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Dose Dependent Effects of Sodium Nitroprusside on Smooth Muscle Mechanics

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Abstract

***Background*:** Sodium nitroprusside (SNP) is a nitric oxide donor (NO) donor used as an antihypertensive agent in emergency. However, it has limited use now because of cyanide poisoning due to breakdown of SNP to NO and five cyanide radicals.

***Objective*:** To evaluate the dose-dependent effects of SNP on active tension (inotropic response) and rate of contraction (chronotropic response) in isolated rabbit intestinal smooth muscle.

***Study Design and Setting*:** Animal study in which experimental study design was used and study was conducted Physiology department of Karachi University.

***Methodology*:** The intestine was isolated from animal and mounted on organ bath assembly. Responses of varying incremental concentrations SNP (0.5 mM, 1.5 mM, 2.5 mM, 5.5 mM, 10.5 mM, 12.5 mM & 14.5 mM) with saline used as control, were observed for two contractile parameters i.e. on active tension and rate of contraction, at 40 min time point. The recordings were analyzed using one way ANOVA followed by Bonferroni post hoc test and student paired t-test.

***Results*:** showed that the doses 0.5 and 1.5 mM. SNP had greater while the doses 2.5, 5.5, 10.5, 12.5 and 14.5 mM caused significant decline in inotropic response compared with control. There was a decline in chronotropic response in a dose dependent manner, albeit insignificant while maximum change of relaxation noted at1.5mM concentration when compared with saline.

***Conclusion*:** SNP produced a dose-dependent effect on isolated rabbit intestinal smooth muscle, with higher concentrations significantly reducing active tension. This overall smooth muscle relaxation response by SNP indicates that SNP is responsible for release of NO.

Keywords: Active tension, ionotropic, nitric oxide, smooth muscle contraction, sodium nitroprusside.

1. INTRODUCTION

Sodium nitroprusside (SNP) is a potent vasodilator that is used in hypertensive crisis and in pediatric vascular and cardiac surgeries [1]. It exerts its effect by releasing nitric oxide (NO) Nitric oxide (NO) is a small, highly reactive gaseous molecule recognized as a crucial biological mediator in various physiological processes, particularly in the nervous and cardiovascular systems. Initially identified as endothelium-derived relaxing factor (EDRF) in 1980, NO is known for its role in facilitating blood vessel dilation. NO mediates several processes such as endothelium-dependent vasorelaxation, platelet adhesion, and regulation of blood pressure. It also plays a role in modulating inflammatory responses [2] SNP binds to oxyhemoglobin, dissociates instantly and forms methemoglobin, thereby releasing nitric oxide (NO) and cyanide [3]. (SNP) primarily induces vasodilation by releasing nitric oxide (NO), which reduces calcium influx through L-type calcium channels, a key factor for muscle relaxation. The function of large-conductance calcium-activated potassium (BK) channels is complex; they can either inhibit or promote NO-induced vasodilation based on the level of contractility. When contractility is elevated, the activation of BK channels may enhance the vasodilatory effects of NO. Furthermore, protein kinase G (PKG), which is activated by cGMP produced from NO, because NO activates guanyl cyclase which produces cyclin GMP, resulting in calcium sequestration and inhibition of cellular contraction [4, 5]. In rat aorta, SNP inhibits smooth muscle contraction by inhibiting

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Potassium induced contraction and by decreasing calcium influx and release of cytosolic calcium from smooth muscle cells [6]. In addition to vascular smooth muscle, SNP also inhibits proliferation of smooth muscle of airway through the thrombin pathway [7].

In the gut, SNP either acts directly through guanyl cyclase or indirectly through cholinergic nerves by inhibiting release of acetylcholine which results in relaxation of colonic smooth muscles [8, 9]. Na^+/K^+ ATPase pump is essential for bidirectional transport of sodium and potassium. In addition to this, it is also involved in maintaining calcium ion concentration across sarcolemma, thus maintaining homeostasis of calcium ions in smooth muscles of the colon through Na^+/Ca^{2+} exchanger. Therefore, any defect in the Na^+/K^+ ATPase leads to partial depolarization of plasma membrane of smooth muscle, leading to disturbance of ionic balance, tone and osmoregulation [10].

