

**Evaluation of analgesic activity of irbesartan in albino mice****P. S. Vaibhavi\*, R. N. Suresha, H. L. Kalabharathi, Siddamma Amogimath,  
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**Received:** 19 January 2014  
**Accepted:** 02 February 2014

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**ABSTRACT**

**Background:** The objective was to evaluate the analgesic activity of irbesartan in albino mice.

**Methods:** Swiss albino mice weighing 25-30 g of either sex were selected for the study. Six animals were allocated to each experimental group. The control group received normal saline (25 ml/kg, p.o.), standard group received pentazocine (10mg/kg, intraperitoneal [i.p.]) and test group received irbesartan (20 mg/kg, p.o.). The above drugs were administered 1 hr prior to the experiments. In case of visceral pain model 0.6% acetic acid was given i.p. 30 mins prior to the experiment to induce writhing, in thermal pain model pretreated mice were placed on Eddy's Hotplate maintained at 55°C and in mechanical stimulus pain model an artery clip was clamped at the base of the tail of pretreated mice. Decrease in total number of writhes in acetic acid induced writhing model and delay in reaction time in both Eddy's hot plate and Tail clip method denoted analgesic activity respectively.

**Results:** The test drug significantly decreased the total number of writhes in acetic acid induced writhing model in mice. The percentage inhibition of writhing was significant which was 84.35% in the standard group and 59.24% in the test group. The test drug significantly delayed the reaction time in both Eddy's hot plate and tail clip method when compared to control group and standard group. Percentage increase in latency period when compared to standard drug was significant and measured 73.11% and 64.31% at 60 min in both Eddy's hot plate and tail clip method, respectively.

**Conclusion:** Irbesartan exhibits analgesic activity in albino mice.

**Keywords:** Angiotensin II, Irbesartan, Analgesia, Proinflammatory molecule

**INTRODUCTION**

Pain is a response to an untoward event associated with tissue damage, such as injury, inflammation or cancer. Pain is associated with electrical activity in afferent fibers of peripheral nerves. These nerves have sensory endings in peripheral tissues which are activated by various stimuli (mechanical, thermal and chemical). These stimuli are sufficient to excite afferent C-fibers to evoke painful sensation. With many pathological conditions, tissue injury is the immediate cause of the pain, and this result in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation.<sup>1</sup> Pain is often associated with inflammation. It is a complex process involving release of chemicals from tissues and migrating cells and various mediators such as substance P, prostaglandins (PGs), leukotrienes, interleukins (IL), and platelet activating factors.<sup>2</sup>

Angiotensin converting enzyme inhibitors and angiotensin receptor antagonists are widely used in various cardiovascular disorders. Since angiotensin converting enzyme (dipeptidyl carboxypeptidase) is also involved in degradation of kinins such as bradykinin and substance P which are potent mediators of pain and inflammation, some investigators have studied the effects of the renin-angiotensin system (RAS) on pain perception. Angiotensin II (Ang II), the key bioactive peptide in RAS, plays an important role in the regulations of blood pressure, cardiac and renal function, and electrolyte homeostasis. Many effects of Ang II take place by the activation of G protein - coupled Angiotensin II type 1 receptor (AT1R) through which Ang II promotes vasoconstriction, oxidative stress, inflammation, cell proliferation and modulation of sympathetic activity after its engagement with AT1R.<sup>3</sup> Ang-II has been studied to increase the transcription cyclooxygenase-2 (COX-2) and activate nuclear factor-kappa B (NF-κB) which in turn increases PG synthesis.<sup>4,5</sup> Thus, it was hypothesized that the analgesic activity of irbesartan, an AT1R antagonist would

be possibly mediated by inhibition of Ang II action by blocking the AT1R and also due to modulation of sympathetic tone.

## METHODS

### *Animals*

Albino mice (25-30 g) of either sex were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed into groups of 6-8 per cage at a temperature of 25°±1° and relative humidity of 45-55%. Animals had free access to food and water. The study was carried out after obtaining the approval of Institutional Animal Ethical Committee (IAEC: 26(A)).

### *Drugs and chemicals*

Pentazocine (Taj Pharmaceuticals, India), irbesartan (Sunpharma, India), acetic acid 0.6% diluted in distilled water were used.

