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Evaluation of analgesic, antipyretic, hypoglycemic and CNS depressant activity of 2-bromopopylamine hydrobromide, 3-bromopopyl ammonium bromide, ortho-amino aniline and benzimidazole-2-thiol in animal model

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ABSTRACT

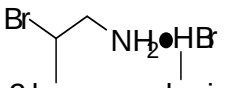
Synthetic compounds are encompassing the major therapeutic classes of drugs which resemblance to the natural or existing therapeutically active compounds. Herewith four synthetic compounds i.e. 2-bromopropylamine hydrobromide, 3-bromopropyl ammonium bromide, ortho-amino aniline and benzimidazole-2-thiol were subjected for analgesic, antipyretic, hypoglycemic and CNS depressant activity in mice and rat model following standard protocol. Among the four supplied synthetic compounds, benzimidazole-2-thiol and 3-bromopropyl ammonium bromide at a dose of 50 mg/kg body wt revealed significant analgesic activity with 37.85% and 42.85% inhibition of writhing (P value < 0.001 in both cases). None of the test samples showed antipyretic activity in rat model at a dose of 50 mg/kg body wt in comparable with standard antipyretic drug Paracetamol. On the other hand benzimidazole-2-thiol reduced blood glucose level with respect to standard drug Glibenclamide having P value 0.00082 and 3-bromopropyl ammonium bromide lengthened the Na-phenobarbitone induced sleeping time having the P value 0.0009 which was statistically significant.

Keywords: Analgesic activity, Antipyretic activity, Hypoglycemic activity, CNS depressant activity, Synthetic compounds.

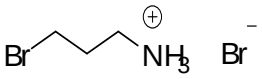
INTRODUCTION

Synthetic drugs are those substances that are produced entirely from chemical reactions in a laboratory. Their chemical structure can be identical to naturally occurring drugs, such as cocaine and opium, but they are often designed to enhance effects from naturally occurring drugs or to prevent side effects that are unwanted. Many purely synthetic compounds with no alternative natural source are classified by the chemical structure of the parent synthetic compound. Drugs that share a common core structure belong to a particular group. But members within a particular group may produce different effects. Pharmacological activity within a group may vary widely [1]. Although natural compounds and herbs were the basis of treatment for earlier days, the synthetic compounds have taken over as the prime source of new drugs in the modern world. Four synthetic compounds were supplied to evaluate their primary biological activity. They are

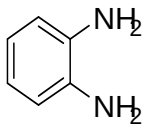
a) 2-bromopopylamine hydrobromide
b) 3-bromopopyl ammonium bromide
c) Ortho-amino aniline
d) Benzimidazole-2-thiol



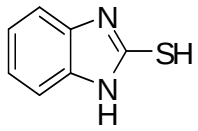
2-bromopropylamine hydrobromide



3-bromo propyl ammonium bromide



O-Amino aniline



Benzimidazole-2-thiol

MATERIALS AND METHODS

Collection of the test samples and experimental animals

Test compounds were 2-bromopropylamine hydrobromide, 3-bromopropyl ammonium bromide, benzimidazole-2-thiol and ortho-amino aniline obtained from the Department of Pharmaceutical Chemistry, University of Osaka, Japan. It was a part of collaborative research work among Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Department of Pharmaceutical Chemistry, University of Osaka, Japan and Department of Pharmacy, ASA University of Bangladesh. The supplied compounds were preserved properly and subjected to assays for different biological activities at suitable dose per kg body weight of experimental animal. Swiss-albino mice (*Mus musculus*) aged 4-5 weeks and albino Wister rats (120-150 g) obtained from the animal house of Jahangirnagar University, Bangladesh was used for the experiment. They were kept in standard environmental condition and fed ICDDRB formulated rodent food and water. The experimental animals were handled according to the animal ethical committee of University of Dhaka properly and strictly.

Evaluation of analgesic activity

Analgesic activity of test samples were evaluated by acetic acid induced Writhing Method [2]. In this method acetic acid was administered intra-peritoneal to the experimental animals to create pain sensation for producing squirms of their body at regular interval out of pain known as writhing. Each writhing was counted and taken as an indication of pain sensation. Any substance that has got analgesic activity was supposed to lessen the number of writhing of animals within a given time frame which was compared with the control and standard group. In the present study, Diclofenac sodium was used as a standard drug.

Experimental design and sample preparation

Swiss albino mice were randomly selected of either sex and divided into six groups denoted as group-I, group-II, group-III, group- IV, group-V and group-VI consisting of 5 mice in each group. Among all the groups, control materials and standard drug were applied to group-I and group-II and rest of the groups were treated with test samples. Standard drug (Diclofenac sodium) and test samples were administered at doses of 50 mg/kg body wt of mice suspending the materials within normal saline and Tween-80. At zero hour test samples, control (1% Tween-80 solution in saline) and Diclofenac sodium were administered orally by means of a long needle with a ball-shaped end. After 40 minutes acetic acid (1%) was administered intra-peritoneal to each of the mouse of all the groups. After 5 minutes of acetic acid administration number of squirms or writhing were counted for each mouse for ten minutes.

