

# Potential role of Thiocolchicoside in anxiety disorder: A pre-clinical study

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## ABSTRACT

**Aim:** The aim of the current study is to evaluate anti-anxiety and potentiating effect of Thiocolchicoside in animal models of anxiety.

**Methodology:** A total of 24 (n=24) Swiss albino mice were procured, and they were divided into four groups of six mice in each. First group of mice (control) received 10 ml/kg-Normal Saline, second group (standard) received 2.0 mg/kg-Diazepam, test-1 received 1 mg/kg-Thiocolchicoside and test-2 received Thiocolchicoside (1mg/kg) + Diazepam (2mg/kg) for seven days per orally. All the mice were evaluated for anti-anxiety activity by Elevated Plus Maze (EPM) 60 minutes after the oral drug administration of drugs on day 1, 3 and 7 and later after a washout period of one month, same four groups of mice were screened by Light and Dark Arena (LDA) model after receiving respective drugs.

**Results:** One-way ANOVA followed by Tukey's Kramer test were applied for inter group comparison and correlation test for intra-group comparison. Results are expressed in mean  $\pm$  SEM. In EPM, time spent in open arm for the control, standard, test-1 and test-2 were 64.5 $\pm$ 25.81, 128.33 $\pm$ 17.6, 138 $\pm$ 10.56 and 168.33 $\pm$ 22.35 seconds respectively. There is statistically significant difference between standard (P=0.05), test-1 (P=0.05) and test-2 (P=0.03) when compared with control group. Similarly, time spent in closed arm for the control, standard, test-1 and test-2 were 238.83 $\pm$ 20.41, 171.67 $\pm$  17.6, 162 $\pm$ 10.56 and 131.67 $\pm$ 22.35 seconds respectively. In this, test-2 is statistically significant from the control group (P=0.05). The number of entries in the open arm for the control group, standard group, test-1 and test-2 were 3.5 $\pm$ 1.64, 13.17 $\pm$ 7.44, 21  $\pm$  4.05 and 13.33  $\pm$  2.16 respectively. There is statistically significant difference between standard (P=0.03), test-1 (P=0.02) and test-2 (P=0.03) with control. Similarly, number of entries to closed arm for the control, standard, test-1 and test-2 were 9.5 $\pm$ 3.62, 16.33  $\pm$  5.65, 16.33  $\pm$  4.23 and 8.17  $\pm$  1.72 respectively. The values obtained for the standard, test-1 and test-2 were not statistically significant (P=0.8). In LDA, time spent in light arena for the control, standard, test-1 and test-2 were 106.83 $\pm$ 18.21, 163.5 $\pm$ 21.66, 105.33  $\pm$

11.57 and 125.17  $\pm$  16.35 seconds respectively. Statistically significant difference between the control and the standard group (P=0.05) is noted. Time spent in dark arena for the control, standard, test-1 and test-2 were 193.17 $\pm$ 18.21, 136.5 $\pm$ 11.66, 194.67 $\pm$ 15.57 and 174.83 $\pm$ 16.35 seconds respectively. Here only standard group is statistically significant when compared with control (P=0.05). The number of entries in the light arena for the control, standard, test-1 and test-2 were 11.67  $\pm$  1.37, 13.17  $\pm$  2.48, 12  $\pm$  2.61 and 11.67  $\pm$  1.03 respectively. The number of entries in the dark arena for the control, standard, test-1 and test-2 were 12.17  $\pm$  1.47, 13.17  $\pm$  2.93, 11.83  $\pm$  2.23 and 11  $\pm$  0.89 respectively. With regard to number of entries in the light and dark arena there was no statistical significant difference between the groups (P>0.05).

**Conclusion:** The study result clearly showed that Thiocolchicoside (1 mg/kg) has anti anxiety and additional potentiating effect when combined with diazepam in EPM and LDA models.