### *Methodology*

Animals were divided into three groups (with 6 mice each). The study groups were divided as follows; Group-1 (control) received normal saline 25 ml/kg (orally), Group-2 (standard) received pentazocine 30 mg/kg/day and Group-3 (test) received irbesartan 20 mg/kg/day (orally) all of which were administered 30 min prior to the test in this acute study.

### *Assessment of analgesic activity*

#### *Acetic acid induced writhing response in mice<sup>6</sup>*

In this method, animals are administered orally with standard drug and test drugs which are suspended in distilled water. Thirty minutes after treatment, the mice were given i.p. injection of 0.6% v/v acetic acid in a volume of 10 ml/kg to induce the characteristic writhings. A stereotyped behavior in mice/rats characterized by constriction of the abdomen, twining of the trunk and extension of hind limbs is called as writhing. The total number of writhings occurring between 5 and 15 mins after the injection was recorded. Percentage of inhibition of writhing was calculated as follows:

$$\% \text{ inhibition of abdominal constrictions between control animals, standard and test drug treated mice using the ratio} = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

#### *Eddy's hot plate method in mice<sup>7</sup>*

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated

analgesic effects. Food was withdrawn on the preceding night of the experiment. Each animal were gently placed individually on the Eddy's hot plate maintained at 55°C. The reaction time or the latency period to exhibit nociceptive responses such as licking paws or jumping off the hot plate were recorded at 0, 30, 60, 120, and 180 min after the administration of the test drug or control drug.

#### *Tail clip method in mice<sup>8</sup>*

The test is done by applying a metal artery clip at the base of the tail with its jaw sheathed with thin rubber tubing after administering control, test and standard drugs. A quick response of the mice was seen as biting of the clip. The time between the application of the clip and the response was noted by a stop watch. Those mice which did not show any effort to dislodge the clip within 15 s were rejected. The tail clip was applied at 30, 60, 90, and 120 min after oral administration of extract. It was considered as a response if there is no attempt by the mouse to dislodge the clip within 15 s.

### *Statistical analysis*

The effects of different drugs under study were calculated by taking the mean and SD of the outcome parameters. Analysis of variance (ANOVA) was applied to compare the effects of drugs under the study. Data were analyzed using ANOVA followed by *post-hoc* Bonferroni's test. Differences were considered as significant at 5% level (\*p<0.05).

## RESULTS

### *Acetic acid induced writhing response in mice*

In this test, control group showed a mean number of writhing to be 35.16±2.85, pentazocine treated group showed 5.5±2.42 and irbesartan treated group showed 14.33±2.16. The mean number of writhings caused by test group was significantly decreased when compared with the control group. The percentage inhibition of writhing as calculated by the above formula in pentazocine treated group was 84.35% while that of the test drug irbesartan treated group was 59.24% (Table 1 & Figure 1).

### *Eddy's hot plate method in mice*

The test drug irbesartan brought about an increase in the latency period at all-time intervals and was significant when compared to control at 60 and 90 min. Percentage increase in latency period when compared to standard drug was significant and measured 73.11% and 80.24% at 60 and 90 min (Table 2 & Figure 2).

