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Pharmacological exploration of anti-arthritic potential of terbutaline through *in-vitro* and *in-vivo* experimental models

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Abstract: Terbutaline have been reported to have anti-inflammatory activity. Present study aimed to check the anti-arthritic activity of terbutaline. The drug was tested using *in vitro* models (bovine serum albumin denaturation, egg albumin denaturation and HRBC membrane stabilization) and *in vivo* (formaldehyde induced arthritis). Results of bovine serum albumin denaturation assay illustrated that terbutaline inhibited 89.54±0.46% denaturation at 6400µg/ml concentration. Terbutaline resulted in dose dependent impediment of protein denaturation in egg albumin denaturation assay with 74.40±0.72% inhibition at concentration of 6400µg/ml. Terbutaline also showed protection of HRBC membrane against hypotonic stress in a dose dependent manner, with maximum 76.45±0.62% prevention at 6400µg/ml concentration. Results of formaldehyde induced arthritis model showed that paw volume was significantly declined by terbutaline with maximum percentage inhibition at 10th day of study period which implies immune inhibitory potential of terbutaline. Findings of present study concluded that terbutaline has arthritis reducing potential possible through inhibitory effects on synthesis and release of inflammatory mediators as well as limiting the formation of autoantigen. Thus, terbutaline might be the potential candidate for use in treatment of arthritis.

Keywords: Rheumatoid arthritis, terbutaline, formaldehyde, bovine serum albumin.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, and progressive inflammatory disorder that affects the synovial joints, that accounts for the deformity, disability, synovial proliferation and fibrosis (Choudhary *et al.*, 2018; Mishra *et al.*, 2011). Arthritis is one of the most common musculoskeletal inflammatory disease involving declined quality of life when compared to patients suffering from other Non-communicable disease (NCDs) (Rudan *et al.*, 2015).

Worldwide prevalence rate of rheumatoid arthritis is believed to be 1% with notable variations among different population groups (Silva-Fernández *et al.*, 2020). The therapies employed currently to treat rheumatoid arthritis comprised of non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and biological response modifiers such as antibodies. Prolonged use of anti-arthritic drugs is found associated with various adverse drug reactions like GI bleeding, hepatotoxicity, osteoporosis, immuno-suppression, and recurrent infectious disease (Gutiérrez-Rebolledo *et al.*, 2018; Uttra *et al.*, 2019). Therefore, it is needed to explore more drugs having low cost and lesser adverse effects in order to gain favorable outcomes in remission and cure of rheumatoid arthritis.

Terbutaline is a short acting sympathomimetic drug used in the treatment of asthma. The mechanism of action of terbutaline involves stimulation of β-2-adrenergic receptors in lungs causing bronchial smooth muscle relaxation (Naik *et al.*, 2017). Terbutaline has found to be an effective drug in controlling inflammation of airways by suppressing the release of inflammatory cells and reducing respiratory resistance thereby enhancing the activity of mucosal cilia (Yu *et al.*, 2021). Terbutaline has shown pain reduction in women having primary dysmenorrhea, pain was relieved in all patients within one minute after injection of terbutaline (Åkerlund *et al.*, 1976). Terbutaline have also been reported for reduced pro-inflammatory activities and neutrophil recruitment in acute lung injury (Keränen *et al.*, 2016; Lu *et al.*, 2019). Current study employees the approach of drug re-purposing for terbutaline with aim to explore the anti-arthritic activity using *in-vivo* and *in-vitro* animal models

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MATERIALS AND METHODS

Drugs and chemicals

Formaldehyde, Bovine serum albumin, Sodium hydroxide, Sodium chloride, Dextrose, Ethanol and Piroxicam were obtained from Sigma Aldrich, USA. Disodium hydrogen phosphate and Sodium citrate were obtained from Merck Darmstadt, Germany. Potassium dihydrogen phosphate, Citric acid and Hydrochloric acid were obtained from Riedel-de Haen, Seelze, Germany while Terbutaline was obtained from Stands Pharma, Lahore, Pakistan.

Experimental animals and housing conditions

For *in-vivo* experiments, Sprague–Dawley rats of both sex weighing (150-250 g) were purchased and kept in animal house of College of Pharmacy, University of Sargodha. Animals were housed in cages at 12 hourly light and dark cycles and maintained at room temperature 23°C to 27°C.The animals were allowed *ad libitum* access to water and food. The experimental protocol was approved from ethical committee college of Pharmacy UOS.

