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Anti-inflammatory and analgesic activities of acetophenone semicarbazone and benzophenone semicarbazone

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ABSTRACT

Objective: To study anti-inflammatory and analgesic activities in swiss albino mice, two schiff bases namely acetophenone semicarbazone (ASC) and benzophenone semicarbazone (BSC) were synthesized and characterized. **Methods:** Two doses of the test compounds 25 and 50 mg/kg (p.o) for each were selected throughout the research work. The anti-inflammatory activity of the test compounds was determined by 'carragenan induced mice paw edema inhibition' method. The analgesic activity was determined by both, 'acetic acid induced writhing' and 'tail immersion' methods. All such data were compared with standard drugs at the dose of 10 mg/kg (p.o.). **Results:** Both ASC and BSC have showed positive effects as anti-inflammatory and analgesic agents. Anti-inflammatory and analgesic activities of the test compounds at 50 mg/kg (p.o.) were quite comparable to those of standard drugs at 10 mg/kg (p.o.). **Conclusion:** Both ASC and BSC can be considered as potent anti-inflammatory and analgesic agents.

1. Introduction

Compounds with the structure of $-C=N-$ (azomethine group) are known as schiff bases, which are usually synthesized from the condensation of primary amines and compounds having active carbonyl groups. The biological activities of schiff bases have attracted considerable attention to organic and medicinal researchers for many years. Schiff bases are now well known for their importance in biological fields such as anticancer^[1,2], antimicrobial^[3,4], anti-inflammatory^[5,6], analgesic^[7], pesticidal^[8] agents. They are also found to increase^[9] the macrophages to the host in most cases. Based on the above-mentioned applications of schiff bases, the present piece of work has been undertaken to study the anti-inflammatory and analgesic profile of acetophenone semicarbazone (ASC) and benzophenone semicarbazone (BSC).

2. Methods and materials

2.1. Chemicals

All chemicals and reagents used to carry out the research work were of reagent grade.

2.2. Experimental animal

Swiss albino mice of 5–7 weeks old, weighing 25–30 g were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) Mohakhali, Dhaka.

2.3. Animal care

Mice were kept in iron cages with saw dust and straw bedding which was changed once a week regularly. Standard mouse diet (recommended and prepared by ICDDR'B) and water were given in adequate.

2.4. Ethical clearance

Protocol used in this study for the use of mice as animal model for research was approved by the University Animal

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Ethical Committee (27/08/RUBCMB).

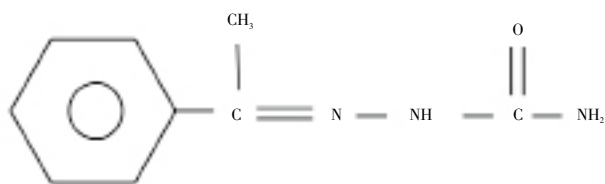
2.5. Synthesis of acetophenone semicarbazone (ASC) and benzophenone semicarbazone (BSC)

The procedure for the synthesis of ASC and BSC was similar to that described in the literature^[10].

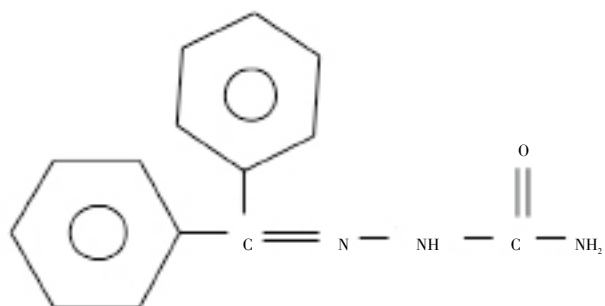
2.6. Characterization

The formation and purity of these compounds have been confirmed by taking melting points, infrared spectra and mass spectral studies.

2.7. Structure of ASC and BSC



Acetophenone semicarbazone (ASC).



Benzophenone semicarbazone (BSC).

2.7. Determination of median lethal doses (LD₅₀)

LD₅₀ values were estimated by the “acute toxicity test” as described elsewhere. The test compounds were dissolved in 3% DMSO administered orally to different groups with increasing doses. Four animals were taken in each group. Mortality was determined after 24 h of treatment. The dose, at which the 50% mice survived, was considered as LD₅₀ value of the compound.

2.8. Study of anti-inflammatory and analgesic activities

2.8.1. Anti-inflammatory activity

The anti-inflammatory activity^[11] of the test compounds was determined using the carrageenan-induced mice paw edema inhibition method employing 1.0% carrageenan solution as the phlogistic agent. The test compounds were administered orally as suspensions in 3% DMSO, 30 min before the injection of the phlogistic agent, at dose level of 25 and 50 mg/kg (p.o.) body weight. Diclofenac sodium was used as a standard at a dose

level of 10 mg/kg (p.o.) body weight. 3% DMSO served as a control. Groups of four swiss albino mice of either sex were used in each experiment. The volume of paw edema was measured with the help of plethysmograph by mercury displacement method at 0 h (immediately after injection of carrageenan). Then, the volume of paw edema was observed at 1, 2, 3, and 4 h. The results are presented in Table 1. The percentage inhibition of paw edema was calculated using the formula.

$$\% \text{ Inhibition} = 1 - V_t/V_c \times 100$$

V_t and V_c are the volumes of paw edema in treated and control groups, respectively.