Gastrointestinal system contains neurons through which enteric system connects to central nervous system. It mainly comprises of extrinsic nerve fibers, the submucosal cells, myenteric plexus and the interstitial cells of Cajal (ICC). (ICC) are located throughout the gastrointestinal (GI) tract and are primarily found at the myenteric and submucosal borders of the circular muscle layer. These specialized ICC are responsible for generating slow waves (SWs) in non-sphincter muscles. Slow wave (SW) activity involves the electrophysiological processes that facilitate phasic contractions in the gastrointestinal (GI) tract. Both SWs and phasic contractions rely on Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels, highlighting the importance of regulating Ca^{2+} entry for maintaining tone in the gut [11]. The motor function of the gastrointestinal tract (GIT) depends on a careful balance of excitatory and inhibitory neurotransmission, mainly facilitated by cholinergic and adrenergic mechanisms. Furthermore, nitric oxide (NO), a vital inhibitory neurotransmitter produced by nitric oxide synthase (NOS), plays a significant role in regulating GIT motility [12]. Neurons that release NO are present throughout the gastrointestinal tract but are more concentrated in the duodenum, ileum and caecum, thus making these segments ideal for research [13].

Although, the effect of SNP on enteric system up to 5mins following administration of SNP have been conducted, there is scarcity of data regarding dose dependent prolonged effect of SNP on the gut. Hence, the aim of this study was to investigate the effect of different gradations of SNP dosing (0 – 14.5 mM) on smooth muscle activity of the rabbit gut (ilium) at multiple time points between 5 and 40 min.

2. METHODOLOGY

This study was carried out on Animalia Rabbit from class Mammalia of kingdom Animalia and order lagomorpha. Animal was purchased from the local market. The animal was received care under guidelines given by International Centre for Chemical and Biological Sciences (ICCBS). Approval was taken from institutional bioethical committee, university of Karachi with ERC # IBC KU-405/2024.

Sodium nitroprusside (SNP), Potassium chloride (KCL), Acetylcholine (Ach). Krebs buffer solution was used in experiment with pH =7.4 and temp = 37℃ (Sodium Chloride (NaCl) 118 mM, KC1 4.80 mM, Calcium Chloride ($CaC1_2$) 2.50 mM, Magnesium Sulphate ($MgSO_4$) 1.20 mM, Sodium Bicarbonate ($NaHCO^{-3}$) 27.20 mM, Potassium dihydrogen phosphate ($KH2PO_4$) 1.00 mM and glucose 200 mg%). The solutions were saturated with 95% O_2 and 5% CO_2 mixture. All Chemicals used in the experiments were obtained from Merck (Germany).

Single animal (rabbit) weighing 1 to 1.7 kg were used in our experiments [13]. After animals were sacrificed, a clean incision was made to visualize the abdominal cavity, and small intestine was isolated and dissected. Ilium was washed and placed in Krebs buffer for oxygenation. Ilium smooth muscle preparations were mounted on an organ bath assembly composed of gut bath containing gut tube and glass coil. Gut bath was filled with distilled water and maintained at 37℃ by the help of thermostat placed inside the gut bath. A 10 ml gut tube was fixed inside the gut bath and oxygen tube was inserted inside the gut tube with the help of clamps. The proximal end of intestinal strip was attached to a hook on the oxygen tube while the distil end of strip was connected to an isometric transducer compatible with the power Lab. In our experiment approximately 2 cm length of intestinal strip was used.

Experiment was started by adjusting the baseline of recordings at zero. After fixation of the gut tube muscle strip, the tissue was stabilized for 30-45 mins. After stabilization, when intestine showed normal activity, contractures were induced using ACh and KCl and muscle recordings were noted. SNP was then infused in the gut tube muscle strip and after 40 mins, contracture was induced again. There were 8 recordings noted every 5mins for a total of 40 mins.

For induction of contracture, 50 mM KCl was infused in gut tube for 30 seconds, followed by washing of the tube and refilling with normal Krebs solution. After stabilization of the strip (20 mins), 1 uM of ACh was added for 30 sec. Strip was again washed 2 to 3 times and

Table 1: Dose dependent response of nitroprusside on active tension.