**KEYWORDS:** Thiocolchicoside, Diazepam, Elevated Plus Maze, Light and Dark Arena

## INTRODUCTION

Anxiety is a normal human emotion that serves an adaptive function from a psycho biological perspective. The distinction between a 'pathological' and a 'normal' state of anxiety is not clear cut but represents the point at which the symptoms interfere with normal productive activities.<sup>[1]</sup> The principal components of anxiety are psychological symptoms like tension, fear, difficulty in concentration, apprehension and somatic like tachycardia, hyperventilation, shortness of breath, palpitations, tremor, sweating. Stress, fear and anxiety all interact with each other.<sup>[2]</sup> The treatment of anxiety disorder generally involves psychological approaches as well as pharmacotherapy. Over the last decade the drug treatment of anxiety has changed from using traditional anxiolytic/hypnotic agents (i.e. benzodiazepines and barbiturates) to using a range of drugs that are also used to treat other central nervous system (CNS) disorders (e.g. antidepressant, antiepileptic

and antipsychotic) or 5 hydroxytryptamine (5-HT)<sub>1A</sub> receptor agonists (e.g. buspirone) that have no hypnotic effect.<sup>[3]</sup> Benzodiazepines, while being effective anxiolytic drugs, have the disadvantages of producing unwanted side effects such as amnesia, sedation, inducing tolerance and physical dependence. They are also ineffective in treating any depression that may coexist with anxiety. Antidepressants and Buspirone require three or more weeks to show therapeutic effect and must be taken continuously. Selective Serotonin Reuptake Inhibitor (SSRI) are being used in many chronic cases, but needs a minimum period for it to be effective. SSRI are also associated with adverse effects like agitation, restlessness, rigidity, hyperthermia, delirium, sweating, twitching and convulsions.<sup>[4]</sup> Therefore, there is a need of alternative drug to be discovered which is having less side effects. Thiocolchicoside is a natural derivative of colchicine and a semisynthetic derivative of the naturally occurring colchicoside extracted from the seeds of *Gloriosa superba* (Liliaceae family). This medicinal plant has been used as a traditional medicinal herb to cure various diseases in Africa and Southeast Asia. The tuberous roots of *Gloriosa superba* are commonly used to cure snakebites, skin diseases and ulcers, or to treat inflammation. Thiocolchicoside is currently used as a centrally acting muscle relaxant. In addition, it also has anti-inflammatory and analgesic actions.<sup>[5]</sup> While the compound has been in use since many years in the European countries, the first formulation containing Thiocolchicoside was approved in India in the year 2008. Being less sedating than other centrally acting muscle relaxants, Thiocolchicoside is commonly used in the treatment of symptomatic spasms and contractures in muscular, rheumatic and neurologic disorders. Thiocolchicoside shows affinity for inhibitory gamma-aminobutyric acid (GABA) and glycinergic receptors. It has an agonistic action at the spinal-strychnine-sensitive receptors which can be the cause for its myorelaxant effect. Since, Thiocolchicoside has affinity on GABA receptors; the present research activity is carried out to evaluate anti-anxiety and potentiating activity of Thiocolchicoside in Swiss albino mice.

## MATERIALS AND METHODS

The present study was conducted after the approval from the Institutional Animal Ethics Committee (Letter No: YU/IAEC/4/2019 dated 28/01/2019). A total of 24 (n=24) adult Swiss Albino mice (3-4 months old) of either sex weighing 25-30 grams having healthy and normal behavior were included in this study. The study was conducted according to CPCSEA guidelines.

Mice were divided into four groups of six mice in each. First group of mice (control) received 10 ml/kg-Normal Saline, second group (standard) received 2.0 mg/kg-Diazepam, test-1 received 1 mg/kg-Thiocolchicoside and test-2 received Thiocolchicoside (1mg/kg) + Diazepam (2mg/kg) for seven days per orally. All the mice were evaluated for their anti-anxiety activity by using Elevated Plus

Maze (EPM) 60 minutes after the oral drug administration of drugs on day 1, 3 and 7 and later after a washout period of one month, same four groups of mice were screened by using Light and Dark Arena (LDA) model after receiving respective drugs.<sup>[6, 7]</sup>

Drugs like, Normal saline was purchased from institutional pharmacy and used as control at the dose of 10 ml/kg. Diazepam 5 mg tablets (Standard) and Thiocolchicoside 8mg tablets (Test drug) were purchased from institutional pharmacy. Dose of Thiocolchicoside in mice was determined by using transforming factor for mice by using adult human dose (8mg). The transforming factor for mice from adult human is 0.026. Hence, dose for 20g mice =  $8 \times 0.026 = 0.02$  mg. Therefore, dose for mice was set as  $0.02/20 = 0.001$  mg/g of mice or 1mg/kg of mice.

