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An Experimental Study to Evaluate Analgesic and Anxiolytic Effects of Doxazosin in Swiss Albino Mice

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ABSTRACT

Introduction: Doxazosin is an alpha blocker. It works to relax certain muscles in your body. It is a quinazoline derivative that acts as a competitive alpha1 antagonist at the post-synaptic receptor. It relaxes your veins and arteries so that blood can more easily pass through them. It also relaxes the muscles in the prostate and bladder neck, making it easier to urinate.

Aim and Objective: An Experimental Study to Evaluate the Analgesic and Anxiolytic Effects of Doxazosin in Swiss Albino Mice

Material and Methods: The study was carried out in the Department of Pharmacology & Therapeutics at King George's Medical University, Lucknow. The present study was designed to evaluate antianxiety and analgesic effect of doxazosin in an experimental model in swiss albino mice. A total number of 36 female Swiss albino mice were included in the study. They were kept in the institutional animal house under standard conditions. They received normal pellet diet and water ad libitum. They were allowed to get acclimatized to the new environment for a period of 2 weeks. Mice were randomly divided into 3 groups for each parameter, each group containing 6 mice.

Results: In the present study in case of analgesic effect, the best effect was present in tramadol which was the standard drug, followed by doxazosin. It was observed that the test drug may exhibit anti-nociceptive behaviour by both central and peripheral mechanisms but it was weaker as compared to the standard drug. It was found that the anti-anxiety effect into consideration where doxazosin was effective in reducing anxiety, however the result was less significant than the standard drug diazepam.

Conclusion: Although doxazosin was found to have a decent antianxiety and analgesic impact, the results were not as significant as those of the standard medicine, diazepam and tramadol leading to suggestions that doxazosin may have some antianxiety and analgesic effects. Doxazosin can be used as a treatment modality against cases that present with co-existence of pain as well as anxiety. Thus, there can be potential in exploring doxazosin more as an analgesic or anti-anxiety medication.

Keywords: Analgesic, Antianxiety, Doxazosin, Tramadol, Diazepam, Elevated plus maze, Swiss albino mice

INTRODUCTION

Researchers and medical professionals alike are still drawn to the new prospects in the field of neuropharmacology. However, the dearth of information about non-communicable diseases has raised questions about worldwide treatment options. These conditions typically impair the patient's quality of life, are usually invisible to the unaided eye, and require more time to initiate treatment [1].

The primary use of sedative–hypnotic and anxiolytic drugs is to encourage calmness (anxiolytics or sedatives) or to produce sleep (sedative–hypnotics). All people are subjected to states of emotional tension and uneasiness. For otherwise healthy individuals, these occasions are usually mild and short that pharmacological intervention is unnecessary [2,3]. Anxiety is frequently a sign of psychiatric disease and virtually always coexists with a variety of medical and surgical diseases. In cases where counselling is insufficient and the symptoms become unbearable or interfere with the underlying disease's therapy, medication may be a viable option for those seeking anxiety relief [4].

Anxiety that results from fear caused by an acute illness or a stressful event, such as loss of a loved one, is usually self-limiting and can be of relatively short duration. The current options include various kinds of psychotherapy and pharmacotherapy such as benzodiazepines, azapirones, and antidepressants and others [5].

Pain as a type of inflammation is suffered by almost everyone throughout their lifetime. Pain is a kind of convoluted unsavoury phenomenon which consists of sensory experiences including time, space, intensity, emotion, cognition and motivation. Inflammation is the defense response of body, characterized by redness, swelling, heat, pain, and loss in function to get rid of or limit the multiplication of an injurious agent. It demands a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, thermal injuries, and antigen-antibody interaction. Cyclooxygenases (COX) or prostaglandin endo-peroxide synthases (PGHS) exists in two isoforms COX-1 and COX-2. They are classified as the key enzymes inside the synthesis of prostaglandins, the leading mediators of inflammation, pain and increased body temperature [6-8]

The agents which are being used to attenuate pain either by acting in the central nervous system (CNS) or by peripheral pain mechanisms except altering the consciousness are called analgesics [6]. Recently attainable analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by repressing the synthesis of prostaglandin or by blocking the action of cyclooxygenase enzymes in inflammatory pathways. On the other hand, opiates act by affecting the central nervous system [9].

Accordingly, anxiety defined operationally in a given model may differ from that generated by other models with respect to drug response, environmental manipulations, and/or neural substrates. Animals, like humans, express different kinds of fear/anxiety in response to different environmental conditions (eg, acute vs chronic stress, spontaneous vs conditioned responses, etc). Only restricted aspects of human psychopathology can be explored and stimulated using animal models with symptoms of these disorders, rather than a complete anxiety subtype [10,11].

It has been suggested that doxazosin has promising qualities as a treatment for symptoms related to bladder diseases, such as benign prostatic hyperplasia. It has been demonstrated that doxazosin administration increases mean urine flow rates, similar to other α1-adrenoceptor antagonists. It also alleviates additional symptoms related to urinary disorders [12].

In terms of long-term research, doxazosin has incidence of side effects comparable to other α1-adrenoceptor antagonists in the same class. Headache, malaise, dizziness, and exhaustion are among the most often reported adverse effects. One serious danger is syncope, which is thought to be the primary postural consequence of the initial dose. You can reduce the likelihood that this side effect will manifest by carefully monitoring the drug's dosage [13,14].