Drug Concentration (mM)		Active Tension (% initial)							
		5 (Min)	10 (Min)	15 (Min)	20 (Min)	25 (Min)	30 (Min)	35 (Min)	40 (Min)
0	N+SEM	94.4 ± 2.3	95.7 ± 6.7	94.4 ± 8.9	92.4 ± 3.7	89.1 ± 4.7	95.0 ± 5.1	92.3 ± 5.8	95.1 ± 5.1
0.5	N+SEM	106.8 ± 13.0	124.0 ± 15.6	119.6 ± 18.7	122.7 ± 21.0	113.9 ± 12.7	114.0 ± 14.5	113.2 ± 14.0	112.1 ± 15. 5
	p	1.000	0.819	1.000	1.000	1.000	1.000	1.000	1.000
1.5	N+SEM	105.6 ± 12.3	101.0 ± 10.5	115.9 ± 12.8	124.0 ± 17.4	110.1 ± 9.6	112.7 ± 9.8	103.7 ± 12.9	106.7 ± 6.6
	p	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2.5	N+SEM	94.1 ± 7.6	77.4 ± 6.2	66.8 ± 3.7	69.3 ± 3.2	69.56 ± 8.5	71.4 ± 9.5	65.2 ± 10.7	60.1 ± 12.7
	p	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
5.5	N+SEM	43.0 ± 7.2	46.7 ± 8.9	38.1 ± 3.5	40.7 ± 4.4	41.8 ± 4.0	43.0 ± 3.7	42.1 ± 3.8	46.5 ± 7.5
	p	***0.004***	***0.015***	***0.034***	0.151	***0.048***	***0.035***	0.087	0.145
10.5	N+SEM	53.7 ± 4.7	58.1 ± 8.8	73.7 ± 17.9	72.7 ± 18.4	77.6 ± 18.4	82.0 ± 19.1	67.0 ± 19.9	51.6 ± 21.3
	p	***0.041***	0.146	1.000	1.000	1.000	1.000	1.000	0.310
12.5	N+SEM	21.0 ± 3.2	14.0 ± 1.9	14.0 ± 1.9	13.4 ± 3.4	13.7 ± 3.4	10.3 ± 2.2	11.2 ± 2.9	8.0 ± 1.9
	p	***<0.001***	***<0.001***	***0.001***	***0.003***	***<0.001***	***<0.001***	***0.001***	***<0.001***
14.5	N+SEM	12.3 ± 3.9	10.4 ± 12.1	9.2 ± 7.5	10.0 ± 6.0	9.1 ± 12.3	9.5 ± 18.6	8.1 ± 5.2	8.9 ± 5.6
	p	***<0.001***	***<0.001***	***<0.001***	***0.002***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

Means have been compared using ANOVA followed by Bonferroni's post hoc analysis.
n=4 for each reading p-value<0.05 is significant, p-value compared to control (0 mM)

allowed to stabilize further. After stabilization of muscle strip, Sodium nitroprusside was added and its effect was observed on force (Inotropic) and rate of muscle contraction (Chronotropic) for 40 mins. The SNP experiments were performed by using eight different concentrations (0.5, 1.5, 2.5, 5.5, 10.5, 12.5, 14.5 mM). For each concentration of SNP, new strips were used and the gut tube was washed with distilled water.

The results of experiments are expressed as means ± SEM of at least eight observation using muscle preparations from rabbit ileum. Statistical significance was assessed by one way ANOVA followed by Bonferroni's post hoc test while rate of relaxation evaluate by student paired t-test $p<0.05$ was considered significant throughout the study.

3. RESULTS

Different gradations of sodium nitroprusside between 0 - 14.5 mM were tested. All readings were compared to control which was set at 0 mM and 4 readings were noted for each recording.

3.1. Dose Dependent Response of Sodium Nitroprusside on Active Tension

There was a significant decline in active tension at a dose of 5.5 mM at 5, 10, 15, 25 and 30 mins. At 10 mM, there was a decline noted at 5 mins but there was no significant difference noted when compared to control at and after 10 mins. At gradations at and above 12.5 mM, there was