## RESULT

Each mouse was placed in the open arm/light chamber and observed for 5 minutes. During the test period, the time spent in open arm/light chamber and closed arm/dark chamber and numbers of entries to open arm/light chamber and closed arm/dark chamber were recorded. Tables 1 and 2 shows effects of various drugs on mice behavior in EPM and LDA models respectively.

[Table 1 about here.]

[Table 2 about here.]

## DISCUSSION

Anxiety and stress are common psychiatric manifestations in the modern world that affects approximately one-eighth of the world population. But when anxiety starts interfering with daily activities it is called an anxiety disorder. In this present study, anxiolytic effect of Thiocolchicoside was compared with control and standard drug (Diazepam) using EPM model with regard to time spent in open and closed arms and number of entries to open and closed arms on day 1, 3 and 7. Increase in the time spent and frequencies of the entries into the open arms in the EPM model had been confirmed as a potent sign of an anxiolytic agent.

In EPM, behaviours such as time spent in the open/close arm, number of entries in open and closed arm (observed in 5 minutes) were used to assess the level of anxiety in mice. A decrease in anxiety-like behavior characterized by reduced frequency of entry into the close arm, increased frequency of entry into open arm entry, in the Test-1 implies the anxiolytic effect of Thiocolchicoside. In Test -2 increase in the time spent in open arm, decrease in the time spent in closed arm and increase in the number of entries in open arm suggest that Thiocolchicoside is having potentiating effect with the

standard drug Diazepam. So Thiocolchicoside can be used as mono therapy as well as an add on drug with the standard drug like diazepam. There is also an increase in the time spent in open arm, decrease in the time spent in closed arm and increase in the number of entries in open arm in case of standard drug Diazepam confirming its anti anxiety property. An increase in the duration of the entries into the open arms is regarded as a powerful marker for an anxiolytic effect.

In the light and dark arena test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions in the light arena and the time spent in the light arena where in increase in these parameters is considered to reflect anxiolytic-like properties. Our results showed that Test-1 has increased time spent in the light arena, suggesting its anxiolytic action. Test-2 showed increase in time spent in the light arena as well as decrease in time spent in dark arena suggests that thiocolchicoside is having potentiating effect with the standard drug Diazepam. Diazepam being standard drug, showed increase in time spent in the light arena as well as decrease in time spent in dark arena confirming its anxiolytic property. Many mechanisms on neurochemical basis are attributed to the cause of anxiety. Literature review suggest that low levels of GABA and involvement of neurotransmitters like noradrenergic, serotonergic, glutaminergic neurons and peptide such as NPY, CCK play a crucial role in causing anxiety. Previous studies shown that oxidative stress- induced damage to the serotonergic and noradrenergic nervous system is one of the contributing factor in causing anxiety which can be correlated to the protective action of antioxidants which has a potential anxiolytic effect.<sup>[8]</sup> It is possible that the chemical components with antioxidant activities play essential roles in the anxiolytic properties as thiocolchicoside as observed in the study. This is in consonance with a report related to the anxiolytic effect of plant extracts.<sup>[9]</sup>

## CONCLUSION

Results of our study showed that Thiocolchicoside is having anxiolytic property at the dose of 1mg/kg in mice in both EPM and LDA models. Moreover, Thiocolchicoside also showed potentiating anti-anxiety effect when combined with diazepam. Hence, Thiocolchicoside can be used as a mono therapy or as an add on drug in the management of

anxiety. However, further preclinical and clinical investigations are needed to elucidate its pharmacokinetic, pharmacodynamic and long term effects in anxiety disorders.