Recent clinical trials have consolidated the place of doxazosin as an effective antihypertensive agent and have expanded its therapeutic potential to include treatment of benign prostatic hyperplasia, PTSD and Chronic Prostatitis/Chronic Pelvic Pain Syndrome.

Therefore the present study was undertaken to study the antianxiety and analgesic effect of doxazosin by an experimental model in swiss albino mice

MATERIAL AND METHODS

The present study was conducted in the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow, after getting the approval from the Institutional animal ethics committee.

Experimental animals:

In the current study a total of 36 adult healthy female Swiss albino mice, weighing 20-30 gm (n=6 mice/group) were used in the study. Mice were obtained from animal house of Indian Institute of Toxicology Research (IITR), Lucknow. IITR is one of the certified center by Committee for Control and Supervision of Experiments on Animals (CCSEA) for breeding and housing of animals. The animals were allowed to access food and water ad libitum and were kept in the institutional animal house under temperature controlled environment [25±2◦C] with 12 hours light/12 hours dark cycle. A minimum of 14 days acclimatization period was allowed before the commencement of the study and their health was monitored regularly by a veterinary physician. The normal pellet diet was procured from Bharat Science Solution Company, Loknagar, Unnao, Uttar Pradesh.

The study was started after the approval of Institutional Animal Ethic Committee (IAEC) of King George's Medical University (K.G.M.U), Lucknow (**Project No 129/IAEC/2020).**

All experiments in the study were conducted as per the guidelines laid down by Committee for Control and Supervision of Experiments on Animals (CCSEA).

DOSAGE FORMS, DOSAGE AND SOURCES OF THE DRUGS

Drugs and chemicals:

All test drugs and chemicals were purchased from Sigma chemical company, USA. Other chemicals were purchased from TCI, Japan.

Drugs

1) **Doxazosin** – Test drug [15]

Administered in a dose of 4 mg/kg body weight.

Route: Intraperitoneal injection

2) **Diazepam** - Standard for anxiety model [16]

Administered in a dose of 2 mg /kg body weight.

Route: Intraperitoneal injection

3) **Tramadol** - Standard for analgesic model [17]

Administered in a dose of 20 mg/kg body weight.

Route: Intraperitoneal injection

All the drugs were dissolved in dimethylsulphoxide (DMSO) and then diluted in distilled water and administered accordingly.

Technique of intraperitoneal injection

The syringe was filled with the calculated dosage of medication. To prevent any trauma during the surgery, the mouse was manually restrained by holding its entire body with all of its fingers, squeezing the loose skin at the nape of its neck, and having its tail confined between its little and ring fingers. An imaginary line was drawn across the naked abdomen, somewhat above the knees. After cleaning the injection site, a 26-gauge needle with the bevel pointing up was placed at an angle of 30 to 40 degrees into the lower right quadrant of the abdomen. This location on the imaginary line was near to the midline. This was carried out to protect the abdominal organs from harm. A gentle aspiration technique was also used to ensure this; if any fluid or blood was aspirated, the contaminated solution was thrown away and the process was repeated.

Figure 1 : Technique of intraperitoneal injection

EXPERIMENTAL PROTOCOL

The present study has been designed to evaluate antianxiety and analgesic property of doxazosin in an experimental model in swiss albino mice.

ANIMAL GROUPS

A total number of 36 female Swiss albino mice were included in the study. They were kept in the institutional animal house under standard conditions. They received normal pellet diet and water ad libitum. They were allowed to get acclimatized to the new environment for a period of 2 weeks. Mice were randomly divided into 3 groups for each parameter, each group containing 6 mice.

Group 1 to 3 were used to evaluate the analgesic effect of doxazosin and the effect was compared with tramadol. [Group 1 (vehicle), Group 2 (doxazosin), Group 3 (tramadol)].

Group 4 to 6 was then used to evaluate the anxiolytic effect of doxazosin and the effect was compared with diazepam. [Group 4 (vehicle), Group 5(doxazosin), Group 6 (diazepam)].**MICE**

MODELS FOR ASSESSMENT OF DIFFERENT PARAMETERS

For assessing analgesic effect:

Eddy's hot plate analgesiometer : It is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat. Behaviours such as jumping and hind paw-licking are elicited following a noxious thermal stimulus [18]. The time of latency is recorded which is defined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal licks its paw or jumps off to avoid thermal pain [19].

Mice were transferred from the animal house to the experimental room 1 hr before testing to acclimatize them to the laboratory environment. In this model before the start of 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 [20]. Reaction to the thermal stimulus which is the time at which animal starts paw licking or jump response was observed and those who showed initial reaction time of 10 sec or less were included in the study. This test was employed for preferential assessment of possible centrally mediated analgesic effect [21]. 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 minutes. The response or reaction time was evaluated as the time at which mice reacted to the pain stimulus either by jumping, withdrawal of the paws, paw licking, which ever appeared first. Cut off time was 15 sec to avoid damage to the paws [22].

Assessment

The mean reaction time for the test group was calculated and compared with the control and standard group. The results obtained were further assessed by single factor ANOVA, followed by post hoc tests for comparison between individual groups [23].

