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A COMPARATIVE ANIMAL STUDY OF ANALGESIC EFFECTS OF TRANDOLAPRIL AND NIMODIPINE IN MICE USING EDDY'S HOT PLATE

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ABSTRACT: Angiotensin II from the renin-angiotensin system is proposed to be pro-inflammatory induces reactive oxygen species, nuclear factor kappa B. It sensitizes pain receptors to mediators of pain (*via* PGE2). ACEI block these actions increase endogenous opioids by inhibiting enzyme enkephalinase. Voltage-gated calcium channels (VGCC) causing calcium influx are involved in exocytosis of synaptic vesicle (glutamate, substance–P) post membrane depolarization while pain processing. N and T types of VGCC are implicated in the central sensitization of pain stimuli. The current study aims at evaluating the analgesic properties of trandolapril and nimodipine in mice. This was an experimental study. Twenty-four swiss albino mice were randomly divided into four groups. Reaction time was assessed on Eddy's hot plate (±55°C) in trandolapril group (5mg/kg), Nimodipine group (2.5mg/kg) and compared with standard (tramadol 20mg/kg) and percentage change was evaluated at 30, 60, 90, 120 min. ANOVA did a statistical evaluation. A significant increase ($p<0.05$) in reaction time was seen in the trandolapril group at 60, 90,120 min whereas at 30, 60, 90,120 min in the tramadol group. No significant change occurred in the control and nimodipine groups. Trandolapril is therefore proposed to possess analgesic activity in mice. However, no effect was seen with nimodipine at this dose.

INTRODUCTION: Pain is one of the most common symptoms in patients who visit a clinician. It is a distressing experience that is associated with actual or potential tissue damage involving sensory, emotional, cognitive, and social components, according to the International Association for the Study of Pain (IASP). Nociceptive pain is caused by tissue damage, while inflammatory pain occurs after activation of the immune system, and both are protective.

Pathological pain or central neuropathic pain, on the other hand, occurs due to damage in the nervous system or altered neural processing and can occur even in the absence of noxious stimuli [1]. Central pain occurs mostly as sequelae of stroke, spinal cord injury, or brain tumors and deleterious influences on quality of life, mood and cognition. Chronic pain per se ranks amongst the top 10 most

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prevalent diseases globally, with a rough estimate of the prevalence of 18% in developing countries as seen in a recent meta-analysis [2]. Treatment after the establishment of central pain is partial and accompanied by tremendous side effects by the currently used drugs, including tri cyclic antidepressants, opioids, anticonvulsants, lidocaine, capsaicin, and others [3]. This indicates the prerequisite for searching the new approaches both for treatment as well as prevention of occurrence in the first place. The Renin-Angiotensin-Aldosterone System (RAAS) is evidenced to be involved in the pathophysiology of pain. Angiotensin II (Ang II), the main bioactive molecule, acts as an analgesic and inflammatory. Pain is commonly correlated with inflammation. Recent studies have found the presence of local and intracellular RAS in many organs, including the heart, brain, kidney, pancreas, adipose tissue, reproductive organs, and circulating RAS [4, 5, 6, 7].

This local RAS is responsible for several non-hemodynamic actions of Ang II as pro-inflammatory pro-fibrotic. Ang II promotes the production of reactive oxygen species induces nuclear factor kappa B (NF-kB), ultimately causing inflammation, apoptosis, cell migration, differentiation, remodeling of extracellular matrix, and activation of several intracellular signaling leading to tissue injury. It activates the COX-2 pathway leading to endothelial dysfunction. Ang II increases the production of PGE2, which sensitizes pain receptors to mediators of pain and thus increases pain sensitivity.

Calcium channels are crucial in the pathophysiology of pain. Voltage-gated calcium channels (VGCC) causing calcium influx are involved in exocytosis of the synaptic vesicle (containing glutamate, substance-P, calcitonin gene-related peptide) post membrane depolarization in the central terminals of sensory afferent nerves while pain processing when the action potential propagates from peripheral nociceptors and relay in the spinal cord. Earlier studies proposed the role of L-type VGCC in pain sensitization [8]. N and T types of VGCC have also been implicated in the central sensitization of pain stimuli [9]. Considering the role of the renin-angiotensin system and calcium in pain, the present study evaluated the analgesic property of angiotensin-converting enzyme inhibitor trandolapril and calcium channel blocker nimodipine in mice using Eddy's hot plate (thermal method).

