SEDATIVE ACTION OF SOME SUBSTITUTED BENZYLAMIDES

O.P. Sethi, M.V. Gupta, O.S. Bhatia and H.R. Derasari

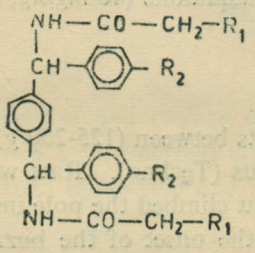
Department of Pharmacology and Physiology,
L.M. College of Pharmacy, Navrangpura, Ahmedabad-380009

Summary: Substituted benzylamide derivatives of amino acylamide (compound A,B,C, & D) were found to be less potent local anaesthetics than lignocaine and procaine. However, the four compounds exhibited sedation without ptosis and reduced spontaneous locomotor activity better than methaqualone. Compound A alone antagonised methylamphetamine induced hypermotor activity. The test compounds potentiated hexobarbitone induced hypnosis. Three compounds antagonised calcium induced stoppage of isolated heart of frog. Except compound C all caused a transitory fall of blood pressure in dog which was not blocked either by atropine or propranolol. These compounds showed neuromuscular blockade and possessed slight analgesic activity but were devoid of anticonvulsant and tranquillizing acitvity. LD_{50} values were calculated to be 164.1 ± 23.0, 229.1 ± 51.0, 181.6 ± 28.18 and 416.9±38.2 *mg/kg* for compounds A,B,C & D respectively.

Key words: basic benzylamides, local anaesthetic, CNS actions, calcium antagonist, blood pressure fall, neuromuscular blockade, anticholinergic, LD_{50}

INTRODUCTION

Four benzylamides, chemically different from lignocaine, which is an aminoacylamide, were recently synthesised by Parikh (9). All compounds possess lipophilic and hydrophilic chains separated by an intermediate chain. (Fig. 1). Patel and Jindal (10) have shown that



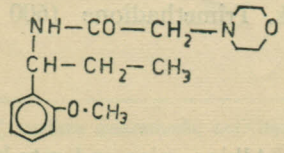
COMPOUND	R_1	R_2	CHEMICAL NAME
A		Cl	1',4'-BIS(-4-CHLORO PHENYL)-1',4'-BIS-N'-PIPERIDINO ACETAMIDO-P-XYLENE.
B		Cl	1',4'-BIS(-4-CHLORO PHENYL)-1',4'-BIS N'-MORPHOLINO ACETAMIDO-P-XYLENE
C		Cl	1'-4'-BIS(-4-METHYL PHENYL)-1',4'-BIS N'-MORPHOLINO ACETAMIDO-P-XYLENE.
D			∝'-N'-MORPHOLINO-N-[∝(2-METHOXY PHENYL)-PROPYL] ACETAMIDE

Fig. 1 : The chemical structure of the test compounds.

piperidinyl-N-(2:4-diethyl amino N-chlorophenyl) acetamide possessed a potent local anaesthetic activity with low toxicity. Grewal *et al.* (7) recently reported local anaesthetic action of new substituted acylamide. In the present study, four compounds were subjected to various pharmacological investigations.

MATERIALS AND METHODS

Local anaesthesia :

Conduction, intradermal and surface anaesthesia : The compounds were tested for conduction anaesthesia in mice by the method of Bianchi (1), intradermal anaesthesia on guinea pig back by the method of Bulbring and Wajda (2), and surface anaesthesia on rabbit cornea by the method of Chance and Lobstein (4).

Central nervous system depression :

Spontaneous locomotor and methylamphetamine induced hypermotor activity : Mice weighing between (18-25 g) were used. Motor activity was recorded before and at 1, 2 and 3 hr intervals after the test compounds (5-50 *mg/kg* i.p.) using 6 beam photoactometer (Techno). Methylamphetamine (4 *mg/kg*, s.c.) was given and interaction with test compounds was studied. Methaqualone (40 *mg/kg*, i.p.) was used as a reference drug.

Hexobarbitone hypnosis : Mice weighing (15-25 *g*) were used. The time of loss and regaining of righting reflex to hexobarbitone (100 and 125 *mg/kg*, i.p.) was observed before and after the test compounds (10-50 *mg/kg*, i.p.). Methaqualone (40 *mg/kg*, i.p.) was used as a reference drug.