2.8.2. Acetic acid-induced writhing test for analgesic activity

The analgesic activity of the test samples was studied^[12] using acetic acid-induced writhing model in mice. Swiss albino mice of either sex were divided into control, standard and different test groups contains four mice in each. The control group received 3% DMSO and standard group was treated with diclofenac sodium at a dose level of 10 mg/kg (p.o.). Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.6% acetic acid but diclofenac sodium was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as ‘writhing’ for the next 30 min. The analgesic activity was expressed as percentage inhibition of writhing in mice. The results are given in Table 2.

2.8.3. Tail immersion test for analgesic activity

The procedure was based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice^[13]. The animals were treated as discussed above. From 1–2 cm of the tail of mice was immersed in warm water kept constant at (54±1) °C. The reaction time was the time taken by the mice to deflect their tails. A cut off period of 5 s was observed to avoid damage to their tail. Reaction time was recorded when animals picked up their tails from hot water at 0, 30, 60 and 90 min after the administration of drugs. The results are shown in Table 3.

2.9. Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis was performed one-way ANOVA followed by Dunnett’s multiple comparison test using sigma stat software (version 2.0, Jandel Scientific Inc. USA).

Table 1.

Anti-inflammatory activity of the test compounds by carrageenan induced paw edema in mice.

Group	Dose mg/kg (p.o.)	% inhibition of paw volume with respect to control (untreated) after inoculation of carrageenan			
		1 h	2 h	3 h	4 h
Standard (treated with diclofenac sodium)	10	14.31	27.37	51.48	70.29
ASC	25	10.30	13.68	31.68	34.65
	50	12.37	25.26	38.61	41.58
BSC	25	10.43	15.79	26.73	36.63
	50	12.37	24.21	33.66	46.63

Numbers of mice in each group were four. Results are shown as mean.

Table 2.

Effect of test compounds on acetic acid induced writhing test in mice.

Group	Doses mg/kg (p.o.)	Frequency of writhing (Mean±SEM)	% inhibition of writhing
Control (untreated)	–	116.00±6.06	–
Standard (treated with diclofenac sodium)	10	25.00±6.06	78.45
ASC	25	35.00±5.82	69.83
	50	33.00±4.44	71.55
BSC	25	34.33±4.92	70.40
	50	29.66±8.73	74.43

Numbers of mice in each group were four. Results are shown as mean±SEM.

Table 3

Effect of test compounds on tail immersion method of analgesic activity test in mice.

Groups	Dose mg/kg (p.o.)	Mean latency period in second at a time of drug administration				% increase of latency period with respect to control		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Control	–	2.38±0.08	1.31±0.10	1.06±0.12	0.91±0.12	–	–	–
Standard (treated with morphine)	10	2.05±0.07	4.04±0.15	3.62±0.04	4.24±0.14	67.57	70.71	78.53
ASC	25	1.43±0.25	1.66±0.03	1.60±0.05	1.35±0.16	21.60	33.96	32.59
	50	1.19±0.23	1.76±0.07	1.66±0.03	1.50±0.05	25.76	36.60	39.33
BSC	25	1.76±0.31	1.87±0.02	1.77±0.09	1.22±0.11	29.93	40.18	25.41
	50	1.17±0.14	1.76±0.12	1.75±0.10	1.24±0.12	25.62	39.61	26.42

Numbers of mice in each group were four. Results are shown as mean±SEM.

3. Results

LD50 value of ASC was found to be 500 mg/kg body weight (p.o.) and that of BSC was 450 mg/kg (p.o.). Two doses for each compound viz. 25 mg/kg (p.o.) and 50 mg/kg (p.o.) have been selected throughout the work.

In the carrageenan-induced mice paw edema test (Table 1) for acute inflammation, the test compound ASC at doses of 25 mg and 50 mg/kg (p.o.) showed 34.65% and 41.58% inhibition of paw edema, respectively, at the end of 4 h. BSC produced almost the same effect at the same dose. Standard drug diclofenac sodium produced 78.45% inhibition in paw volume at 10 mg/kg (p.o.).

Table 2 shows the effect of the test compounds on acetic acid-induced writhing in mice. The oral administration of test compounds significantly inhibited writhing response induced by acetic acid in a dose dependent manner. ASC produced 69.83% and 71.55% inhibition of writhing at doses 25 mg/kg (p.o.) and 50 mg/kg (p.o.) respectively; BSC produced almost the same effect at the same dose. Standard drug diclofenac sodium produced 78.45% inhibition of writhing at 10 mg/kg (p.o.).

The tail withdrawal reflex time following administration

of the test compounds was found to increase with increasing dose of the samples. The results were statistically significant and comparable to that of the reference standard drug morphine. The data are shown in Table 3.

4. Discussion

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3–4 h after carrageenan injection) kinin and prostaglandins are involved[14]. Our results revealed that administration of test compounds inhibited the paw edema starting from the first hour and during all phases of inflammation, which is probably due to inhibition of different aspects and chemical mediators of inflammation.

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free

arachidonic acid from tissue phospholipid. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells^[15], acid sensing ion channels^[16] and the prostaglandin pathways.

The tail immersion test is considered to be selective to examine compounds acting through opioid receptor; the test compounds increased mean basal latency which indicates that they may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain^[17]. Though the test compounds inhibited both mechanisms of pain; these are more active in peripherally than centrally.

Based on the results of the present study, it can be concluded that the test compounds possess strong analgesic and anti-inflammatory potential. However, further studies are necessary to examine the underlying mechanisms of analgesic and anti-inflammatory effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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