Table 1: Test materials used in the evaluation of analgesic activity

Code	Test Samples	Group	Identification	Dose (mg/kg)	R/A
C	1% Tween-80 in normal saline	I	Control	0.1ml/10 g of body wt	Oral
S	Diclofenac sodium	II	Standard	50	Oral
AA	Ortho Amino aniline	III	Test Sample	50	Oral
BPAB	3-bromopropyl ammonium bromide	IV	Test Sample	50	Oral
BPAH	2-bromopropylamine hydrobromide	V	Test Sample	50	Oral
BT	Benzimidazole-2-thiol	VI	Test Sample	50	Oral

Evaluation of antipyretic activity

Antipyretic activity 0f the test samples was done by yeast induced pyresis in rat [3] Pyrexia was induced in albino Wister rats by the injection of 15% Brewer's yeast solution. The study was only carried out in two compounds 3-Bromopopyl ammonium bromide and Benzimidazole-2-thiol respectively. The other two compounds were left out because according to the general perception both analgesic and anti-pyretic activity is mediated by the same pathway by inhibiting the production of prostaglandins and Postacyclin [4], [5].

Experimental design and sample preparation

Albino Wister rats weighing 120-150g was randomly selected and divided into four groups containing 5 rats in each group for test and measured their basal rectal temperature by using a clinical digital thermometer by insertion of thermometer to a depth of one inch into the rectum before inducing pyrexia. After taking the temperature Pyrexia was induced by injecting subcutaneously 15% w/v suspension of Brewer's yeast in distilled water at a dose of 10ml/kg body weight in the back below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin and returned to their cage and allowed to feed. After 18 hrs of Brewer's yeast injection the rise in rectal temperature was recorded. Test samples at a dose of 50 mg/kg body wt were administered orally by making a suspension with Tween-80, DMSO and normal saline. On the other hand standard paracetamol administered orally by making a suspension by normal saline at a dose of 100 mg/kg body wt of rat.

Table 2: Test materials used in the evaluation of analgesic activity

Code	Samples	Group	Identification	Dose (mg/kg)	R/A
C	Yeast, Tween-80 and DMSO in normal saline	I	Control	10 ml/kg body wt	Oral
S	Paracetamol	II	Standard	100	Oral
BPAB	3-bromopropyl ammonium bromide	IV	Test Sample	50	Oral
BPAH	2-bromopropylamine hydrobromide	V	Test Sample	50	Oral

Evaluation of hypoglycemic activity

Hypoglycemic activity of the test samples were evaluated by glucose tolerance test (GTT) [6] which is one of the acceptable methods to evaluate the hypoglycemic activity. It is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism. Glibenclamide was used as a standard drug to compare the results of the experiment.

Experimental design and sample preparation

Swiss albino mice were randomly selected and divided into six groups consisting five mice in each group. Samples and standard were administered orally at a dose of 25 mg/kg and 10 mg/kg body wt respectively. At zero hour test samples, control (1% Tween-80 solution in saline) and Glibenclamide (standard) were administered to the respective groups orally by means of a long needle with a ball-shaped end. After 60 minutes, all groups were treated with 10% glucose solution (2gm/kg body wt.) After 30, 90 & 150 minutes of glucose loading, blood samples were collected from tail vein and blood glucose level was measured by glucometer.

Table 3: Test materials used in the evaluation of hypoglycemic activity

Code	Samples	Group	Identification	Dose (mg/kg)	R/A
C	1% Tween-80 in normal saline	I	Control	-	Oral
S	Glibenclamide	II	Standard	100	Oral
AA	Ortho Amino aniline	III	Test Sample	25	Oral
BPAB	3-bromopropyl ammonium bromide	IV	Test Sample	25	Oral
BPAH	2-bromopropylamine hydrobromide	V	Test Sample	25	Oral
BT	Benzimidazole-2-thiol	VI	Test Sample	25	Oral
	10% glucose		-	-	2gm/kg body wt

Evaluation of CNS depressant activity

Evaluation of CNS depressant activity of the test samples was done by phenobarbitone induced sleeping time test [7]. The test animals (Swiss albino mice) received the supplied synthetic compounds at the dose of 25 mg/kg body weight and control with vehicle (1% Tween-80 in normal saline). After thirty minutes of sample administration, phenobarbitone (25 mg/kg body wt) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