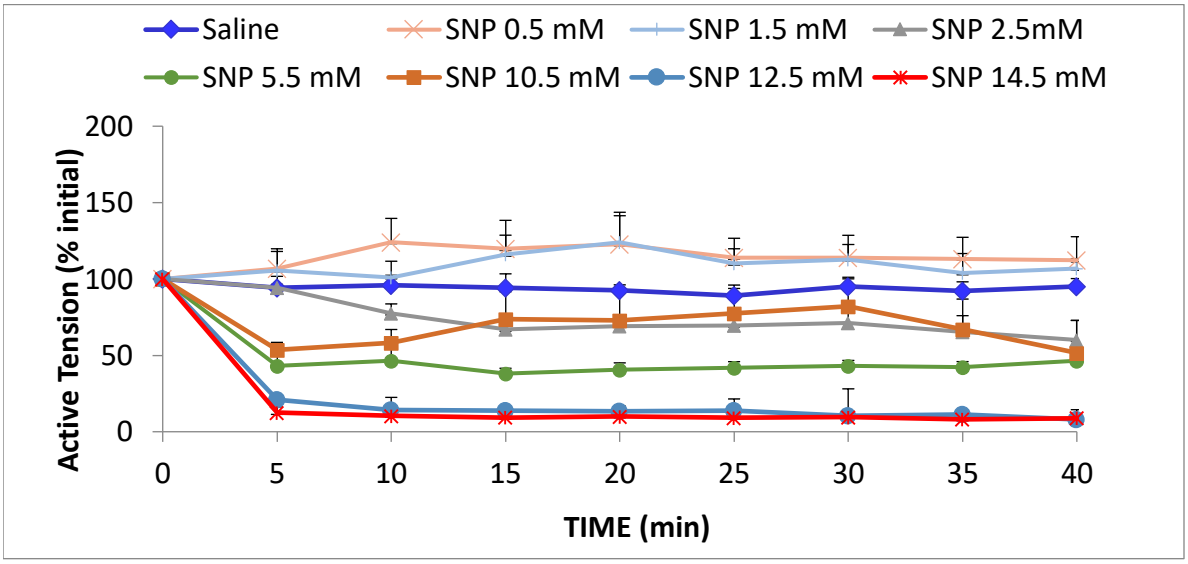


Figure 1: demonstrates the dose dependent effect of Sodium nitroprusside at 5min intervals over 40 mins on active tension of isolated Rabbit Intestinal Nerve Muscle preparation. *Concentrations of SNP used are 0.5 mM, 1.5 mM, 2.5 mM, 5.5 mM, 10.5 mM, 12.5 mM & 14.5 mM, saline was used as control, n=4 for each recording. Error bars = SEM*