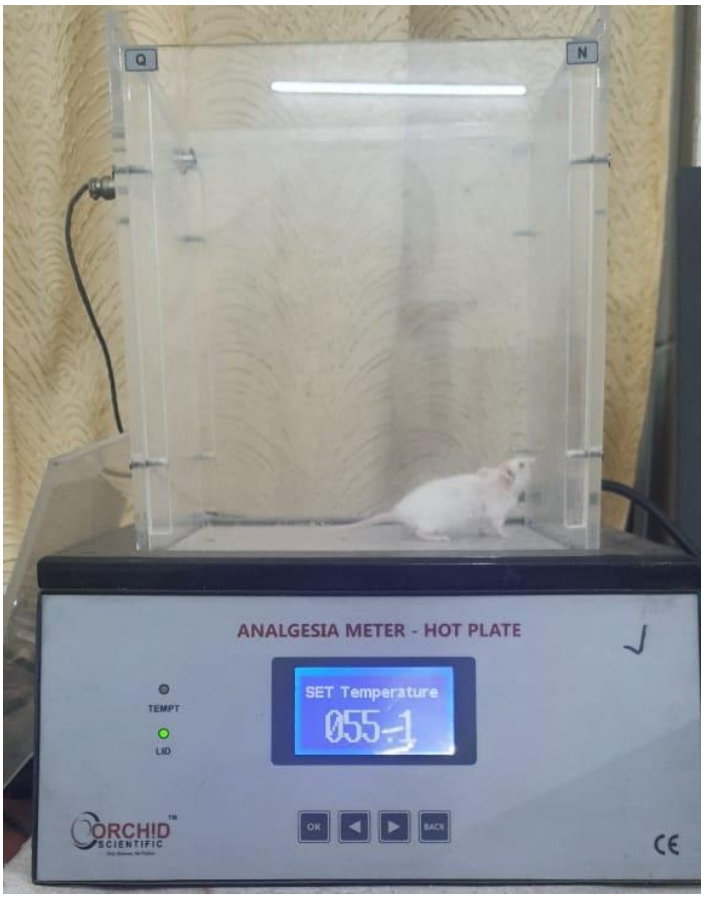


Figure 2 : Eddy's Hot Plate Analgesiometer

For assessing anti-anxiety effect:

Elevated plus maze : The elevated plus maze has been described as a simple method for assessing anxiety and locomotor responses of rodents. It has 4 arms that are arranged to form a plus shape. The instrument is 25 cm elevated from the floor. Dimensions of each open arm of elevated plus maze for mice are: 16 cm length ×5 cm width [24] Dimensions of each closed arm of elevated plus maze for mice are: 16 cm length × 5 cm width × 12 cm height. 30 min after i.p. administration of the test drug or the standard, the mice were placed in the center of the maze facing one of the enclosed arms [25] During a 5 min test period the following measures were taken:

1. No of entries in the open arm
2. No of entries in the closed arm
3. Total time spent in the open arm
4. Total time spent in the closed arm

Behaviour of animals were measured according to the protocol of Walf and their co-workers [26,27].

Figure 3 : Elevated Plus Maze

Statistical Analysis: The results of tests are expressed as Mean ± SD and analysed using single factor ANOVA. Further statistical analysis for individual groups was carried out by Tukey HSD post hoc test. The criterion for statistical significance is taken as $p< 0.05$ in all statistical evaluations.

RESULTS

The present study was conducted to evaluate the role of antianxiety and analgesic properties of doxazosin using an experimental models in Swiss albino mice and assessed against vehicle and standard drug used for antidepressant activity. Group wise distribution was done as given in Table

Table 1: Groupwise distribution of experimental mice

SN	Activity	Group	Group name	Number of mice
1	Analgesic effect	Group 1	Vehicle	6
		Group 2	Doxazosin	6
		Group 3	Tramadol (Standard)	6
3	Anxiolytic effect	Group 4	Vehicle	6
		Group 5	Doxazosin	6

		Group 6	Diazepam (Standard)	6

Assessment of analgesic activity

Analgesic activity was observed by the duration of stay on Eddy's hot plate. Each activity was conducted on 18 mice, 6 mice in each group (Group 1: Vehicle, Group 2: Doxazosin, Group 3: Tramadol).

Table 2: Intergroup comparison of analgesic reaction time (seconds)

at 0, 30, 60, 90, 120 minutes

S. No.	Group	0 min	30 min	60 min	90 min	120 min
1	Control (Vehicle i.p.)	4.92±0.33	5.70±0.55	6.08 ± 0.3	7.02±0.38	6.32±0.45
2	Doxazosin (4 mg/kg i.p.)	4.48±0.53	6.30±0.31[c]	6.92±0.61[b,c]	7.85±0.4[b,c]	7.17±0.43[b,c]
3	Tramadol (20 mg/kg i.p.)	5.07±0.48	8.62±0.53[a]	9.72±0.39[a]	9.98±0.53[a]	8.63±0.45[a]
	ANOVA	F = 2.7 p = 0.09	F = 61.92 p < 0.001*	F = 107.07 p < 0.001*	F = 71.87 p < 0.001*	F = 41.54 p < 0.001*

*Statistically significant [a] p<0.001 as compared to control, [b] p<0.05 as compared to control, [c] p<0.001 as compared to tramadol (20 mg/kg), [d] p<0.05 as compared to tramadol (20 mg/kg)

