, the obtained liquid was centrifuged at 3000 rpm for 20 minutes which yielded crude 2,5-diketo piperazine which was yellowish white crystal. Then it was crystallized using water and very few amount of decoloursing carbon to yield pure white crystalline 2, 5-diketo piperazine.

Step 2: Preparation of intermediate compound:

For the compound INT-4, 0.02 mole of o-phenylene diamine (2.16g) and 0.02 moles of 3, 5-dinitro salicylic acid (4.56 gm) were refluxed in presence of 4N HCl at 100^0C for 3 hours and 10 minutes. Then completion of reaction was checked by TLC. Then 10% NaOH solution was added to make the solution alkaline. The reaction mixture was cooled and allowed to stand for 5 minutes which yield crude 2-(2-hydroxy-3,5-dinitro phenyl)-1H-benzo[d] imidazole which is then recrystallized from ethanol. For INT-5, 2.76 gm of salicylic acid was taken and procedures were same. The compound formed was 2-(2-hydroxy phenyl)-1H-benzo[d] imidazole.

Step 3: Preparation of final product (PZ4, PZ5):

The intermediate compound (INT-4, INT-5) (0.01 mole) was dissolved in ethanol (15ml) followed by addition of 2, 5-diketo piperazine (0.01mole) (1.1 gm) and formaldehyde solution (40% w/v) (0.015mole) (1.13 ml) to undergo Mannich reaction. The reactants were refluxed for 6 hrs with continuous stirring at 70-75˚C. The completion of reaction was checked by TLC. After completion, the reaction mixture was kept in a refrigerator overnight. The product precipitated out and was filtered, dried and recrystallised from ethanol to give 1-((2-(2-hydroxy - 3, 5 - dinitrophenyl)-1-H-benzo[d]imidazole-1-yl)methyl) piperazine - 2, 5-dione[PZ4], and 1-((2-(2-hydroxy phenyl)-1-H-benzo[d]imidazole-1-yl)methyl) piperazine - 2, 5-dione and 1-((2-(2-hydroxy phenyl)-1-H-benzo [d]imidazole-1-yl)methyl) piperazine - 2, 5-dione.[PZ5].



Anthelmintic Evaluation:

The synthesized compounds (PZ1-PZ5) were evaluated in vitro for their antihelmintic activities according to the protocol mentioned below. The earthworms were collected from fish farm, Tararha. Sunsari, Nepal and species identification was done from Biology Department of Central Campus of Technology, Hattisar, Dharan, Nepal.

The anthelmintic activity was evaluated on adult Indian earthworms by Mathew et al method [15]. For preliminary evaluation of anthelmintic activity test samples of synthesized compounds was prepared at the concentration of 1000, 800, 600, 400 µg/ml in DMSO (6%) with normal saline and 6 worms *Pheretima posthuma* of 8-10cm were placed in petridish containing 25 ml of above test solutions of synthesized compounds. Mebendazole (4mg/ml) was used as positive control and normal saline with DMSO (6%) is used as negative control. All the test solutions and standard solutions were prepared freshly before starting the experiment. Observations are made for the time taken for paralysis when movement was lost or no movement. Worms should not relieve even in normal saline. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water and fading of color of worms was observed.

Preparation of stock solution and test solution of piperazine derivatives:

It was prepared by dissolving 100mg of synthesized compound in 100 ml of 6%DMSO with normal saline, which served as a stock solution. From stock solution 1000ug/ml, 800ug/ml, 600ug/ml, 400ug/ml, were prepared. Standard drug solution was prepared by dissolving 100mg of mebendazole in 25 ml of 6%DMSO with normal saline. 6 earthworms were placed in each petridish containing 25 ml of solution of different concentration of test and standard and paralyzing and death times are noted.