Assessment of in-vitro anti-arthritic activity

Bovine Serum Albumin assay

The anti-arthritic activity of terbutaline was assessed through bovine serum albumin denaturation as adopted by (Alamgeer *et al.*, 2015). In this experiment, product control solution contained 0.05 ml of drug concentration and 0.45 ml distilled water. Test control solutions contained 0.05 ml distilled water and 0.45 ml of bovine serum albumin (5% aqueous solution). The test solutions comprised of 0.45 ml of bovine serum albumin and 0.05 ml of different drug concentrations (50-6400 µg/ml). Piroxicam served as standard drug. The solutions were maintained at pH 6.3. Samples were then incubated at 37°C for 20 minutes. After that temperature was raised to 57°C for 30 minutes. After cooling, 2.5 ml of phosphate buffer of pH 6.3 was added to each solution. After wards, at 660 nm absorbance was measured using spectrophotometer (UV-1700 UV-Visible Spectro-photometer, Shimadzu). The percentage of protein denaturation inhibition was calculated using formula as under:

$$\text{Percentage Inhibition} = \frac{100 - (\text{Absorbance of test solution} \;\; \text{Absorbance of product control})}{\text{Absorbance of test control}} \times 100$$

Egg albumin denaturation assay

The reaction mixture (5ml) was consist of 2.8ml of phosphate buffer (pH 6.4), 0.2ml of fresh egg albumin and 2ml of different drug concentrations (50- 6400 µg/ml). Control solution comprised phosphate buffer, egg albumin and distilled water instead of drug concentration. Piroxicam was used as standard drug. The reaction solutions were then incubated at temperature 37±2°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660nm (Mahnashi *et al.*, 2021). The percentage inhibition was then calculated by using the formula as under:

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Red blood cell (HRBC) membrane stabilization assay

The blood sample was taken from healthy human individual who had not taken NSAID at least 2 weeks prior to study. Blood was mixed with equal volume of Alsevier's solution and then centrifuged at 3000 rpm for 20 minutes. The packed cells were removed and washed three times with iso-saline solution (0.85%) and a 10% v/v suspension was made in iso-saline. The sample mixtures were contained 2ml of hypotonic saline, 1ml of phosphate buffer, 0.5ml of 10% of HRBC suspension & 1 ml different drug concentrations (50-6400µg/ml). Piroxicam served as standard drug. Control solution consisted of distilled water, Phosphate buffer and 10% HRBC. All samples were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. After that, absorbance of supernatant solution was measured at 560nm (Alamgeer *et al.*, 2017b). The percentage HRBC membrane stabilization was estimated by the formula:

$$\text{Percentage membrane stabilization} = 100 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Assessment of in-vivo anti-arthritic activity

Formaldehyde induced arthritic rats

Animals were divided into 5 groups with five rats in each group. Group I was served as arthritic control and received normal saline (10ml/kg) orally, group II, III, IV were received terbutaline (5, 10, 20mg/kg oral dose respectively) and group VIII received standard drug piroxicam (10 mg/kg orally) for 10 days. Arthritis was induced by sub planter injection of 0.1ml of 2% formaldehyde solution on 1st day and was repeated on 3rd day. Arthritis was evaluated by measuring paw volume on 2nd , 4th, 6th, 8th and 10th days by using digital Plethysmometer (LE7500, Panlab Harvard Apparatus) (Alamgeer *et al.*, 2017a). Percentage inhibition of paw volume was calculated by formula:

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Vc = Paw volume of control Vt = Paw volume of treated

STATISTICAL ANALYSIS

The results were presented as mean ± SEM. Paw volume data were interpreted statistically by two-way ANOVA followed by Bonferroni post hoc test using Graph Pad prism 7. $p<0.05$ was considered significant.

RESULTS

Terbutaline exhibited dose dependent inhibition of protein denaturation

The inhibitory effects of terbutaline on protein denaturation are shown in fig. 1. Terbutaline exhibited dose dependent inhibition of protein denaturation that was comparable to standard drug piroxicam. The results of present study demonstrate that terbutaline produced a maximum anti-denaturation activity on BSA such as 89.54±0.46% at 6400 µg/ml and these results were higher in comparison to standard drug piroxicam 88.64 ± 0.63 % at same concentration.

