ORIGINAL ARTICLE

Screening of Analgesic Activity and Adverse Effects of Bisthiazolidine in Male Albino Rats

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Abstract

Introduction: *This study was carried out to screen the analgesic efficacy and adverse effects of bisthiazolidines in male albino rats.* ***Materials & Methods:*** *The study was conducted using male Albino rats (130-175gm.) housed in polypropylene cages. The animals were divided into 5 groups (n=6) each receiving different treatments. Both central visceral and peripheral somatic pain were screened using radiant heat method, hot plate method and writhing test. The first group of animals was taken as control, the second group was given the reference standard drug and the other groups received bisthiazolidines at different doses. For sub-acute toxicity study, bisthiazolidines was given per orally (P.O) daily for 14 days at the dose level of 75 mg/kg. Biochemical analysis of blood and histopathological study of GI mucosa was done after 14 days.* ***Results****: Bisthiazolidines showed significant analgesia compared to control. The results were significant (p<0.05) in radiant heat method and also in hot plate method (p<0.05) at the dose of 75 mg/kg. Writhing test showed highly significant result with maximum inhibition (51.17%) at the dose of 75 mg/kg. No significant adverse effects on renal and hepatic functions were found with bisthiazolidine. Histopathological study of GI mucosa showed preservation of normal architecture with bisthiazolidine.* ***Conclusion:*** *Bisthiazolidine showed significant peripheral somatic analgesia and also proved to be safe in respect of the renal and hepatic functions along with no adverse effects on GI mucosa.*

Keywords*: Albino rats, adverse effects, analgesic efficacy radiant heat method, bisthiazolidines, hot plate method and writhing test*

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Introduction

The Non-steroidal anti-inflammatory drugs (NSAIDs) are the mainstay of treatment in pain and inflammation. They act by inhibiting the enzyme cyclooxygenase (COX) which is responsible for biosynthesis of prostaglandins and certain related autacoids. These drugs usually are effective against pain of low to moderate intensity. Gastrointestinal erosions and ulcerations, disturbances of platelet activation, changes in renal function etc. are the known adverse effects of different NSAIDs though they are widely used in patients[1].

Also these (NSAIDs) are a class of pharmacologically active compounds widely used in osteoarthritis (OA) and other diseases. In vivo studies suggest that some NSAIDs accelerate the progression of OA; on the other hand, in vitro methods using cartilage tissue or differentiated chondrocytes can provide much information on the adverse effects of NSAIDs on chondrocyte functions. Thus, the modern medicinal research is interested in the development of new anti-inflammatory compounds endowed with anti-degenerative activity on cartilage to block the tissue

destruction during the inflammatory process [2].
For a long time we investigated bisthiazolidine-4-ones in their chemistry, stereochemistry and anti-inflammatory activity reaching in most derivatives interesting in vivo activity as well as toxicity levels lower than those of well-known NSAIDs used as reference drugs [3].

Materials & Methods

Animals
Animals were kept in polypropylene cages under controlled room temperature (23°±3°C) having relative humidity of 62-72%. The animals were given standard laboratory diet and water. Animals were deprived of food but not water for four hours prior to experiment. They were divided into 5 groups each containing 6 rats. The first group served as a control group which received distilled with 2% solution of gum acacia suspended in water. The second group received the reference standard drug. The third, fourth and fifth groups were given bisthiazolidines suspended in 2% solution of gum acacia inwater at the dose level of 25, 50 and 75 mg/kg orally respectively. The study protocol was approved by the Institute animal ethics committee.
Drugs, Aspirin, bisthiazolidines and Pethidine were used for the study. Aspirin suspended in 2% solution of gum acacia in water was given orally by gastric cannula fitted with a syringe. Bisthiazolidines (2-Phenylimino-5-arylidene-4-thiazolidine-diones) at doses of 25, 50 and 75 mg/kg were similarly administered per orally.