Tranquillizing activity :

Conditioned avoidance response (CAR): Rats between (125-250 *g*) were trained by placing a single rat in Cook's pole climbing apparatus (Techno). Rats were subjected to shock and buzzer at various intervals for 30 sec till the rat climbed the pole immediately. Rats were considered trained if they climbed the pole with the onset of the buzzer. Compounds A,B, C & D (30-50 *mg/kg*, i.p.) were tested. Chlorpromazine (8 *mg/kg*, i.p.) was used as a reference drug.

Anticonvulsant activity :

Electrochock and chemoshock convulsions: Mice between (15-25 *g*) were used. Shocks (60 cycle, 24 mA, and 0.2 sec duration) were delivered through the ear electrodes to mice before and after the compounds (30 to 50 *mg/kg*, i.p.). Diphenylhydantoin (2.5 to 15 *mg/kg*) was used as a reference drug. Pentylenetetrazol (80 *mg/kg*, s.c.) was used before and after the compounds (30-50 *mg/kg*, i.p.) for chemoshock. Trimethadione (600 *mg/kg*, i.p.) was used as a reference drug.

Analgesic activity:

Mice tail clip and rat tail flick methods : Albino mice and rats between (18-30 *g*) and (125-200 *g*) were used for analgesic activity according to the methods described by Turner (13).

Cardiovascular system :

Isolated heart of frog : Frogs were pithed. Isolated heart of frog was perfused through Cyme's cannula. Responses to adrenaline, potassium chloride, calcium chloride and acetylcholine were elicited before and after the test compounds (10-50 *µg/ml*).

Blood pressure of dog : Dogs of either sex were anaesthetised with pentobarbitone (30 *mg/kg*, i.v.). Blood pressure was recorded with pressure displacement transducer using Encardiorite polygraph from the common carotid artery. The test compounds (6-10 *mg/kg*) were given after the control panel of adrenaline, noradrenaline, isoprenaline, acetylcholine and histamine. The modifications of responses were recorded.

Skeletal and smooth muscles :

Isolated rectus abdominis muscle of frog and rat ileum : Rectus was mounted in 15 *ml* organ bath according to the method of Burn (3). Acetylcholine was used before and after the test compounds (15-50 *µg/ml*). Rat's ileum was mounted and modifications of acetylcholine responses by the test compounds were recorded.

Acute toxicity :

LD_{50} estimation : Mice (18-25 *g*) were given graded doses of the test compounds intraperitoneally. Mice were observed for 24 hrs. LD_{50} values were calculated according to Miller and Tainter method (8).

RESULTS

Local anaesthesia :

Conduction, intradermal and surface anaesthesia: Compounds A. B. C & D showed 30-100% conduction, 30-98% intradermal and 25-85% surface anaesthetic activity respectively. These were less potent than lignocaine and procaine (Table I).

TABLE I : Local anaesthetic activity.

Drugs & compounds	*% concentration showing 100% or nearly 100% local anaesthetic activity*		*% concentration giving 50% or nearly 50% local anaesthetic activity*
	Intradermal	*Conduction*	*Surface*
Lignocaine	0.3	0.33	0.27*
Procaine	0.5	0.66	
Compound A	1.0	1.50	1.50
Compound B	3.0	4.00	3.00
Compound C	2.0	3.0	2.00
Compound D	1.0	2.0	1.00

*Lignocaine showed 100% surface anaesthetic activity.

Central nervous system depression :

Spontaneous locomotor and methylamphetamine induced hypermotor activity : Compounds A, B, C & D exhibited a dose dependant reduction of locomotor activity recorded in 3 hrs (Table II). Table III summarizes the antagonism of the test compounds A,B,C & D against methylamphetamine and comparison with methaqualone.

TABLE II : Spontaneous locomotor activity. Locomotor activity recorded for 10 min. (Five mice in each group).