RESULT AND DISCUSSION:

TABLE 1: PHYSICAL PARAMETER OF SYNTHESIZED FINAL PRODUCTS

S.no	Product code	Name	Structure	Physical state	Yield value (gm)	Yield%
1	PZ1	1,4-Bis-(4-dimethyl amino benzyl)-piperazine-2,3-dione.		Orange coloured powder	1.72	60.35%
2	PZ2	1,4-Bis-(4-chloro benzyl)-piperazine-2,3-dione.		Brown coloured powder	2.2	80.8%
3	PZ3	1,4-Bis-(2-hydroxy benzyl)-piperazine-2,3-dione.		Yellowishwhite powder Hygroscopic in nature	1.4	57.26%
4	PZ4	1-((2-(2-hydroxy-3,5-dinitro phenyl)-1-H-benzo[d]imidazole-1-yl) methyl) piperazine -2,5-dione.		Brown crystal	2.54	59.24%
5	PZ5	1-((2-(2-hydroxy phenyl)-1-H-benzo[d]imidazole-1-yl) methyl) piperazine -2,5-dione.		Faint greenish powder	2.5	75.30%

All the ccompounds were soluble in water, while none was soluble in diethyl ether and PZ-2, PZ-3 and PZ-5 were soluble in benzene while others were insoluble in benzene.

Melting Points: The melting points of synthesized organic compound were determined by open capillary tube method in a heavy liquid paraffin bath.

Melting point is valuable criterion of purity for an organic compound as pure crystal is having definite and sharp melting point.

TABLE 2: MELTING POINT OF SYNTHESIZED COMPOUND

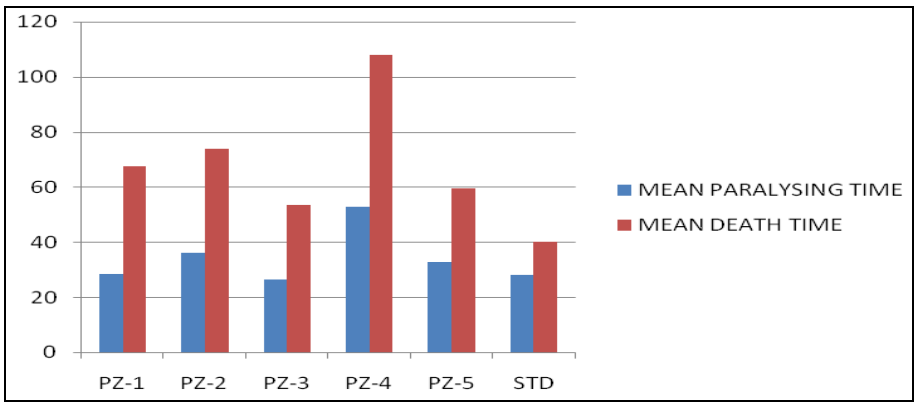
S.No	Product Code	IUPAC Name	Melting Point(°C)
01.	PZ-1	1,4-Bis-(4-dimethyl amino benzyl)-piperazine-2,3-dione	250
02.	PZ-2	1,4-Bis-(4-chloro benzyl)-piperazine-2,3-dione.	240
03.	PZ-3	1,4-Bis-(2-hydroxy benzyl)-piperazine-2,3-dione	235
04.	PZ-4	1-((2-(2-hydroxy-3,5-dinitro phenyl)-1-H-benzo[d]imidazole-1-yl) methyl) piperazine -2,5-dione.	200
05.	PZ-5	1-((2-(2-hydroxy phenyl)-1-H-benzo[d]imidazole-1-yl) methyl) piperazine -2,5-dione..	210

Anti helminthes screening:

TABLE 3: DATA FOR ANTIHELMINTHIC ACTIVITY

Compound code	Antihelmintic activity				
	Concentration (ug/ml)	Paralyzing time(min)	Death time(min)	Mean paralyzing time(min)	Mean death time(min)
PZ1	1000ug/ml	15	59	28.25	67.5
	800ug/ml	22	64		
	600ug/ml	30	70		
	400ug/ml	46	77		
PZ2	1000ug/ml	25	63	36	73.75
	800ug/ml	32	69		
	600ug/ml	38	79		
	400ug/ml	49	84		
PZ3	1000ug/ml	18	42	26.25	53.5
	800ug/ml	22	52		
	600ug/ml	29	57		
	400ug/ml	36	63		
PZ4	1000ug/ml	37	82	52.75	108
	800ug/ml	46	98		
	600ug/ml	60	120		
	400ug/ml	68	132		
PZ5	1000ug/ml	27	42	32.75	59.5
	800ug/ml	30	55		
	600ug/ml	34	67		
	400ug/ml	40	74		
***Std.**	4mg/ml	28	40	28	40
6% DMSO	-	-	-	-	-

*standard = mebendazole for antihelmintics activity, the antihelmintics activity were reported as mean paralyzing time and mean death time.



DISCUSSION: Various piperazine derivatives were prepared by two different schemes viz. **scheme 1** and **scheme 2**. In **scheme 1**, ethylene diamine was reacted with substituted benzaldehyde in presence of ammonium purpurate (reductive alkylation reaction) followed by reaction with oxallic acid (cyclization process) to afford 1, 4-Bis-(4-substituted benzyl)-piperazine-2,3-dione. All the compounds were purified through recrystallization by ethanol. All the synthesized compound have been characterised by physico chemical datas such as melting point, colour, %yield, solubility etc.compunds PZ-2 and PZ-3 were synthesized earlier and were found to be active against *Enterobious vermicularis* and *Fasciola hepatica* [3].

PZ-1 was synthesized for the first time by substituting *p*-Cl, *o*-OH by N-(CH3)2 in the benzyl ring of Bis phenyl piperazines derivatives. The synthesized derivatives(PZ1-3) posess 2,3 dione moiety as common, as it has been reported earlier 2,3-dione moiety in the structure of piperazine was considered as promising candidates as an antihelminthic agent [3, 16]. In scheme 2, the targeted compounds were synthesized fusing benzimidazole and piperazine through Mannich reaction. The synthesized compounds were subjected to antihelminthic activity as per referred protocol using mebendazole as standard drug against *Pheretima posthuma.*

The anti helmenthic activity data focus that PZ-1(mean paralyzing time 28.25 minute and mean death time 67.5 minute respectively) and PZ-3(mean paralyzing time 26.25 minute and mean death time 53.5 minute respectively)showed better activity among all the synthesized compounds and even in comparison to benzimidazole linked piperazine derivatives i,e PZ-4(mean paralyzing time 52.75 minute and mean death time 108 minute respectively) and PZ-5(mean paralyzing time 32.75 minute and mean death time 59.5 minute respectively) as it was anticipated that benzimidazole ring if incorporated in pierazine ring may exert better antihelminthic activity than other piperazine derivatives, but here the result does not comply with this above justification. Bis benzylderivatives were found to have better antihelminthic activity than bezimidazole derivatives.

CONCLUSION: Compared to two different schemes, the compound produced by scheme: PZ-1 got more yield value. Among them PZ-2 yield percentage was high, 80.8%. Scheme 2 had good yield value but took comparatively longer time of reaction as it produced complex or higher molecular weight piperizine derivatives. It proves that ethylene diamine is the good precursor for synthesizing piperazine and its derivatives.

The synthesized compounds were screened for antihelmintic and antioxidant activity. From scheme 1: PZ1, PZ3 were found to have very good anti helminthic activity. It indicates that the presence of 4-dimethyl amino group and 2-hydroxy group at para position of benzyl moiety attached to the piperazine ring enhanced the activity compared to other groups like 4-chloro derivatives at the same position. Similarly from scheme 2: PZ-5 was potent which had 2-hydroxy benzyl group attached to benzimidazole. It shows that incorporation of 2-hydroxy benzyl group in drug structure was found to be active against helminthes (earthworms).

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