MATERIAL AND METHOD:

Animals: The study was performed in the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow. Ethical clearance was approved by the Institutional Animal Ethics Committee (IAEC). Approval number-(118/IAEC/2019). 24 adult healthy swiss albino mice, weighing 20-30 gm, were selected for the procedure and obtained from CPSCEA – certified animal house [IITR, Lucknow]. They were housed in appropriate-sized cages under 25±2°C and maintained at 12 hours light / 12 hours dark cycle. They were provided with adequate food and water.

Drugs: Trandolapril was obtained from Sigma Aldrich. It was solubilized in DMSO (Dimethyl Sulfoxide) and dissolved in normal saline, and administered per-orally (p.o.) in a dose of 5 mg/kg[10]. Nimodipine was given 2.5 mg/kg intraperitoneally (i.p.) [11], tramadol [12] 20 mg/kg i.p. was purchased from an authorized medical store.

Groups: 24 swiss albino mice were randomly divided into 4 groups (n = 6). Group1-normal saline, Group 2-trandolapril, Group 3-nimodipine, Group 4-tramadol. These mice were evaluated for the central analgesic property on Eddy's hot plate and 'reaction time' time at baseline and 30, 60, 90, 120 min were recorded. Statistical evaluation was done by ANOVA followed by a post hoc test. $P < 0.05$ was considered statistically significant.

Behavioral Test: Analgesic activities were studied using Eddy's hot plate (thermal method) [13]. This method was first described by Eddy and Leimbach (1953). In this model, before the start of the experiment, the hot plate was set for a temperature 55±1°C. The mice were then gently placed on the hot plate, maintaining the temperature at 55±1°C. Reaction to the thermal stimulus, which is when an animal starts paw licking or jump response, was observed, and those who showed an initial reaction time of 10sec or less were included in the study. This test was employed for preferential assessment of possible centrally mediated analgesic effects [14]. Control reaction time was taken by testing each

animal at least twice before the experiment **(**at 0 min**)**. The mice in the test, standard, and control group were then treated with respective drugs, and reaction time was again assessed at 0, 30, 60, 90, and 120 min. The response or reaction time was evaluated as when mice reacted to the pain stimulus either by jumping, withdrawal of the paws, paw licking, whichever appeared first [15]. Cut off time was 15 seconds to avoid damage to the paws [16].

Statistical Analysis: Data were expressed as Mean ± Standard deviation. Results were analyzed using SPSS **(**Statistical Package for Social Sciences**)** Version 21.0 statistical analysis software by ANOVA single factor followed by post hoc Tukey's HSD test. An intragroup comparison was done using the **'**paired-t test**'**. $p < 0.05$ was considered to be significant.

RESULT: The analgesic effect was observed by the duration of stay on Eddy's hot plate at 0, 30, 60, 90, 120 min. ANOVA of the results was found to be comparable at baseline ($p>0.05$); however, it was statistically significant at 30, 60, 90, 120 minutes ($p < 0.05$). Between-group comparison at 30 sec was significantly higher only in the standard group. At 60 min and 90 min, post-administration of the drug, the analgesic reaction time of the standard group was maximum, followed by trandolapril group, while at 120 min reaction time of trandolapril group was maximum followed by standard group.

The percentage change in baseline upon intragroup comparison is shown in **Table 1**. The control and nimodipine groups did not show any statistically significant change in reaction time at any of the observation periods, suggesting no analgesic effect.

Trandolapril group showed a significant percentage increase from baseline at 60, 90, and 120 min with the maximum increase at 90 min, and the standard group showed significant change at 30, 60, and 90 min. **Fig. 1** shows the trend of analgesic reaction time of the different groups at various time intervals.