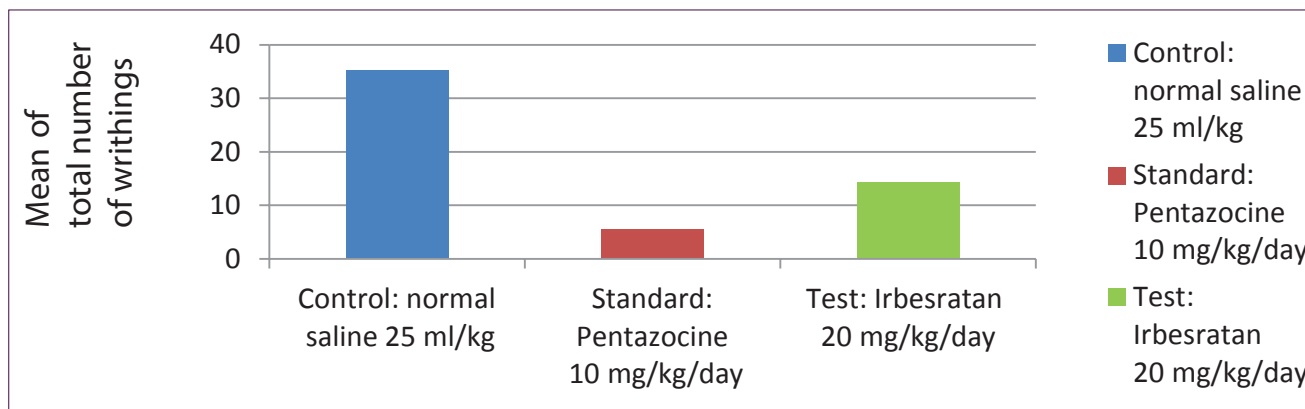
### *Tail clip method in mice*

The test drug irbesartan brought about an increase in the latency period at all-time intervals and was significant when

**Table 1: Acetic acid induced writhing response in mice.**

Groups	Dosage	Mean no. of writhings	% Inhibition	% Writhing caused
Control	25 ml/kg	35.16±2.85	0	100
Standard (pentazocine)	10 mg/kg/day	5.5±2.42	84.35	15.65
Test drug (irbesratan)	20 mg/kg/day	14.33±2.16*	59.24	40.76

Values are expressed as mean±SD from six observation. \*p<0.001

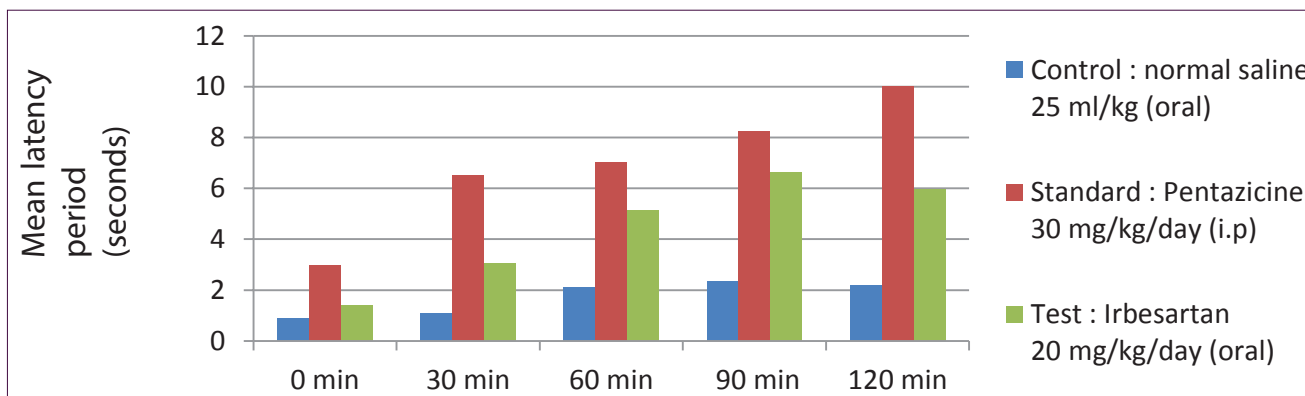


**Figure 1: Acetic acid induced writhing response in mice.**

**Table 2: Eddy's hot plate method in mice.**

Groups	Dosage	0 min	30 min	60 min	90 min	120 min
Control	25 ml/kg	0.89±0.15	1.09±0.15	2.11±0.20	2.32±0.26	2.20±0.24
Standard (pentazocine)	10 mg/kg/day	2.95±0.14	6.49±0.17	7.03±0.42*	8.25±0.40*	10.02±0.36*
Test drug (irbesartan)	20 mg/kg/day	1.38±0.43	3.03±0.23	5.14±0.24*	6.62±0.38*	5.94±0.49

Values are expressed as mean±SD from six observation. \*p<0.001



**Figure 2: Eddy's hot plate method in mice.**

compared to control at 60 and 90 min. Percentage increase in latency period when compared to standard drug was significant and measured 64.31% and 63.70% at 60 and 90 min (Table 3 & Figure 3).