No. of groups	*Drugs & dose (mg/kg, i.p.)*	*Count before*	*% inhibition after drug*			*mean % inhibition in 3 hr*
			1 hr	*2 hr*	*3 hr*	
2	Normal saline 0.1 *ml*/10 *g*	650	10	20	27	19.7
2	Methaqualone 40	590	60	68	77	68.3
	Compound A					
2	10	490	50	75	78	67.7
2	20	530	60	79	85	74.7
2	30	600	78	84	90	84.0
2	Normal saline 0.1 *ml*/10 *g*	456	15	27	32	24.7
2	Methaqualone 40	402	75	78	83	78.7
	Compound B					
2	10	380	36	45	63	48.0
2	30	379	56	69	82	69.0
2	50	341	83	84	82	83.0
2	Normal saline 0.1 *ml*/10 *g*	750	25	34	40	33.0
2	Methaqualone 40	685	56	72	76	68.0
	Compound C					
2	5	835	60	73	56	53.0
2	10	800	82	55	44	60.3
2	30	1015	85	83	84	84.0
2	Normal saline 0.1 *ml*/10 *g*	700	20	30	36	28.7
2	Methaqualone 40	650	58	75	79	70.7
	Compound D					
2	10	590	54	74	80	69.3
2	30	568	63	73	87	74.3
2	50	574	76	89	93	86.0

Methaqualone caused sedation & ptosis. Compounds A,B,C, & D caused sedation alone.

TABLE III: Methylamphetamine induced hypermotor activity. Locomotor and methylamphetamine (4 *mg/kg*, subcut) induced hypermotor activity was recorded for 10 min. Each group had 5 mice.

No. of groups	*Drugs*	*Dose (mg/kg, i.p.)*	*Cumulative locomotor activity for 10 min (expressed as % of control)*	*% Inhibition*
2	Normal saline*	0.1 *ml*/10 *g*	100	....
2	Normal saline	0.1 *ml*/10 *g*	198	....
2	Methaqualone	40	102	96
2	Compound A	10	138	60
2		20	133	65
2		30	110	88
2	Normal saline*	0.1 *ml*/10 *g*	100	....
2	Normal saline	0.1 *ml*/10 *g*	220	....
2	Methaqualone	40	105	115
2	Compound B	10	219	1
		30	215	5
		50	215	5
2	Normal saline*	0.1 *ml*/10 *g*	100	....
2	Normal saline	0.1 *ml*/10 *g*	180	....
2	Methaqualone	40	83	97
2	Compound C	5	175	5
		10	168	12
		30	160	20
2	Normal saline*	0.1 *ml*/10 *g*	100	....
2	Normal saline	0.1 *ml*/10 *g*	183	....
2	Methaqualone	40	80	103
2	Compound D	10	178	5
		30	175	8
		50	173	10

*Methylamphetamine was not given .

Hexobarbitone hypnosis : Compounds A,B, C & D afforded slight to highly significant potentiation of hexobarbitone hypnosis. Fig. 2 shows comparison with methaqualone.

Tranquillizing activity :

Conditioned avoidance response (CAR) : None of the compounds showed any tranquillizing activity, which was evident from CAR study.

Anticonvulsant activity :

Electroshock and chemoshock convulsions : None of the test compounds showed any significant anticonvulsant activity. Diphenylhydantoin and trimethadione afforded complete protection against MES and chemoshock convulsions.