Radiant Heat method
Animals were held in suitable restrainer with the tail protruding out. Radiant heat was applied over the tail on a single spot over the proximal one third with the help of analgesiometer. The time taken by the animal to withdraw (flick) the tail was taken as the reaction time. Before administration of the test compound or the standard drug, the normal reaction time was recorded. Animals were subjected to a preliminary screening and rats showing tail flick response in 9-11 seconds were selected. The animals were submitted to the same testing procedure after 30, 60, 90 and eventually 120 minutes after administration of the drug and test compound. For each individual animal, the reaction time was noted. Pethidine (12 mg/Kg. SC.) was given as reference standard.

Hot Plate method
The hot plate was heated and maintained at 56.0±2C. The time taken to cause a reaction (licking paws or jumping) was recorded as reaction time. Before administration of the test compound or the standard, the normal reaction time was determined. The animals are submitted to the same testing procedure after 30, 60, 90 and eventually 120 minutes after administration of the drug and test compound. For each individual animal the reaction time was recorded. Pethidine (12mg./Kg SC.) was given as reference standard. A cut off time of 30 seconds was followed to avoid any thermal injury .[6]

Writhing induced with 0.6% solution of acetic acid
The albino rats were pretreated with drugs 50 minutes before induction of writhing. The animals received the standard drug aspirin (30mg./Kg P.O.) which served as reference standard. Analgesic activity of bisthiazolidine (25, 50, 75 mg/kg P.O.) was assessed by counting the number of writhes induced by intra-peritoneal injection of 1ml. /kg of 0.6% solution of acetic acid. The rats were placed individually into glass beakers and five minutes were allowed to elapse. The rats were then observed for a period of ten minutes and the number of writhes was recorded for each animal.[4] Percentage protection against abdominal constriction was taken as an index of analgesia. It was calculated as:

No. of writhing in control group – No. of writhing in treated group X 100 / No. of writhing in control group

Testing of Subacute Toxicity
From rat tail vein blood sample was taken prior to experiment for estimation of liver function test, urea, and creatinine in the control group of animals. For testing subacute toxicity, 3 rats receiving aspirin and 3 rats receiving bisthiazolidines in the dose 75mg. /kg were to be given the drug daily for 14 days. The biochemical tests were repeated after 14 days in those receiving bisthiazolidine. Among those receiving aspirin one animal died after 7days.

Immediate postmortem was done and on macroscopic examination of the stomach mucosa, thickening and paleness with presence of greyish white necrotic foci were detected. GI tissue was taken in 10% formalin for histopathological examination. Aspirin was discontinued in the other 2 animals. After 14 days, 3 rats which received 75 mg/kg of bisthiazolidine were sacrificed by euthanasia with ketamine (200 mg/kg body weight). Macroscopic examination of gastrointestinal mucosa did not show any obvious abnormality. Specimen of stomach and intestine were collected for histopathological study.

Statistical analysis

All the three methods results were expressed as mean ± SEM. Statistical analysis was determined using one way analysis of variance (ANOVA) followed by Dunnett's *test*.

Results

Pain induced by application of radiant heat in rats. Bisthiazolidine in dose of 75mg./kg showed some significant increase in latency of tail flick response compared to control at 60 minutes onwards following administration of drug. Similar result was seen after 90 minutes. The doses of 75 mg/kg mildly increased the latency of tail flick response (p<0.05) table- 1.

Hot plate method

Bisthiazolidines at the dose of 25 and 50mg. /kg did not show any significant increase in the mean basal reaction time in hotplate method compared to control. The dose of 75 mg/kg showed some significant increased basal reaction time (p<0.05). The inhibition was observed at 60mins. at 75 mg/kg dose (p<0.05) table- 2.

Writhing induced by 0.6% solution of acetic acid

The bisthiazolidines at doses of 50 and 75mg. /kg reduced significantly (P<0.001) the number of abdominal constrictions induced 0.6% solution of acetic acid by compared to control group. Maximum inhibition of writhing response with bisthiazolidines was 51.17% with 75 mg/kg, which was comparable to aspirin [Table- 3]. For sub-acute toxicity study, 9 rats were equally divided into 3 groups containing 3animals each. First group was kept for control and received 2% gumacacia solution in distilled water daily for 14 days, while animals of second group were administered aspirin daily at 20mg. /kg for14 days. Bisthiazolidines was given at 75mg. /kg daily for 14 days to animals of group 3. Blood samples were collected from orbital plexus prior to experiment and after 14 days post dosing of drugs for AST, ALT, BUN and CRT analysis.