**DISCUSSION**

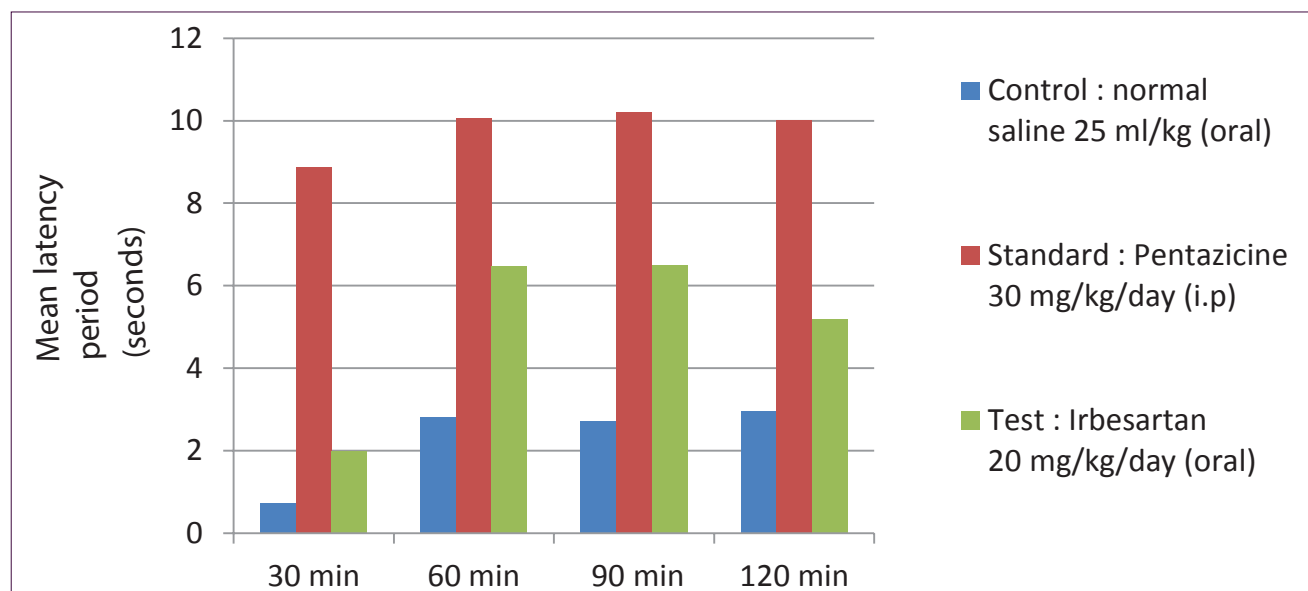
Pain is an unpleasant sensation and is always a subjective feeling. Pain arising from any noxious stimuli is called as nociceptive pain. Opioids and non-steroidal anti-inflammatory drugs are the main drugs used for symptomatic

relief of pain, but they exert a wide array of adverse effects especially on the long-term use. Conditions like neuropathy, migraine or arthritis require a longer term of drug treatment.<sup>9</sup> The importance of Ang II in regulating cardiovascular function has led to the development of non-peptide antagonists of the AT1 Ang II receptor for clinical use. Angiotensin receptors blockers such as losartan, candesartan, olmesartan, telmisartan, irbesartan acts by blocking at angiotensin receptors (AT II type 1 receptors). By antagonizing the effects of Ang II, these agents relax smooth muscle and thereby promote vasodilation, increase

**Table 3: Tail clip method in mice.**

Groups	Dosage	30 min	60 min	90 min	120 min
Control	25 ml/kg	0.73±0.23	2.81±0.22	2.71±0.16	2.95±0.21
Standard (pentazocine)	10 mg/kg/day	8.84±0.12	10.06±0.12	10.2±0.07	10.01±0.09
Test drug (irbesartan)	20 mg/kg/day	2.03±0.11	6.47±0.21	6.5±0.18	5.18±0.41

Values are expressed as mean±SD from six observation. \*p<0.001

**Figure 3: Tail clip method in mice.**

renal salt and water excretion, reduce plasma volume, and decrease cellular hypertrophy.