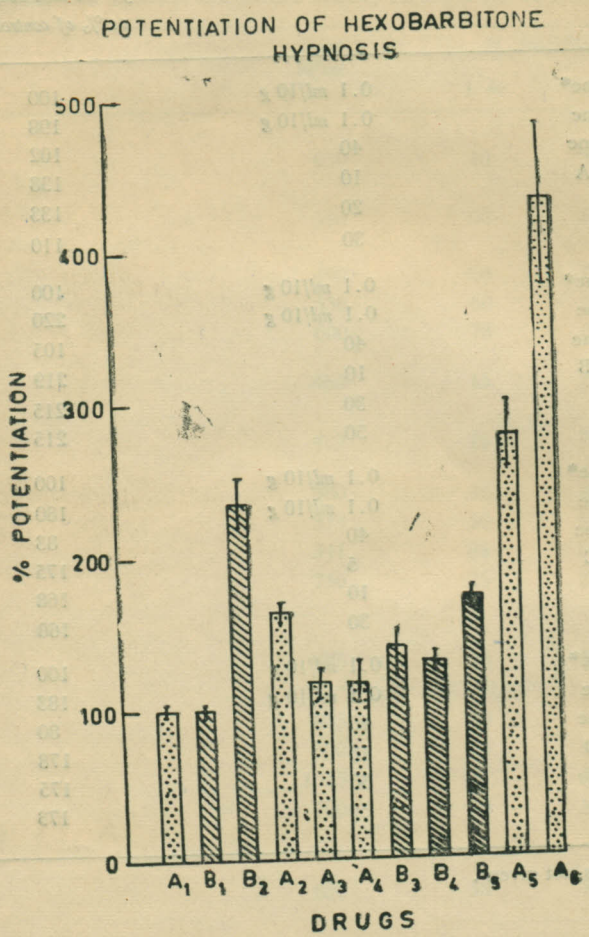


Fig. 2 : Potentiation of hexobarbitone hypnosis by the test compounds. A_1 : hexobarbitone 100 *mg/kg*; A_2 : hexobarbitone 100 *mg/kg* + Methaqualone 40 *mg /kg*; A_3 : hexobarbitone 100 *mg/kg* + compound A 30 *mg/kg*; A_4 : hexobarbitone 100 *mg/kg* + compound A 50 *mg/kg*; A_5 : hexobarbitone 100 *mg/kg* + compound D 30 *mg/kg*; A_6 : hexobarbitone 100 *mg/kg* + compound D 50 *mg/kg*; B_1 : hexobarbitone 125 *mg/kg*: B_2 : hexobarbitone 125 *mg/kg* + Methaqualone 40 *mg/kg*: B_3 : hexobarbitone 125 *mg/kg* + compound B 30 *mg/kg*; B_4 : hexobarbitone 125 *mg/kg* + compound C 10 *mg/kg*; B_5 : hexobarbitone 125 *mg/kg* + compound C 30 *mg/kg*.

Analgesic activity :

Mice tail clip and rat tail flick methods : Compound B exhibited slight analgesic activity, whereas compounds A,C & D were devoid of it.

Cardiovascular system :

Isolated heart of frog : Compounds A,B, C & D showed slight bradycardia in isolated frog heart. Compounds A, C & D antagonised the calcium induced stoppage of heart (Fig. 3), whereas compound B was devoid of it.

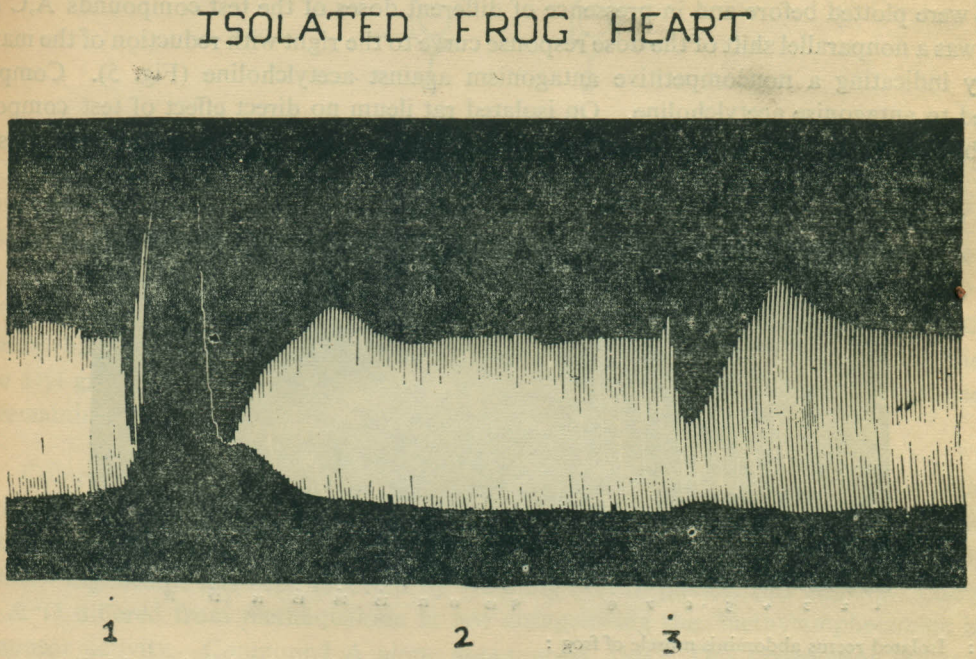


Fig. 3 : Isolated heart of frog.
At 1 : calcium chloride 1 *mg/ml;* 2: compound C 20 μg/*ml;* 3: calcium chloride 1 *mg/ml.*

Blood pressure of dog : Compounds A,B & D (6-10 *mg/kg*) respectively exhibited a transitory fall of blood pressure which was not blocked either by atropine or propranolol (Fig.4).