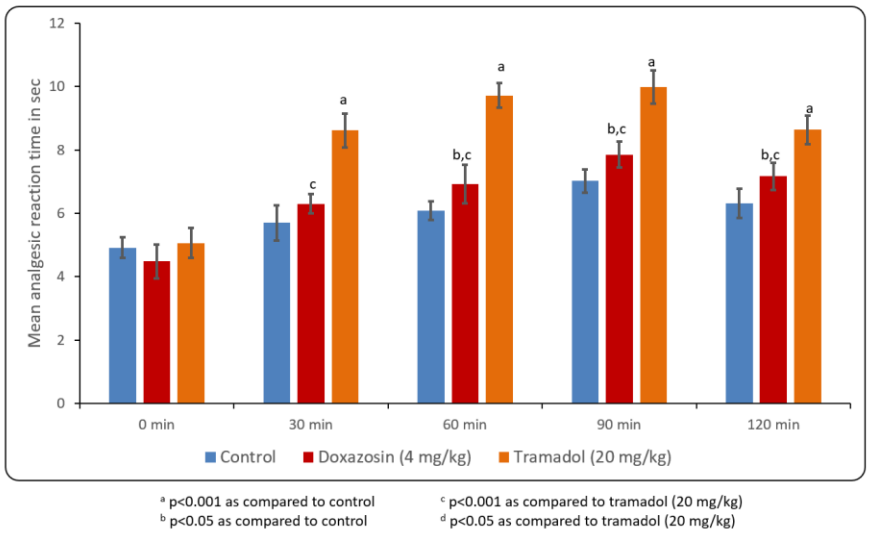


Figure 4: Analgesic reaction time of different groups

Intergroup comparison:

At 0 min (immediately after administering drugs), Group 2 mice had the quickest reaction time (4.48±0.53) followed by control (vehicle) (4.92±0.33) and maximum reaction time was recorded for tramadol (20 mg/kg) (5.07±0.48). On comparing the intergroup and between group differences of analgesic reaction time, none of the differences amongst the groups were significant. Therefore at 0 min, analgesic reaction time of mice in the above 3 groups was found to be comparable.

At 30 mins after administration of the drugs, analgesic reaction time was maximum for the tramadol (20 mg/kg) (8.62±0.53) group followed by doxazosin (4 mg/kg) (6.30±0.31) group and minimum for control (vehicle) (5.70±0.55) group. Intergroup difference was found to be significant ($p<0.001$). The difference in reaction time in Eddy's hot plate was highly significant ($p<0.001$) between control (vehicle) (5.70±0.55) group and tramadol (20 mg/kg) (8.62±0.53) group, with tramadol (20 mg/kg) group showing the most delay in reaction time. The difference in reaction time was highly significant ($p<0.001$) between doxazosin (4 mg/kg) (6.30±0.31) and tramadol (20 mg/kg) (8.62±0.53) group, with tramadol (20 mg/kg) (8.62±0.53) group showing the most delay in reaction time indicating that doxazosin (4 mg/kg) (6.30±0.31) group may have some weak analgesic activity.

At 60 mins after administration of the drugs, analgesic reaction time was maximum for the tramadol (20 mg/kg) (9.72±0.39) group followed by doxazosin (4 mg/kg) (6.92±0.61) group and least for control (vehicle) (6.08 ± 0.3) group. Intergroup difference was found to be significant ($p<0.001$). The difference in reaction time in Eddy's hot plate was highly significant ($p<0.001$) between control (vehicle) (6.08 ± 0.3) group and tramadol (20 mg/kg) group, with tramadol (20 mg/kg) (9.72±0.39) group showing the most delay in reaction time. The difference in reaction time was highly significant ($p<0.001$) between doxazosin (4 mg/kg) (6.92±0.61) group and tramadol (20 mg/kg) (9.72±0.39) group and less significant ($p<0.05$) between control (vehicle) (6.08 ± 0.3) group and doxazosin (4 mg/kg) (6.92±0.61) group with doxazosin (4 mg/kg) (6.92±0.61) group showing slight delay in reaction time indicating that doxazosin (4 mg/kg) may have some weak analgesic activity.

At 90 mins after administration of the drugs, analgesic reaction time was maximum for the tramadol (20 mg/kg) (9.98±0.53) group followed by doxazosin (4 mg/kg) (7.85±0.4) group and least for control (vehicle) (7.02±0.38) group. Intergroup difference was found to be significant ($p<0.001$). The difference in reaction time in Eddy's hot plate was highly significant ($p<0.001$) between control (vehicle) (7.02±0.38) group and tramadol (20 mg/kg) (9.98±0.53) group, with tramadol (20 mg/kg) (9.98±0.53) group showing the most delay in reaction time. The difference in reaction time was highly significant ($p<0.001$) between doxazosin (4 mg/kg) (7.85±0.4) group and tramadol (20 mg/kg) (9.98±0.53) group and less significant ($p<0.05$) between control (vehicle) (7.02±0.38) group and doxazosin (4 mg/kg) (7.85±0.4) group with doxazosin (4 mg/kg) (7.85±0.4) group showing slight delay in reaction time indicating that doxazosin (4 mg/kg) may have some weak analgesic activity.