Anti-denaturation effect of terbutaline on egg albumin denaturation assay

The anti-denaturation effect of terbutaline was evaluated using egg albumin denaturation assay. The results summed in fig. 2 depicts that terbutaline resulted in dose dependent impediment of protein denaturation i.e. 25.68±0.24% at 50µg/ml that was exceedingly elevated to 74.40±0.72% at 6400µg/ml. Comparing the results with standard piroxcam implicit anti-denaturation activity of terbutaline throughout concentration range with elevated level at higher concentrations.

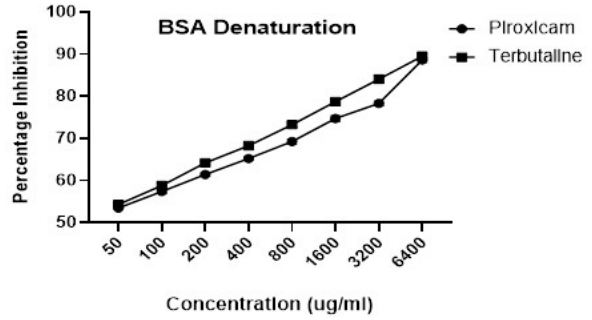

Fig. 1: Inhibitory effect of terbutaline on bovine serum albumin denaturation. Values are expressed as mean ± SEM (n=3).

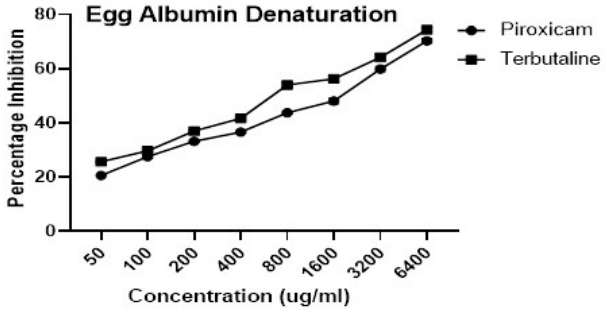

Fig. 2: Terbutaline inhibited denaturation of egg albumin. Values are expressed as mean ± SEM (n=3).

Terbutaline exhibited protection against RBC lysis in HRBC membrane stabilization method

In HRBC membrane stabilization method, terbutaline exhibited 76.45±0.62% protection against lysis of RBC at 6400µg/ml that was compared to 73.47±0.57% inhibition exhibited by standard drug Piroxicam at 6400µg/ml (fig. 3).

Terbutaline prevented formaldehyde induced arthritis

Figure 4 and table 1 summarizes the activity of terbutaline (5, 10, 20mg/kg) in comparison with standard drug (Piroxicam 10mg/kg) against formaldehyde induced arthritis in rats. On day 1st and 3rd, injection of formaldehyde in sub plantar region of right hand paw resulted in paw swelling of all the rats. Increment in paw volume tended to subside in a dose dependent manner in treatment groups. Maximum inhibitory effect was observed on 10th day of study period where terbutaline at dose of 5 mg/ kg, 10 mg/kg and 20 mg /kg caused notable reduction in paw edema by 40.79%, 42.26% and 62.88% respectively. Instead, treatment with standard drug (Piroxicam) at dose of 10mg/Kg presented 65.93% decline in paw volume after 10 days of treatment period (fig. 4 & table 1). These outcomes indicate that terbutaline manifested significant effects in inhibiting paw edema as compared to arthritic control.

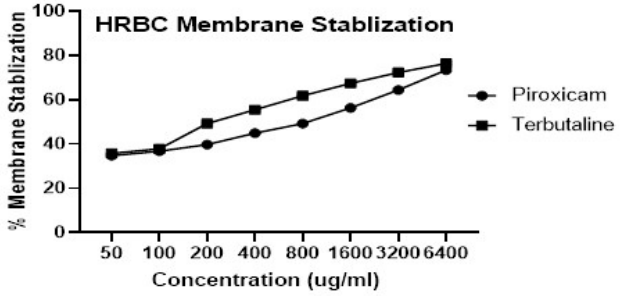

Fig. 3: Terbutaline causes stabilization of HRBC membrane. Values are expressed as mean ± SEM (n=3).