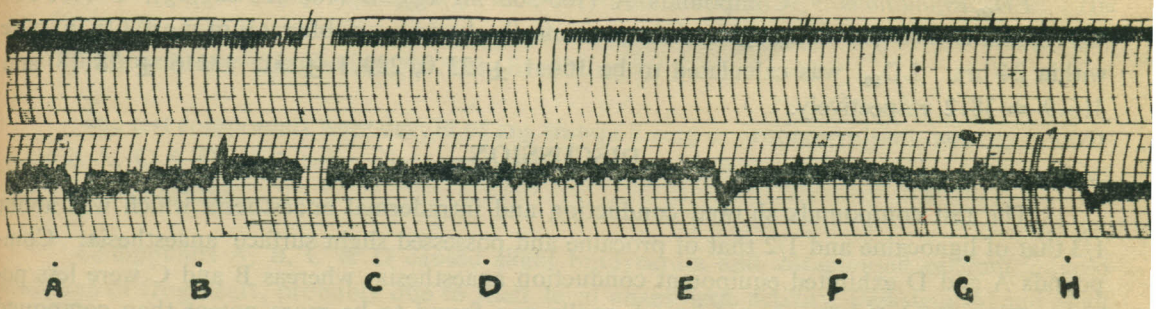


Fig. 4 : Dog's blood pressure and respiration.
Upper tracing : Respiration rate
Lower tracing : Blood Pressure At
A : compound D (10 *mg/kg*); B: Adrenaline (2 μg/*kg*); C: Atropine suplhate (1 *mg/kg*); D: Acetylcholine (2 μg/*kg*) ; E : compound D (10 *mg/kg*); F : Propranolol (40 μg/*kg*); G : Isoprenaline (2 μg/*kg*) and H : compound D (10 *mg/kg*) .

Compound C did not cause fall of blood pressure. None of the compounds altered the responses to adrenaline, noradrenaline, acetylcholine and histamine.

Skeletal and smooth muscles :

Isolated rectus abdominis muscle of frog and rat ileum : Acetylcholine dose response curves were plotted before and in pressence of different doses of the test compounds A,C & D. There was a nonparallel shift of the dose response curve to the right with reduction of the maxima, thereby indicating a noncompetitive antagonism against acetylcholine (Fig. 5). Compound B failed to antagonise acetylcholine. On isolated rat ileum no direct effect of test compounds was observed. All compounds (5-25 $\mu g/ml$) caused reduction of acetylcholine responses.