At 120 mins after administration of the drugs, analgesic reaction time was maximum for the tramadol (20 mg/kg) (8.63±0.45) group followed by doxazosin (4 mg/kg) (7.17±0.43) group and least for control (vehicle) (6.32±0.45) group. Intergroup difference was found to be significant ($p<0.001$). The difference in reaction time in Eddy's hot plate was highly significant ($p<0.001$) between control (vehicle) (6.32±0.45) group and tramadol (20 mg/kg) (8.63±0.45) group, with tramadol (20 mg/kg) (8.63±0.45) group showing the most delay in reaction time. The difference in reaction time was highly significant ($p<0.001$) between doxazosin (4 mg/kg) (7.17±0.43) group

and tramadol (20 mg/kg) (8.63±0.45) group and less significant ($p<0.05$) between control (vehicle) (6.32±0.45) group and doxazosin (4 mg/kg) (7.17±0.43) group with doxazosin (4 mg/kg) (7.17±0.43) group showing slight delay in reaction time further indicating that doxazosin (4 mg/kg) may have some weak analgesic activity.

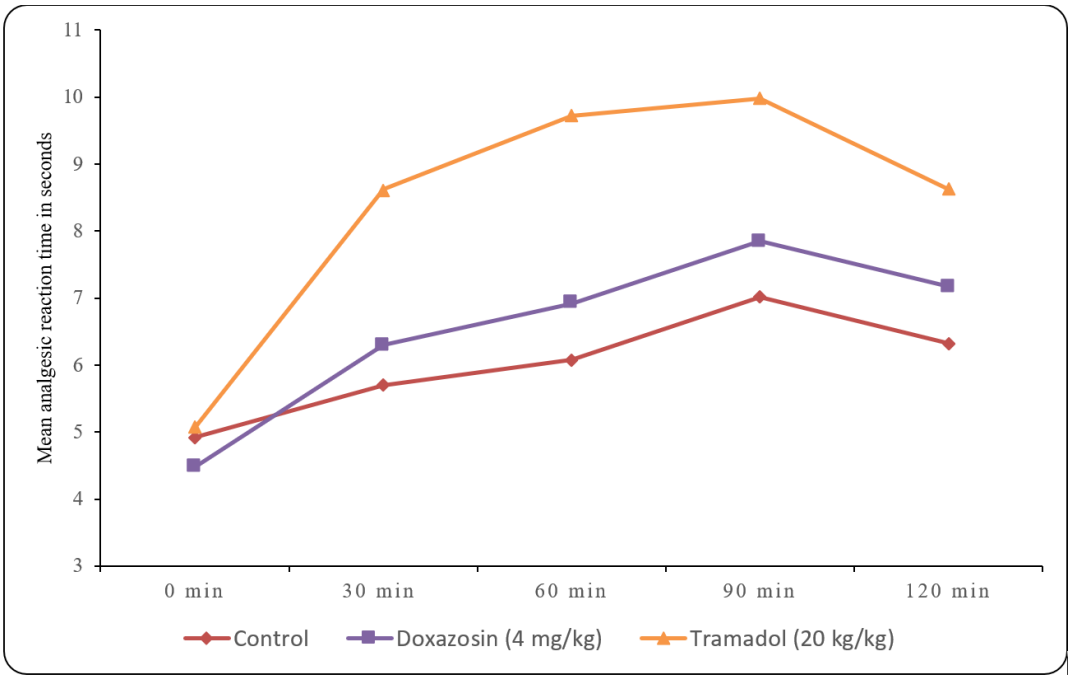


Figure 5: Trend of analgesic reaction time of different groups

Assessment of anxiolytic activity-

Anxiolytic activity was observed by total entry of closed arm, total entry in open arm, total time spent in closed arm, total time spent in open arm in elevated plus maze. Each activity was conducted on 18 mice, 6 mice in each group (Group 1: Vehicle, Group 2: Doxazosin, Group 3: Diazepam).

Table 3: Intergroup comparison of total entries in closed arms, total entry in open arm, total time spent in closed arm, total time spent in open arm in elevated plus maze

S.No	Group	Total entry in closed arm	Total entry in open arm	Total time spent in closed arm (sec)	Total time spent in open arm (sec)
1	Control (Vehicle i.p.)	11.67 ±1.63	4.00 ±1.1	194.38 ±12.96	31.82 ±4.19
2	Doxazosin (4mg/kg i.p.)	10.50 ±1.87	5.50 ±1.05[b]	180.73 ± 10.45[d]	36.87 ±3.58[c]
3	Diazepam (2mg/kg i.p.)	8.33 ±1.51[b]	6.83 ±0.75[a]	161.68 ±9.91[a]	48.65 ±5.74[a]
	ANOVA	F = 6.11 p = 0.01*	F = 12.62 p < 0.001*	F = 12.94 p < 0.001*	F = 21.2 p < 0.001*

[*] Statistically significant [a] $p<0.001$ as compared to control, [b] $p<0.05$ as compared to control, [c] $p<0.001$ as compared to Diazepam (2 mg/kg), [d] $p<0.05$ as compared to Diazepam (2 mg/kg)

a) Effect of doxazosin on total entries in closed arms and total entry in open arm in elevated plus maze