Experimental design and sample preparation

The experimental animals were randomly selected and divided into five groups consisting of 5 mice in each group. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. At zero hour test samples (25 mg/kg body wt) and control (1% Tween-80 solution in saline) were administered orally by means of a long needle with a ball-shaped end. After 30 minutes, all groups were

treated with sodium phenobarbitone at a dose of 25 mg/kg body wt. After that the onset of sleep and the total time of sleep or the time between the onset of sleep and the waking up was recorded

Table 4: Test materials used in the evaluation of CNS depressant activity

Code	Test Samples	Group	Identification	Dose (mg/kg)
C	1% Tween-80 & DMSO in normal saline	I	Control	Blank
S	Phenobarbitone	All groups	Standard	25
AA	Ortho amino aniline	III	Test Sample	25
BPAB	3-bromopropyl ammonium bromide	IV	Test Sample	25
BPAH	2-bromopropylamine hydrobromide	V	Test Sample	25
BT	Benzimidazole-2-thiol	VI	Test Sample	25

RESULTS

Analgesic activity

The effects of the test samples to subside the pain caused by acetic acid were observed and data were evaluated statistically. Statistical data evaluation confirmed that the compound benzimidazole-2-thiol and 3-bromopopyl ammonium bromide at a dose of 50 mg/kg body wt revealed significant analgesic activity with 37.85% and 42.85% inhibition of writhing (P value < 0.001 in both cases). Table 5

Table 5: Analgesic activity of the test samples with P values

Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing	P value
Control	28±0.32	-	-
Control	28±0.32	-	
Standard	14±0.32	50	Less than 0.0001
Ortho amino aniline	27.2 ± 0.97	2.857	0.4554
3-bromopropyl ammonium bromide	17.4± 1.29	37.85	Less than 0.0001
2-bromopropylamine hydrobromide	23±0.98	17.85	0.0014
Benzimidazole-2-thiol	16 ± 0.71	42.85	Less than 0.0001

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): P<0.05.All values are means of individual data obtained from five mice (n = 5)

Antipyretic activity

Antipyretic activity of the test samples was done by yeast induced pyresis in rat at a dose of 50 mg/kg body wt in comparable with standard antipyretic drug paracetamol. Statistical evaluation proved that test samples did not show any anti-pyretic activity after its administration in rats. Table 6

Hypoglycemic activity

All the test samples were submitted to hypoglycemic test following standard glucose tolerant test. Among all the samples benzimidazole-2-thiol reduced blood glucose level in comparable with standard drug Glibenclamide having P value 0.00082.Table 7

CNS depressant activities

All test samples were subjected to CNS depression test by phenobarbitone induced sleeping time test. Among all the samples, 3-bromopopyl ammonium bromide lengthened the Na-phenobarbitone induced sleeping time having the P value 0.0009 which was statistically significant. Table 8

Table 6: Effects of control, standard and test samples on yeast-induced pyrexia in rats at different time intervals with P values

Group	Dose (mg/kg body wt)	Initial Rectal Temp. in ºF before Yeast Injection	Rectal Temperature in ºF after 18 hrs of Yeast Injection (Mean ± SEM)									
			0 hour	P value	1 hour	P value	2 hour	P value	3 hour	P value	4 hour	P value
Control	-	99.0 ± 0.173	100.6 ± 0.25	-	100.7±0.28	-	100.7 ± 0.2	-	100.68 ± 0.19	-	100.7 ± 0.19	-
Standard	100	98.56 ± 0.10	100.56 ± 0.33	0.7053	99.82 ± 0.12	0.0222	99.2 ± 0.12	<0.0001	98.86 ± 0.051	<0.0001	98.32 ± 0.1	<0.0001
BPAB	50	98.32 ± 0.23	100.58 ± 0.23	0.6106	100.48 ± 0.27	0.7473	100.44 ± 0.21	0.3720	100.3 ± 0.26	0.1816	100.51 ± 0.18	0.5921
BT	50	98.4 ± 0.17	100.24 ± 0.28	0.6776	100.52 ± 0.16	0.2039	100.44 ± 0.25	0.3255	100.56 ± 0.17	0.6504	100.46 ± 0.15	0.4422

BPAB = 3-bromopropyl ammonium bromide and BT = Benzimidazole-2-thiol

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): P<0.05.All values are means of individual data obtained from five rats (n = 5)