Tests for subacute toxicity

Histopathological examination of Gastro-intestinal tract mucosa of rat showed preservation of normal architecture with bisthiazolidines of 75mg. /kg [Figure- 1] whilst the rat G.I. tract mucosa showed proliferation, desquamation and coagulative necrosis of the lining mucosal epithelium with cellular infiltration and atrophy of secretary glands which received aspirin [Figure- 2]. Estimation of blood for AST, ALT, urea and creatinine showed no significant difference between the control group of animals and those receiving bisthiazolidines at 75mg. /kg table- 4.

Table- 1: Effect of Bisthiazolidines on radiant heat induced tail flick response in rats

Group	Pre-treatment	30 mins.	60 mins.	90 mins.	120 mins.
Control	10 ± 0.34	10 ± 0.6	10.29±0.38	9.78±0.48	10.28±0.51
Pethidine (12mg/kg)	10 ± 0.58	19.8±0.59***	20 ±0.85***	18.52±0.48***	17.16±0.36***
Bisthiazolidines (25mg/kg)	9.83±0.47	10.9±0.42	10.94±0.36	10.99±0.31	10.89±0.3
Bisthiazolidine (50mg/kg)	9.83±0.6	10.66±0.33	10.96±0.3	11.02±0.44	11.±0.24
Bisthiazolidine (75mg/kg)	10.5±0.42	11.02 ± 0.36	12.90±0.55*	12.88±0.53*	11.01±0.33

The mean±SEM; n=6, *P<0.05, **P<0.01, ***P<0.001 when compared with control group

Table- 2: Effect of bisthiazolidines on hot plate response in albino rats

Group	Pre-treatment	30 mins.	60 mins.	90 mins.	120 mins.
Control	10.16±0.47	9.5±0.43	9.5±0.42	9.66±0.49	9.33±0.33
Pethidine (12mg/kg)	10.66±0.88	15.33±0.42***	16.66±0.66***	16.16±0.47***	15.16±1.3***
Bisthiazolidines (25mg/kg)	10.5±0.42	9.66±0.49	9.66±0.42	10 ± 0.57	10 ± 0.57
Bisthiazolidines (50mg/kg)	11±0.36	10.5±0.76	10.83±0.7	10.5±0.43	11.03±0.55
Bisthiazolidines (75mg/kg)	10.3±0.49	12.49±0.6*	12.80±0.34*	12.76±0.49*	12.5±0.42*

The mean±SEM; n=6, *P<0.05, **P<0.01, ***P<0.001 when compared with control group

Table- 3: Effect of Bisthiazolidines on writhing induced by 0.6% solution of acetic acid

Significant	Number of writhes ± SEM	% of inhibition
Control	5.83±0.47	0 NS
Pethidine (12mg/kg)	1.8±0.3	68.96 P<0.001
Bisthiazolidines (25mg/kg)	4. 8±0.30	17.24 P> 0.05
Bisthiazolidine (50mg/kg)	3.66±0.21	37 P<0.001
Bisthiazolidine (75mg/kg)	2.83±0.30	51.17 P<0.001

Table- 4: Biochemical parameters in rats receiving bisthiazolidine at 75mg/kg

	Control	Bisthiazolidines (75mg/kg)
SGOT(AST)IU/L	59.83±2.104	60.33±1.85
SGPT(ALT)IU/L	34.33±1.9	36.66±2.07
BUNgm/dl	10.66±0.66	11.66±0.55
Creatinine gm/dl	1.09±0.08	1.11±0.04

Figure- 1: Architecture of mucosa of G.I. Tract was preserved with Bisthiazolidines at 75mg/kg.