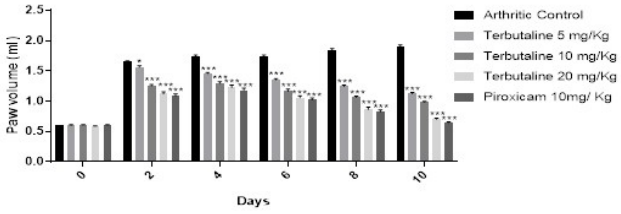

Fig. 4: Terbutaline inhibited paw volume in formaldehyde induced arthritis. Results were expressed as mean ± SEM (n=5). *p < 0.05, **p < 0.01, ***p < 0.001 compared to arthritic control analyzed by two-way ANOVA followed by Bonferroni post hoc test.

DISCUSSION

Current study was aimed to examine anti-arthritic potential of terbutaline using different *in vitro* and *in vivo* models. Denaturation of proteins is considered as one of the well documented cause of rheumatoid arthritis. Auto antigen production in various rheumatic diseases may be caused by *in vivo* denaturation of proteins. Various anti-inflammatory drugs have presented dose dependent effect

Table 1: Effect of terbutaline on percentage inhibition of paw edema against formaldehyde induced arthritis in rats

Day	Percentage inhibition			
	Terbutaline 5mg/Kg	Terbutaline 10mg/Kg	Terbutaline 20mg/Kg	Piroxicam 10mg/kg
2	18.30%	23.87%	31.88%	34.06%
4	16.28%	25.68%	29.47%	32.34%
6	22.33%	33.21%	39.29%	41.35%
8	32.28%	42.06%	52.82%	55.00%
10	40.79%	48.26%	62.88%	65.93%

to protect against thermally induced denaturation of proteins in different anti-inflammatory and anti-arthritic models (Qasim *et al.*, 2020). Present study employed egg albumin and BSA to carry out protein denaturation assay. Result of the present study makes it evident that terbutaline is capable of inhibiting the production of auto antigen because of *in vitro* protein denaturation inhibition in a dose dependent manner. This implies that terbutaline may possess disease reducing potentials for inflammation and arthritis. Exposure of red blood cells (RBC) to hypotonic stresses cause excessive fluid accumulation within the cells resulting in lysis of the membranes accompanied with hemolysis and hemoglobin oxidation (Mahnashi *et al.*, 2021). Damages to lysosomal membrane stimulate the release of lysosomal contents like phospholipase A2, proteases and other enzymes leading to hydrolysis of phospholipids and subsequent inflammatory responses (Alamgeer *et al.*, 2015; Mounnissamy *et al.*, 2007). Findings of current study highlighted that terbutaline protected against HRBC hemolysis in a dose dependent manner. These results imply significant potential for membrane stabilization and possible anti-inflammatory effect of terbutaline. Formaldehyde induced arthritis is an extensively used model for non-immunological acute arthritis that is employed for investigation of the anti-inflammatory and anti-arthritic potential of various drugs. Inoculation of formaldehyde in hind paw of rats provokes a localized bi-phasic inflammatory reaction. The early neurogenic phase involves release of substance P whereas the late inflammatory phase involves the discharge of histamine, bradykinin, serotonin and prostaglandins which produce a striking vasodilation and vascular permeability leading to edema formation and analgesic stimulation (Alamgeer *et al.*, 2017b). The results obtained as shown in fig. 1 and table 4 indicate that terbutaline at doses of 5mg/kg, 10 mg/kg & 20mg/kg has significantly declined the paw edema when compared to arthritic control. Result of the present study showed that terbutaline (20mg/kg) significantly subdued the paw edema induced in formaldehyde injected paws. This decline in inflammation by treatment of terbutaline might be due to inhibition of protein denaturation and decreased release of inflammatory mediators like prostaglandins, histamine and serotonin that are responsible for inflammation (Uttra *et al.*, 2019). Terbutaline inhalation has been previously shown to improve lung liquid clearance by decreasing levels of inflammatory cytokines TNF-α and IL-1β in endotoxin induced lung injury model of neonatal rats. Both TNF-α and IL-1β have shown to possess potential role in inflammation (Lu *et al.*, 2019).

CONCLUSION

Keeping in mind the above discussion, it is concluded that terbutaline possess remarkable anti-inflammatory and anti-arthritic potential as expressed by results of *in-vitro* and *in-vivo* models. Terbutaline presented these results by increased inhibition of protein denaturation and elevated HRBC membrane stabilization. Results of formaldehyde induced arthritis model depicted reduction in paw volume of rats. The current study suggests that terbutaline may have significant usefulness in treatment of rheumatoid arthritis, however, further detailed studies may unveil more precise mechanisms and proven efficacies.

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