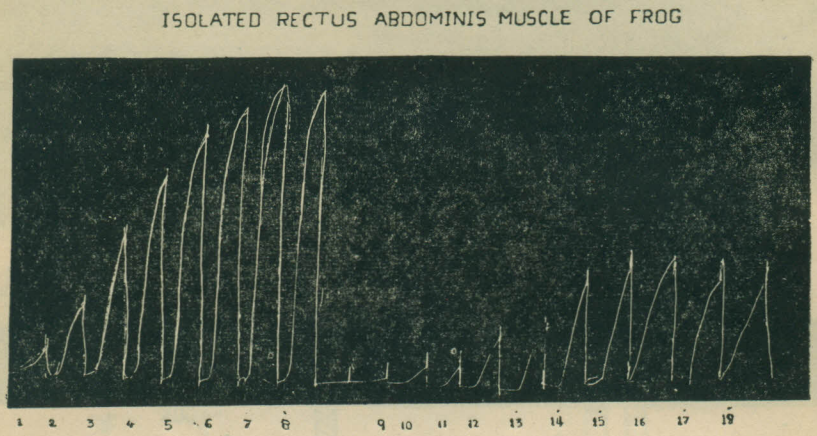


Fig. 5 : Isolated rectus abdominis muscle of frog :
At 1, 2, 3, 4, 5, 6, 7, 8 administration of 5, 10, 20, 30 , 50, 70, 90, 100 $\mu g/ml$ of acetylcholine.
At 9, 10, 11, 12, 13, 14, 15, 16, 17, & 18 administration of 5, 10, 10, 50, 100, 200, 300, 400, 600, & 900 $\mu g/ml$ of acetylcholine, in presence of compound A (10 $\mu g/ml$).

Acute toxicity :

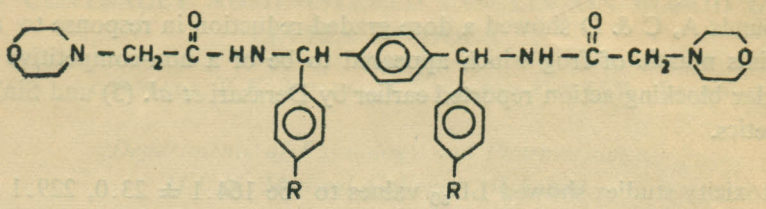
LD_{50} estimations : Compounds A (100-300 *mg/kg*), B (100-400 *mg/kg*), C (100-300 *mg/kg*) and D (300-600 *mg/kg*) given intraperitoneally showed variable rates of mortality in mice within 24 hrs. LD_{50} was calculated to be 164.1 ± 23.0, 229.1 ± 51, 181.6 ± 28.18 and 416.9 ± 38.2 respectively.

DISCUSSION

The test compounds showed conduction and intradermal anaesthesia which was about 1/3 that of lignocaine and 1/2 that of procaine and possessed slight surface anaesthesia. Compounds A and D exhibited equipotent conduction anaesthesia whereas B and C were less potent. Compound D when tested intradermally was found to be more potent than compound A, followed by C and B respectively. Compounds B and C possessed the basic structure I.

Compound C (R_2=CH_3) was more potent as intradermal anaesthetic than compound B (R_2 = Cl). The difference in activity could be explained by different substitutions at R_2. The compound in which aryl group was substituted with electron donating groups (ROR. NR,

R_2, NHCOR, etc) at para-position might be more potent than those in which aryl group was substituted by electron withdrawing groups (RNO, COR, Cl, etc.)



I

Out of compounds A and C, A was more active than C as it possessed piperidino, whereas compound C possessed morphilino moiety. This observation confirms the finding of Grewal *et al.* (7) and Weidman *et al.* (14), who showed that the compounds with piperidino moiety are more potent than those with morphilino moiety as local anaesthetics. Compound D with one aryl group showed the maximum local anaesthetic activity. This could be explained as the optimum balance between the lipophilic and hydrophilic chains is obtained, when there is a single aryl group in the molecule as in compound D than when there are more than one, as in the remaining compounds.

Compounds A, B C & D caused a dose graded reduction in the locomotor activity of the mice whereas compounds A,B & D were more potent than methaqualone. This indicates that the three compounds possess better sedative activity or CNS depressant action than methaqualone. Lignocaine has been reported by Weidling (15) to possess this activity. Compounds B, C & D differed from methaqualone in not antagonising the methylamphetamine induced hypermotor activity. Compound A alone antagonised this. This observation indicates that the compound A may be acting at the sites where methylamphetamine is acting, whereas B, C & D depresses the CNS at other sites. Further, support for its sedative action comes from the potentiation of hexobarbitone hypnosis by all the compounds. Compound D exhibited a highly significant potentiation (4 to 5 times) than methaqualone while others were less effective. Wilder Smith *et al.* (16) showed potentiation of hexobarbitone induced hypnosis by lignocaine. The present study does not provide evidence to show possible interaction of the test compounds with hexobarbitone metabolism in the liver.

The test compounds were devoid of any tranquillizing or anticonvulsant activity. Compounds A, C & D exhibited a slight analgesic action which is also in accordance with a similar effect of lignocaine.