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Group	DAY 1				DAY 3				DAY 7			
	Time spent in		No of entries		Time spent in		No of entries		Time spent in		No of entries	
	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm
Control (NS 10 ml/kg)	64.5 ± 25.81	238.83 ± 20.41	3.5 ± 1.64	9.5 ± 3.62	94.83 ± 29.3	205.17 ± 29.3	10.67 ± 5.35	11 ± 3.63	100 ± 22.97	199 ± 22.97	10.17 ± 3.49	13.67 ± 3.08
Standard (Diazepam 2mg/kg)	128.33 ± 17.6*	171.67 ± 17.6	13.17 ± 7.44*	16.33 ± 5.65	134.83 ± 23.88	165.17 ± 13.88	18.67 ± 5.13*	18.83 ± 3.49	172.17 ± 18.33*	127.83 ± 18.33*	17.83 ± 2.86*	12.83 ± 4.45
Test-1 Thiocolchicoside (1mg/kg)	138 ± 10.56*	162 ± 10.56	21 ± 4.05*	16.33 ± 4.23	170.67 ± 13.2*	129.67 ± 17.75*	18.17 ± 4.22*	14.67 ± 6.12	161.33 ± 20.12	138.33 ± 19.58	14 ± 2.76	11.17 ± 2.64
Test -2 (Diazepam 2mg/kg + Thiocolchicoside 1mg/kg)	168.33 ± 22.35*	131.67 ± 22.35*	13.33 ± 2.16*	8.17 ± 1.72	161.83 ± 12.7*	138.17 ± 12.7*	18.83 ± 2.93*	13.17 ± 2.64	167.17 ± 25.65	132.83 ± 25.65	17 ± 4.05*	11.17 ± 2.64

N=6; Mean ± SEM, \* P ≤ 0.05 on comparing Standard, Test group 1 & 2 with control, One-way ANOVA followed by Tukey's Kramer test for intergroup comparison and correlation test for intragroup comparison.

**Table 1: Effect of various drugs on mice behavior in EPM model**

Group	DAY 1				DAY 3				DAY 7			
	Time spent in		No of entries		Time spent in		No of entries		Time spent in		No of entries	
	Light area	Dark area	Light area	Dark area	Light area	Dark area	Light area	Dark area	Light area	Dark area	Light area	Dark area
Control (NS 10 ml/kg)	106.83 ± 18.21	193.17 ± 18.21	11.67 ± 1.37	12.17 ± 1.47	63.17 ± 19.51	236.83 ± 19.51	10.83 ± 1.47	11.17 ± 0.98	105.17 ± 16.61	194.5 ± 17.05	9.67 ± 3.78	10 ± 3.63
Standard (Diazepam 2mg/kg)	163.5 ± 21.66*	136.5 ± 11.66*	13.17 ± 2.48	13.17 ± 2.93	123.5 ± 11.09*	176.5 ± 21.09	15.17 ± 1.94	14.67 ± 1.97	148.67 ± 13.32	151.33 ± 13.32	14.67 ± 3.78	14.33 ± 3.33
Test-1 Thio-colchico-side (1mg/kg)	105.33 ± 11.57	194.67 ± 15.57	12 ± 2.61	11.83 ± 2.23	146.5 ± 10.49*	153.5 ± 20.49	15.33 ± 1.63	14.83 ± 1.33	165.67 ± 12.41*	134.33 ± 12.41	14 ± 3.63	13.67 ± 3.83
Test -2 (Diazepam 2mg/kg + Thio-colchico-side 1mg/kg)	125.17 ± 16.35	174.83 ± 16.35	11.67 ± 1.03	11 ± 0.89	156.83 ± 18.01*	143.17 ± 18.01*	15.83 ± 1.47	15.5 ± 2.07	157.5 ± 16.69	142.5 ± 16.69	15.83 ± 2.64	15.33 ± 3.08

N=6; Mean ± SEM, \* P ≤ 0.05 on comparing Standard, Test group 1 & 2 with control, One-way ANOVA followed by Tukey's Kramer test for intergroup comparison and correlation test for intragroup comparison

**Table 2: Effect various drugs on mice behavior in LDA model**