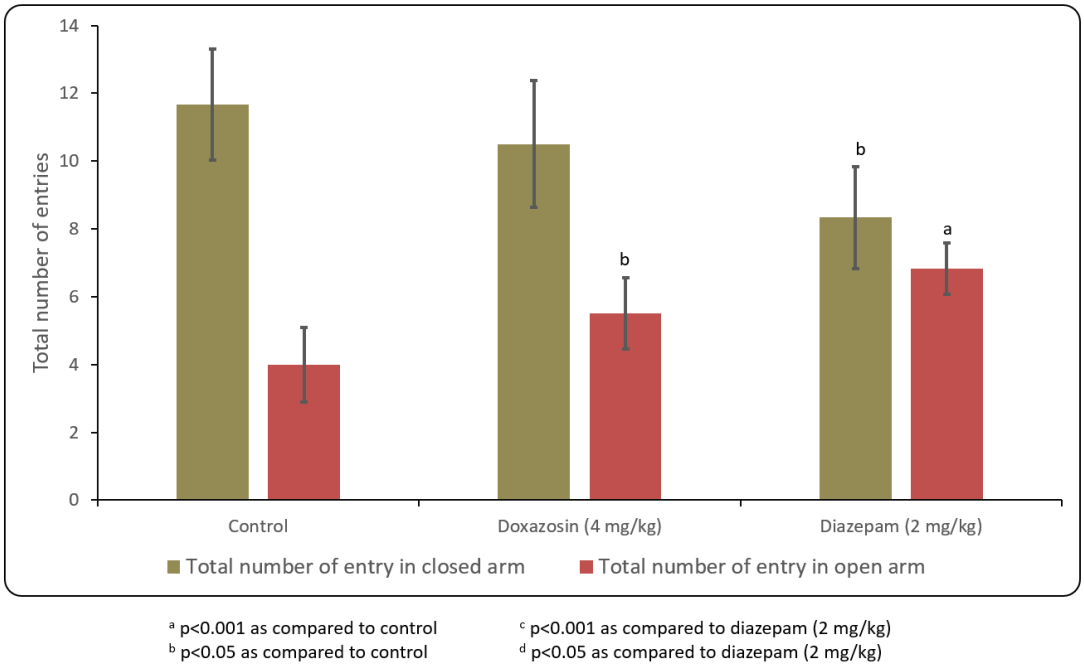


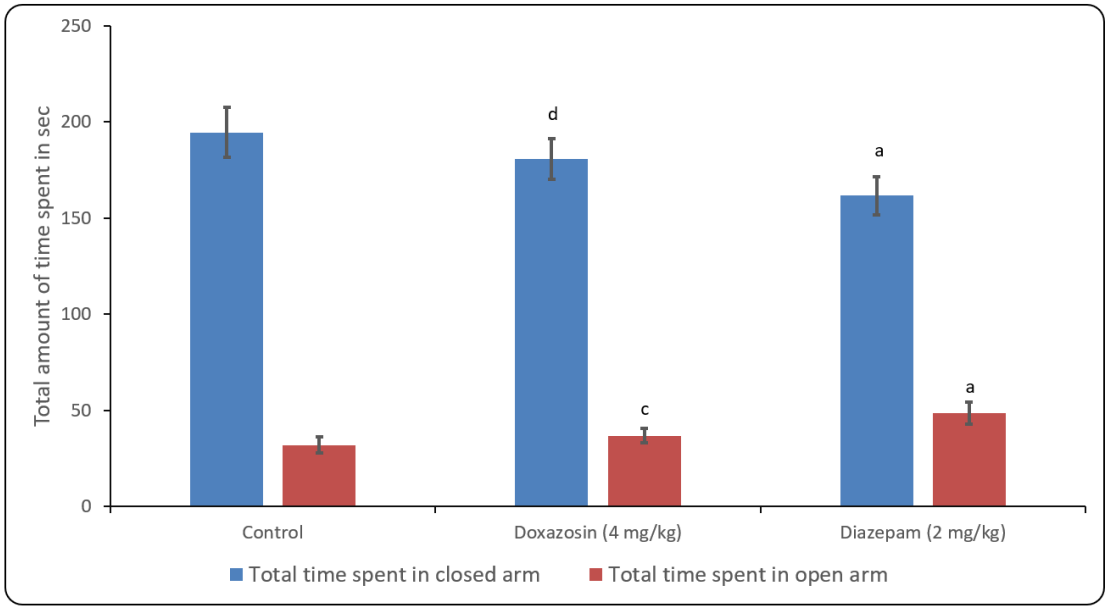
Figure 6: Mean total entries in closed and open arms in elevated plus maze

Intergroup comparison

In regards to total entries in closed arms, it was least for the diazepam (2 mg/kg) (8.33 ±1.51) group followed by doxazosin (4 mg/kg) (10.50 ±1.87) group and most for control (vehicle) (11.67 ±1.63) group. Intergroup difference was found to be significant ($p<0.01$). The difference in total entries in closed arms was significant ($p<0.05$) between control (vehicle) (11.67 ±1.63) group and diazepam (2 mg/kg) (8.33 ±1.51) group, with diazepam (2 mg/kg) (8.33 ±1.51) group showing the most reduction in total entries in closed arms. The difference in total entries in closed arms was not significant between doxazosin (4 mg/kg) (10.50 ±1.87) group and diazepam (2 mg/kg) (8.33 ±1.51) group and control (vehicle) (11.67 ±1.63) group and doxazosin (4 mg/kg) (10.50 ±1.87) group with doxazosin (4 mg/kg) (10.50 ±1.87) group showing slight decrease in total entries in closed arms indicating that doxazosin (4 mg/kg) may have some weak anxiolytic activity.