Compounds A, B, C & D caused slight bradycardia and antagonised calcium induced stoppage of frog heart, probably due to their local anaesthetic activity, Feinstein (6) and Shah *et al* (11). Compounds A, B & D exhibited a transitory fall of blood pressure in dogs, whereas, compound C did not. This fall may have been due to a direct vasodilator or histamine like action on the blood vessels, as it was not blocked either by atropine or propranalol. Responses to

adrenaline, nor-adrenaline, isoprenaline, acetylcholine and histamine on blood pressure were not modified by these compounds.

Compounds A, C & D showed a dose graded reduction in response to acetylcholine on rectus abdominis muscle of frog which appeared to be of a non-competitive type, indicating a neuromuscular blocking action reported earlier by Derasari *et al.* (5) and Sinha *et al.* (12) for local anaesthetics.

Acute toxicity studies showed LD_{50} values to be 164.1 ± 23.0, 229.1 ± 51.0, 181.6± 28.8 and 416.4 ± 38.2 for compounds A,B, C & D respectively.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. C.S. Shah, Principal, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380009, for providing facilities to carry on this study. We are grateful to Dr. J.J. Trivedi, Principal, M.G. Science College, Navrangpura, Ahmedabad-380009, for the supply of test compounds. Generous gift of methaqualone by Mr. K.C. Jain, Roussel Pharmaceuticals (India), Bombay, is highly appreciated.

REFERENCES

1. Bianchi, C. A simple new quantitative methods for testing local anaesthetics. *Br. J. Pharmac.*, **11** : 104-106, 1956.
2. Bulbring, E. and I. Wajda. Biological comparison of local anaesthetics. *J. Pharmac. Exp. Ther.*, **85** : 84-87, 1945.
3. Burn, J.H. Practical Pharmacology. *Blackwell Scientific Publications, Oxford*, P. 30, 1952.
4. Chance, M.R.A. and H. Lobstein. The value of the guinea pig corneal reflex for test of surface anaesthesia. *J. Pharmac. Exp. Ther.*, **82** : 203-211, 1944.
5. Derasari, H.R. and M.N. Patel. Screenig of some basic amides for local anaesthetics and neuromuscular blocking activity. *Ind. J. Phsiol. Pharmac.*, **17** : 55-62, 1973.
6. Feinstein, M.B. Inhibition of contraction and calcium exchangeability in rat uterus by local anaesthetics. *J. Pharmac. Exp. Ther.*, **152** : 516-524, 1966.
7. Grewal, M.S., Saroj Sanan and G.C. Mittal. Local anaesthetic activity of some new substituted acylamides II. *Ind. J. Physiol. Pharmac.*, **19** : 76-80, 1975.
8. Miller, L.C. and M.L. Tainter. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proc. Soc. Expt. Bio. Med.*, **57** : 261-264, 1944.
9. Pariekh, S.P. Ph. D. Thesis, Gujarat University, 1975.
10. Patel, M.A. and M.N. Jindal. Local anaesthetic activity of some lignocaine analogues. *Arch internat. Pharmacodyn.*, **156** : 118-129, 1965.
11. Shah, D.S., P.R. Raghunath, A.K. Gupta, R. Mokal and O.D. Gulati. Neuromuscular blocking action of 2-(2-(3 pyridyl) Vinyl) -3-0- toly1-3, 4-dihydroguinazoline-4-One (SRC 909). *Arch. internat. Pharmacodyn.*, **209** : 283-295, 1974.
12. Sinha, Y.K. Studies on local anaesthetic drugs. *J. Pharm. & Pharmac.*, **5** : 620-625, 1953.
13. Turner, R.A. "Screening methods in Pharmacology." by Turner, R.A. *Academic press, New York*, p. 167. 1965.
14. Weidmann, H. and P.V. Peterson. The local anaesthetic action of a series of diamino propionic acid anilides. *J. Pharmac. Exp. Ther.*, **115** : 246-250, 1955.
15. Weidling, S. Xylocaine, the Pharmacological basis of its clinical uses. *Almquist & Wiksell, Uppsala*, 1959.
16. Wilder Smith, A.E., Edouard Frommel and R.W. Morris. Effect of local anaesthetics on barbiturate sleeping time. *J. Pharm. Pharmac.*, **11** : 600-606, 1959.