TABLE 1: INTRAGROUP COMPARISON OF 'ANALGESIC REACTION TIME' (SECONDS) ON EDDY'S HOT PLATE

Groups Change	Change	Time (min) Mean SD	% Baseline
NS (control)	30	-0.02	0.12-0.38
	60	-0.09	0.13-1.43
	90	-0.130.14	-2.13
	120	-0.050.10	-0.81
Trandolapril (test)	30	0.230. 35	3.49
	60	2.700.584	1.22**
	90	3.430.62	52.46**
	120	3.200.87	48.84**
Nimodipine (test)	30	0.030.12	-0.42
	60	-0.070.33	-1.13
	90	-0.070.21	-1.22
	120	-0.060.21	-0.99
Tramadol (standard)	30	4.810.677	1.47**
	60	5.570.528	2.83**
	90	6.150.579	1.43**
	120	1.200.731	7.84*

Level of significance-*denotes $p<0.05$, ** denotes $p<0.01$.

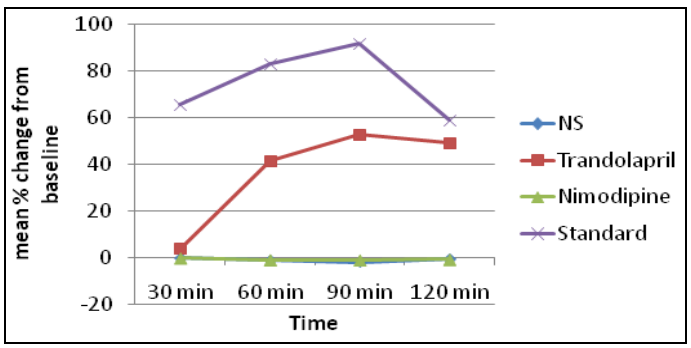

FIG. 1: MEAN PERCENTAGE CHANGE IN ANALGESIC REACTION TIME OF DIFFERENT GROUPS. Values are mean ± SD of analgesic 'reaction time' ■ NS (control); ■ Trandolapril; ■ Nimodipine; ■Tramadol (standard**)**.

DISCUSSION: The method of evaluation of pain by a thermal stimulus applied to the paws of rodents by using a hotplate is very sensitive and specific for the screening of drugs with central analgesic activity, as also demonstrated by Nemirovsky *et al.* (2001). These responses are, however, not demonstrated in peripherally acting analgesics [17]. The reaction time significantly increased in the trandolapril group suggesting an analgesic effect, and this effect was lower than the standard tramadol group. However, the nimodipine group does not show any significant increase in reaction time, suggesting no analgesic effect. Tramadol, used as a standard drug, is a centrally acting analgesic. Antinociception mechanism is considered both opioid-related (mu) and non-opioid related by inhibiting the reuptake of norepinephrine and serotonin as it is only partially antagonized by naloxone [12].

The analgesic effect due to trandolapril may be possible due to inhibition of excess release of Ang II, thereby decreasing the production of reactive oxygen species, NF-kB, NADPH oxidase, and pro-inflammatory cytokines downregulating several intracellular signaling leading to tissue injury. This may have resulted in inhibition of pain and inflammation. It suppresses sympathetic activation, the COX-2 pathway, and decreases the production of PGE2 [18]. Another possible mechanism is that inhibiting the enzyme enkephalinase, it must have increased the levels of endogenous opioids. The results in our study were consistent with findings of previous studies and one by Suresha and Amoghimath *et al.* (2014) that suggest both central and peripheral mechanisms for antinociception [18].

Nimodipine did not show significant improvement in reaction time in the present study, and the reason may be due to the difference in subtype selectivity as L type calcium channels are of four subtypes (CaV 1.1, CaV 1.2, CaV 1.3, CaV 1.4) and T types (CaV 3.1,CaV3.2, CaV3.3). Another reason may be the difference in pain model and dosing duration, as some studies showed potentiation of morphine analgesia upon chronic nimodipine and morphine coadministration [19, 20].

CONCLUSION: Trandolapril showed an increase in reaction time as demonstrated in Eddy's hot plate at a dose of 5 mg/kg BW, so it can be concluded that it has central analgesic property, but the effect was lesser than tramadol. On the other hand, Nimodipine did not show any increase in reaction time at a dose of 2.5 mg/kg BW on a hotplate so it can be concluded that nimodipine does not possess the analgesic property at this dose. Therefore, it can be concluded that trandolapril has a central analgesic effect. However, further reinforcing these results using more animal models and biochemical specifications are required.

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CONFLICTS OF INTEREST: None declared

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