In regards to total entries in open arms, it was most for the diazepam (2 mg/kg) (6.83 ±0.75) group followed by doxazosin (4 mg/kg) (5.50 ±1.05) group and least for control (vehicle) (4.00 ±1.1) group. Intergroup difference was found to be significant ($p<0.001$). The difference in total entries in open arms was highly significant ($p<0.001$) between control (vehicle) (4.00 ±1.1) group and diazepam (2 mg/kg) (6.83 ±0.75) group, with diazepam (2 mg/kg) (6.83 ±0.75) group showing the most increase in total entries in open arms. The difference in total entries in open arms was not significant between doxazosin (4 mg/kg) (5.50 ±1.05) group and diazepam (2 mg/kg) (6.83 ±0.75) group whereas that between control (vehicle) (4.00 ±1.1) group and doxazosin (4 mg/kg) (5.50 ±1.05) group was less significant ($p<0.05$) with doxazosin (4 mg/kg) (5.50 ±1.05) group showing slight increase in total entries in open arms indicating that doxazosin (4 mg/kg) may have some weak anxiolytic activity.

b) Effect of doxazosin on total time spent in closed arm and total time spent in open arm in elevated plus maze



[a] $p<0.001$ as compared to control
[b] $p<0.05$ as compared to control
[c] $p<0.001$ as compared to diazepam (2 mg/kg)
[d] $p<0.05$ as compared to diazepam (2 mg/kg)

Figure 7: Mean total time spent in closed and open arms in elevated plus maze

In regards to total time spent in closed arms, it was least for the diazepam (2 mg/kg) (161.68 ±9.91) group followed by doxazosin (4 mg/kg) (180.73 ± 10.45) group and most for control (vehicle) (194.38 ±12.96) group. Intergroup difference was found to be significant ($p<0.001$). The difference in total time spent in closed arms was significant ($p<0.05$) between control (vehicle) (194.38 ±12.96) group and diazepam (2 mg/kg) (161.68 ±9.91) group, with diazepam (2 mg/kg) (161.68 ±9.91) group showing the most reduction in total time spent in closed arms. The difference in total time spent in closed arms was less significant ($p<0.05$) between doxazosin (4 mg/kg) (180.73±10.45) group and diazepam (2 mg/kg) (161.68 ±9.91) group with doxazosin (4 mg/kg) (180.73 ± 10.45) group showing slight decrease in total time spent in closed arms indicating that doxazosin (4 mg/kg) may have some weak anxiolytic activity.

In regards to total time spent in open arms, it was most for the diazepam (2 mg/kg) (48.65±5.74) group followed by doxazosin (4 mg/kg) (36.87±3.58) group and least for control (vehicle) (31.82 ±4.19) group. Intergroup difference was found to be significant ($p<0.001$). The difference in total time spent in open arms was highly significant ($p<0.001$) between control (vehicle) (31.82 ±4.19) group and diazepam (2 mg/kg) (48.65 ±5.74) group, with diazepam (2 mg/kg) (48.65 ±5.74) group showing the most increase in total time spent in open arms. The difference in total time spent in open arms was highly significant ($p<0.001$) between doxazosin (4 mg/kg) (36.87 ±3.58) group and diazepam (2 mg/kg) (48.65 ±5.74) group with doxazosin (4 mg/kg) (36.87 ±3.58) group showing slight increase in total time spent in open arms indicating that doxazosin (4 mg/kg) may have some weak anxiolytic activity.

DISCUSSION

Globally, non-communicable diseases are impacting a sizable portion of the population and are becoming more common. Additionally, it might lead to a significant drop in life quality and an increase in mortality. Risk factors that lead to the same include unhealthy lifestyle choices, inactivity, and chronic stress. Subsequent symptoms including diabetes, hypertension, and obesity may then follow suit. Serious psychological problems like depression and anxiety may take a long time to manifest when chronic illnesses and ongoing stress are coupled. Anomalies pertaining to

the HPA axis, RAAS, and general sympathetic nervous system have also been linked to elevated stress.

Doxazosin has been used in combination with β-blockers, calcium channel antagonists, diuretics and angiotensin-converting enzyme (ACE) inhibitors in patients with hypertension that is not controlled with monotherapy.

Standard medications for conditions like anxiety & pain are expensive and can have major adverse effects like NSAID-induced erosive gastritis. Many of them only work to offer momentary comfort, and over time, they may cause a host of dangerous adverse effects. Drugs in the opioid class have the potential for abuse, dependency, and habituation. In light of the aforementioned considerations, it is imperative that we search for better, safer substitutes that are affordable, non-habit forming, and safe.

The dose and dosage form chosen for the administration of doxazosin was as per previous studies, Doxazosin (4 mg/kg) [28] was first solubilized in DMSO and then administered intraperitoneally through injection after diluting in normal saline.