The two major classes of angiotensin receptors are AT1 and AT2. Actions of Ang-II and Ang-III are mediated by the AT1 receptor. AT1 receptors are up-regulated in hyperangiotensinergic states and are normally present in blood vessels, heart, kidney, adrenal cortex and lung & brain and mediate vasoconstrictor effects. ATR 1 antagonists displace Ang II and thereby inhibit Ang II mediated actions.<sup>10</sup>

Direct evidence to suggest that Ang II is involved in central antinociceptive modulation came from studies in which Ang II administered by intracerebroventricular route attenuated morphine induced analgesia in the hot plate mice. (Kaneko et al. 1985).<sup>11</sup> Ang-II brings about increase in the release of reactive oxygen species (ROS) which in turn activate NF-κB (known to initiate inflammatory process) that increases the transcription of pro-inflammatory cytokines, adhesion molecules, and NADPH oxidase. Ang-II has been studied to increase the transcription COX-2 and activate NF-κB which in turn increases PG synthesis.<sup>4</sup> Studies have shown that Ang-II infusion elevate the synthesis and concentrations of tumor necrosis factor-α (TNF-α), IL-6 which is an earliest marker in thermal pain, chemokine monocyte chemoattractant protein-1 (MCP-1), elevate tissue levels of NF-κB, and inflammatory cell infiltration.<sup>5,12</sup> Thus, Ang II itself behaves as a proinflammatory molecule

which plays a vital role in both pain and inflammation. Earlier studies on valsartan, an Ang-II receptor blocker have demonstrated suppression of ROS generation in leukocytes, NF-κB binding activity and the expression of total cellular p65 expression (a protein component of NF-κB) in mononuclear cells and plasma C-reactive protein concentrations in normal subjects.<sup>13,14</sup>

Studies have shown that mononuclear phagocytes synthesize angiotensinogen, Ang I and AII and express receptors with high affinity for AII. AII on binding these receptors modulate mononuclear phagocyte function and stimulates TNF-α production. In this study losartan, a selective inhibitor of subtype AT1 receptors for Ang II showed inhibition of N-formylmethionylleucyl-phenylalanine binding to neutrophil receptors.<sup>15</sup> In another study, candesartan inhibited LPS-induced increased expression of toll-like receptor 4 (TLRs) and downstreamed inflammatory factors likely through Ang II type 1 receptor independent pathway in human renal tubular epithelial cells. TLRs are widely distributed on immune cells, mucosal epithelial and endothelial cells which play a crucial role in immune and inflammatory processes. Its activation leads to increase in the expression of inflammatory mediators such as IL-1β, IL-6, TNF-α, MCP-1, through activation of NF-κB. Candesartan brought about a dose dependent anti-inflammatory action by suppression of NF-κB and chemokine expression.<sup>3</sup> There is also evidence to suggest the role of Ang II in central nociception where increased



hot plate latency in mice by repeated administration of ART1 antagonist losartan was reversed by the opioid receptor antagonist naloxone. ART1 antagonists also cause modulation of sympathetic neurotransmission by preventing the pressor and sympathoexcitatory responses normally evoked by exogenous or endogenous Ang II.<sup>16</sup>

Data presented here suggests that irbesartan an angiotensin receptor blocker possesses analgesic activity. The test drug was shown to possess analgesic activity evident in the above analgesic models, signifying it possesses both central and peripherally mediated activities. Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, PGs, bradykinins and substance P, which stimulate nerve endings.<sup>17</sup> The significant reduction in acetic acid-induced writhes by irbesartan suggests that the analgesic effect may be peripherally mediated through the inhibition of synthesis and release of PGs by inhibition of NF- $\kappa$ B mediated COX-2 up-regulation. The hot-plate and tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level.<sup>18</sup> The analgesic activity of irbesartan was consistently significant at 60 and 90 minutes when compared to control and standard drug in both eddy's hot plate and tail clip method which coincides with the peak action of irbesartan. The significant increase in pain threshold as seen by an increase in the latency period produced by irbesartan in these models suggests inhibition of NF- $\kappa$ B & ILs particularly IL-6 which is an earliest mediator involved in thermal pain and also possible opioid mediated action.

## CONCLUSION

Thus, irbesartan possesses analgesic activity demonstrated by significantly decreasing the total number of writhes in acetic acid induced writhing model and significantly delaying the reaction time in both Eddy's hot plate and tail clip method respectively when compared to control and standard group.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Animal Ethical Committee*

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doi: 10.5455/2319-2003.ijbcp20140414

**Cite this article as:** Vaibhavi PS, Suresha RN, Kalabharathi HL, Amogimath S, Jayanthi MK, Shetty KP. Evaluation of analgesic activity of irbesartan in albino mice. *Int J Basic Clin Pharmacol* 2014;3:331-5.