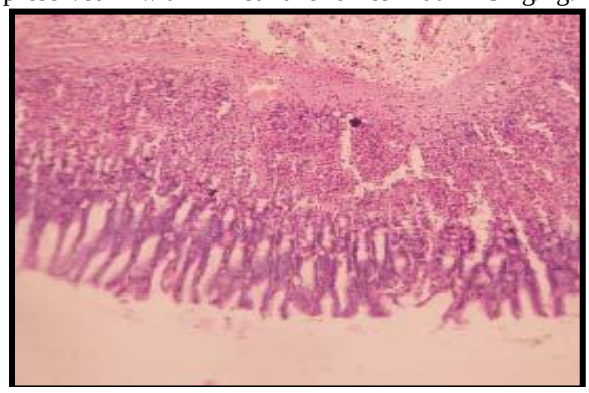
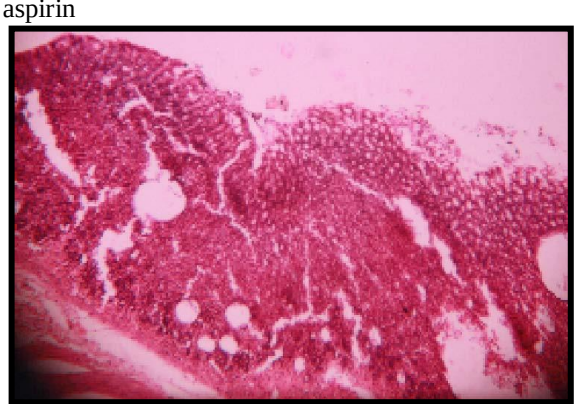


Figure- 2: Necrosis & sloughing of epithelium by aspirin



Discussion

A study of analgesic efficacy and adverse effects of bisthiazolidines in male albino rats has been done in various models of pain for study of both visceral and somatic pain. The stimulus may be thermal (tail flick, tail immersion, hot plate tests), mechanical (tail or paw Pressure tests), electrical stimulation of (paw, tail) or chemical (writhing test).[7]

NSAIDs act primarily on peripheral pain mechanisms but also in CNS to raise pain threshold.[8] They are the most commonly used anti-inflammatory, antipyretic, analgesic drugs. Most NSAIDs block prostaglandin synthesis by inhibiting COX1 and COX2 non-selectively, but

now some selective COX2 inhibitors has been developed. Of the common toxicities caused by NSAIDs due to inhibition of prostaglandin synthesis, gastric mucosal damage is most troublesome. This sometimes limits the use of this group of drugs in patients with chronic pain.

The hot plate and radiant heat methods are suitable for evaluation central visceral pain. Centrally acting analgesics like pethidine is used as reference standard for this purpose.[5] In the method of pain induction by application of radiant heat on rat tail & hot plate, bisthiazolidines at the dose of 75mg. /kg showed some significant increase in latency of tail flick. The bisthiazolidines at a dose of 75mg. /kg showed some significant increase in basal reaction time in radiant heat & hot plate method. It was thus found to be effective as an analgesic in the models applied for study of central visceral pain at higher doses.

Writhing was induced by intraperitoneal injection of 0.6% solution of acetic acid for study of peripheral somatic pain. The reference drug (aspirin) offers relief from inflammatory pain by inhibiting the formation of pain mediators in the peripheral tissues. On intra-peritoneal injection of 0.6% solution of acetic acid, the nociceptive response is due to release of endogenous substances such as bradykinin and prostaglandins, which stimulate the nociceptive endings. A highly significant reduction in the number of abdominal constrictions with bisthiazolidines was observed at 50 and 75mg. /kg compared to control indicating good analgesic activity in visceral pain.

The histopathological examination of rat G.I. tract mucosa showed preservation of normal architecture with bisthiazolidines at a dose of 75 mg/kg. While desquamation and coagulative necrosis of the lining mucosal epithelium with cellular infiltration and atrophy of secretary glands were observed with the use of aspirin. Bisthiazolidines was thus found to have no detrimental effects on mucosa of the gastro-intestinal tract even on prolonged use at a dose of 75mg. /kg.

Conclusion

It can be concluded that bisthiazolidines has efficacy as analgesic more on somatic and to some extent on visceral components of pain at a dose of 75mg/kg having no adverse effects as well as on hepatic and renal functions on the gastrointestinal mucosa.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

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