Current study comprised a total number of 36 female Swiss albino mice randomly divided into 6 groups with 6 mice in each group. All the animals were allowed to get acclimatized to the new environment for 2 weeks. Groups 1 to 3 were used to assess the effect of doxazosin on analgesic activity and Groups 4 to 6 were used to assess the effect of doxazosin on anxiolytic activity.

Pain is defined as a distressing experience associated that can be associated with actual or potential tissue damage with emotional, sensory, cognitive and social components. The free nerve endings also known as nociceptors have the ability to respond to extremes of temperature and chemicals that are released by damaging cells.

In our study, we have used the method of Eddy and Leimbach (1953) [29] and measured the increase in reaction time that occurs after giving drugs having analgesic properties at various time intervals. This particular method of assessment of pain by the help of thermal stimulus applied to the paws of rodents by using hot plate is very sensitive as well as specific for the screening of

drugs that are thought to possess central analgesic activity. These responses are not demonstrated in peripherally acting analgesics.

Tramadol used as a standard drug, is known to act as a centrally acting analgesic. The mechanism of how it acts as an analgesic has been attributed to be associated to its activity on both opioid related (mu) and non-opioid activity. It inhibits the reuptake of norepinephrine and serotonin and it is only partially antagonized by naloxone. This study was in support to the study performed by the other research investigator R. N. Suresha et al., where decrease in number of writhes, the delay in reaction time in tail clip and Eddy's hot plate method denoted the analgesic activity. Perindopril decreased the number of writhes, delayed the reaction time in tail clip and Eddy's hot plate method considerably when compared with control (normal saline), but less when compared with standard (pentazocine) [30].

In the present study, the increase in reaction time was observed with doxazosin at 30, 60, 90 and 120 min and with standard drug tramadol at 30, 60, 90 and 120 min which was statistically significant ($p<0.001$) as compared with the control group. The analgesic effect with doxazosin was lower as compared with the tramadol group. (Table 2 and Figure 4). Intergroup differences were significant at 30, 60, 90 and 120 mins following administration of drugs. The effect of doxazosin was less pronounced as compared to tramadol and the order of increase in analgesic reaction time was Group 3 > Group 2 > Group 1.

Anxiety is encountered very commonly in everyday life. It is the feeling of fear about any impending danger which is usually out of proportion and unusual than normal and is commonly accompanied by physical symptoms. It interferes with daily work of the patient, alters sleep and hampers the quality of life.

The number of entries in closed arms and total time spent in closed arms was least in diazepam group, followed by doxazosin group and most was for control group. The difference between doxazosin group and diazepam group was statistically significant ($p<0.001$) and total number of entries and total time spent in closed arms was decreased in doxazosin group but the decrease was not as much as in the diazepam group.

The number of entries in open arms and total time spent in open arms was most in diazepam group, followed by doxazosin group and least was for control group. The difference between doxazosin group and diazepam group was statistically significant ($p<0.001$) and total number of

entries and total time spent in open arms was increased in doxazosin group but the increase was not as much as in the diazepam group.

It is a well known fact that diazepam acts by GABA facilitatory effect on GABA receptors and hence lead to anxiolysis.

In the current study Doxasozin showed action as a weak anxiolytic and analgesic. It's effects in these parameters was not as significant as the standard drugs of the respective groups but it had a modulatory action on both parameters of pain and anxiety.

In terms of analgesic effect, the best effect was present in tramadol which was the standard drug, followed by doxazosin. It can be inferred from the findings that the test drug may exhibit anti-nociceptive behaviour by both central and peripheral mechanisms but it was weaker at best.

When we take the anti-anxiety effect into consideration, doxazosin was effective in reducing anxiety, however the result was less significant than the standard drug diazepam. Doxazosin (4 mg/kg) was not able to decrease any parameter more than the respective standard drugs of the above areas of research but in one instance where it shined was that it was seen in this study that it had an impact on all the above areas and can be used to as a treatment modality against cases that present with co-existence of pain and anxiety, after further testing in the future.

Anxiety is a common issue at present, especially since the novel coronavirus disease (Covid-19) pandemic has increased its levels one step further worldwide [33]. In the central nervous system, neurotransmitters responsible for the occurrence of symptoms of anxiety disorders include norepinephrine, serotonin, dopamine and gamma-aminobutyric acid (GABA) .

Anxiety disorders are among the most common mental, emotional, and behavioral problems and affect one-eighth of the total population worldwide and they have a substantial negative impact on the quality of life [35]. Hence treatment of anxiety is an important area of research interest in psychopharmacology.

CONCLUSION

The main purposes of anxiolytic medications are to induce sleep (sedative-hypnotics) or to promote serenity (anxiolytics). Although doxazosin was found to have a decent antianxiety and analgesic impact, the results were not as significant as those of the standard medicine, diazepam

and tramadol leading to suggestions that doxazosin may have some antianxiety and analgesic effects. When looking at doxazosin's anti-anxiety effects, it was discovered to be helpful in reducing anxiety; nevertheless, the difference in results was not as great as that of diazepam, the standard medicine as Doxazosin works by blocking certain chemicals, which helps to widen blood vessels and relax muscles.

Declarations:

Conflicts of interest: There is not any conflict of interest associated with this study

Consent to participate: There is consent to participate.

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