Table 7: Hypoglycemic activity of the test samples

Group	Plasma level of glucose (Mean ± SEM)			
	0 min	30 min	90 min	150 min
Control	5.98 ± 0.191	10.34 ± 0.214	7.46 ± 0.226	6.08 ± 0.348
Standard	5.92 ± 0.265	3.76 ± 0.194	3.56 ± 0.221	3.44 ± 0.147
AA	5.8 ± 0.089	9.7 ± 0.230	6.96 ± 0.250	5.74 ± 0.172
BPAB	6.22 ± 0.18	9.944 ± 0.262	7.02 ± 0.213	6.04 ± 0.268
BPAH	6.52 ± 0.46	9.34 ± 0.238	6.62 ± 0.208	5.84 ± 0.098
BT	5.76±0.46	8.44 ± 0.196	6.48± 0.169	5.22 ± 0.102

AA = Ortho amino aniline, BPAB = 3-bromopropyl ammonium bromide, BPAH = 2-bromopropylamine hydrobromide and BT = Benzimidazole-2-thiol

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): P<0.05.All values are means of individual data obtained from five rats (n = 5)

Table 8: The onset of sleeping time and the total sleeping time of each mouse with P values

Compound	No. of mice	Onset of sleeping time	Average onset time (min)	Sleeping time	Average sleeping time(min)
1) Control	1)Mice 1	15 min	24 ± 2.65	110 min	108 ± 2.81
	2)Mice 2	27 min		115 min	
	3)Mice 3	29 min		98 min	
	4)Mice 4	21 min		107 min	
	5)Mice 5	28 min		110 min	
2) Ortho amino aniline	1)Mice 1	25 min	23.8 ± 1.77 P value = 0.9515	90 min	103.6±3.65 P value = 0.3668
	2)Mice 2	29 min		103 min	
	3)Mice 3	18 min		106 min	
	4)Mice 4	23 min		108 min	
	5)Mice 5	24 min		111 min	
3)3-bromopopyl-ammonium- bromide	1)Mice 1	25 min	24.8 ±1.28 P value = 0.7924	147 min	132.4±3.85 P value = 0.0009
	2)Mice 2	28 min		130 min	
	3)Mice 3	21 min		124 min	
	4)Mice 4	27 min		130 min	
	5)Mice 5	23 min		131 min	
4)2-bromo propylamine hydro bromide	1)Mice 1	21 min	21.6 ± 1.36 P value = 0.4434	106 min	105.2±2.13 P value = 0.4502
	2)Mice 2	23 min		111 min	
	3)Mice 3	26 min		108 min	
	4)Mice 4	18 min		99 min	
	5)Mice 5	20 min		102 min	
5) Benzimidazole-2-thiol	1)Mice 1	26 min	21.6±1.36 P value= 0.4434	140 min	126.4±6.62 P value = 0.0801
	2)Mice 2	18 min		128 min	
	3)Mice 3	21 min		130 min	
	4)Mice 4	23 min		108 min	
	5)Mice 5	20 min		126 min	

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): P<0.05.All values are means of individual data obtained from five mice (n = 5)

DISCUSSION

Synthetic compounds were screened for analgesic, antipyretic, hypoglycemic and CNS depressant activity in animal model. Statistical evaluation of the experimental data showed that benzimidazole-2-thiol and 3-bromopopyl ammonium bromide showed analgesic activity due to inhibition of prostaglandin synthesis in mice. 3-bromopopyl ammonium bromide also showed CNS depressant activity in mice model. So it could be concluded that 3-bromopopyl ammonium bromide could be used as narcotic analgesic. On the other hand the experimental synthetic compounds could not alter the thermo regulatory set point of hypothalamus in rat model though they could block the prostaglandin synthesis. Among all compounds, benzimidazole-2-thiol reduced blood glucose level in mice probably by increasing uptake and utilization of glucose.

CONCLUSION

The obtained experimental data concluded that the synthetic compounds under the investigation revealed important therapeutic application in animal model. So it is necessary to do further investigation to explore the safety use of these compounds as drug in human subjects.

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REFERENCES

[1] Thomas G. Medicinal chemistry, 2nd edition, England, **2007**, pp 76-92.
[2] M Ahmed, HA Shikha, SK Sadhu, MT Rahman and BK Datta. *Pharmazie*. **2001**, 56, 657-660.
[3] Tk Chattergee. Handbook of Laboratory Mice and Rats, 1st Ed. Kolkata, Department of Pharmaceutical Technology, Jadavpur University, **1993**, pp 157.
[4] Sshid Gul Khattak, S Naeemuddin Gilani and M Ikram. *J. Ethnopharmacol*, **1985**, 14 (1), 45-49.

[5] K Hirose, H Jyoyama, Y Kojima, M Eigyo and H Hatakeyama. *Drug Research*, **1984**, 34, 280-286.
[6] P Trinder. *Clin. Biochem*, **1969**, 6, 24-27.
[7] EM Williamson, DT Okpako, FJ Evans. Pharmacological methods of phytotherapy research: selection preparation and pharmacological evaluation of plant material, 1st edition England, **1996,** pp 184-186.