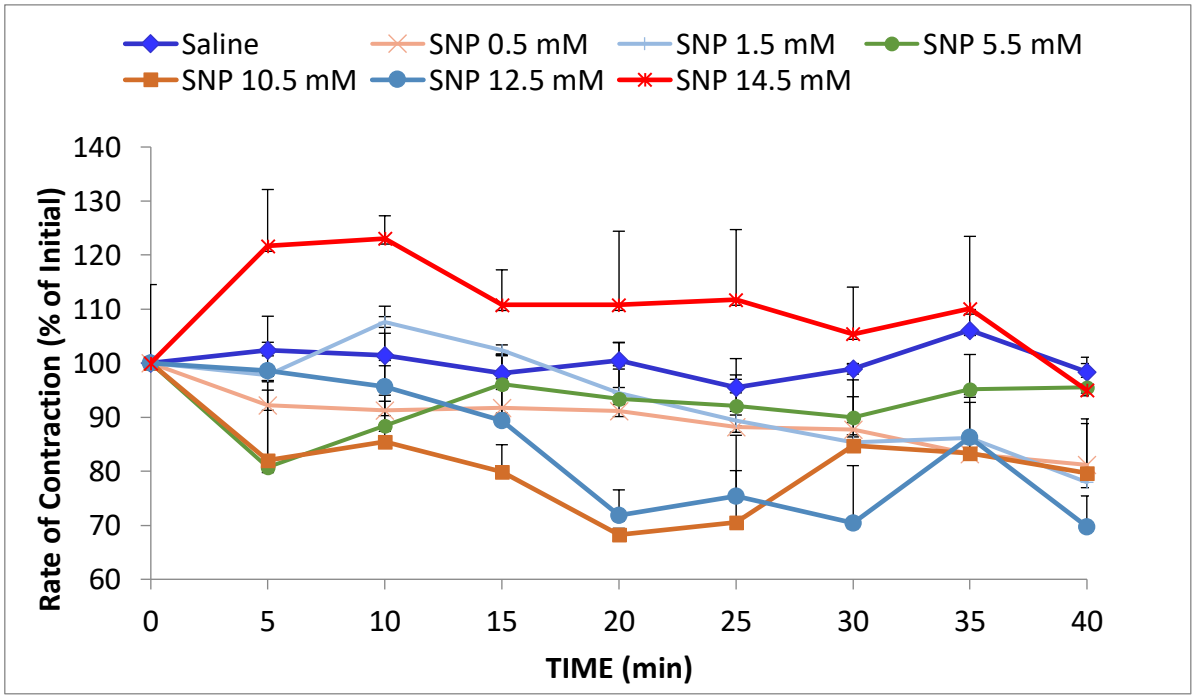


Figure 2: demonstrates the dose dependent effect of Sodium nitroprusside at 5min intervals over 40 mins on rate of muscle contraction of isolated Rabbit Intestinal Nerve Muscle preparation. *Concentrations of SNP used were 0.5 mM, 1.5 mM, 2.5 mM, 5.5 mM, 10.5 mM, 12.5 mM & 14.5 mM; saline was used as control, n=4 for each recording. Error bars = SEM*

Table 2: Dose dependent response of nitroprusside on rate of contraction.

Drug Concentration (mM)	Rate of Contraction (% initial)							
	5 (Min)	10 (Min)	15 (Min)	20 (Min)	25 (Min)	30 (Min)	35 (Min)	40 (Min)
0	102.4 ± 1.5	101.5 ± 4.0	98.1 ± 3.3	100.5 ± 3.4	95.5 ± 1.5	99 ± 1.0	106.1 ± 3.8	98.4 ± 2.7
0.5	92.3 ± 4.5	91.3 ± 8.2	91.8 ± 9.9	91.1 ± 12.7	88.2 ± 12.6	87.7 ± 12.1	83.2 ± 9.5	81.2 ± 8.5
1.5	97.8 ± 9.4	107.6 ± 10.5	102.4 ± 8.3	94.5 ± 6.0	89.4 ± 5.0	85.3 ± 7.2	86.1 ± 6.9	78 ± 7.4
2.5	142.9 ± 29.7	207.2 ± 103.9	235.9 ± 124.3	282.7 ± 199.3	268.5 ± 179.6	262.5 ± 184.5	263.6 ± 185.3	296.3 ± 212.4
5.5	80.7 ± 14.3	88.4 ± 4.6	96.1 ± 5.5	93.4 ± 5.6	92.1 ± 5.7	90 ± 3.8	95.2 ± 6.4	95.5 ± 4.5
10.5	82 ± 14.6	85.5 ± 8.6	79.8 ± 5.1	68.2 ± 1.2	70.5 ± 9.6	84.7 ± 12.2	83.3 ± 3.0	79.7 ± 9.1
12.5	98.6 ± 10.1	95.6 ± 14.9	89.4 ± 8.9	71.8 ± 4.7	75.4 ± 11.3	70.4 ± 10.7	86.3 ± 7.4	69.7 ± 5.7
14.5	121.7 ± 14.5	123.1 ± 10.4	110.8 ± 4.2	110.8 ± 6.5	111.7 ± 13.7	105.4 ± 13.0	110.1 ± 8.7	95 ± 13.4

Values are expressed as mean + SEM; n=4 for each reading, p-values were insignificant compared to controls (0 mM)

a remarkable decrease in active tension recordings at all-time points. There was however no statistically significant difference at doses lower than 5.5 a mM when compared to control (Table **1**). A pictorial representation of the results is displayed in Fig. (**1**).

3.2. Dose Dependent Response of Sodium Nitroprusside on Rate of Contraction

There was no significant difference is rate of contraction reported with increasing gradations of SNP at 5 mins intervals when recorded for a total of 40 mins, thereby suggesting no effect of SNP on chronotropy of the gut musculature (Table **2**, Fig. **2**).

3.3. Dose Dependent Response of Sodium Nitroprusside on Rate of Relaxation

In our study we had observed significant change in rate of relaxation of gut of rabbit shown in Fig. (**3**) via graph. This change in rate of relaxation of muscle was observed due to change in time and concentration of drug. The concentration of drug was (0.5, 1.5, 2.5, 5.5, 10.5, 12.5, 14.5 mM). The experiment of SNP was run for 40 min for both with drug and drug free Krebs solution, after those results were compared with control group.

3.4. Dose Dependent Response of Sodium Nitroprusside on Rate of Rise of Contraction

We observed the significant change in the rate of rise of contraction in the gut of rabbit shown in Fig. (**3**) via graph. This change is observed due to the change in concentration of drug and time. The concentration of drug was (0.5, 1.5, 2.5, 5.5, 10.5, 12.5, 14.5 mM). The experiment of SNP was run for 40 min with drug and drug free kreb's solution, after those results were compared with control group.

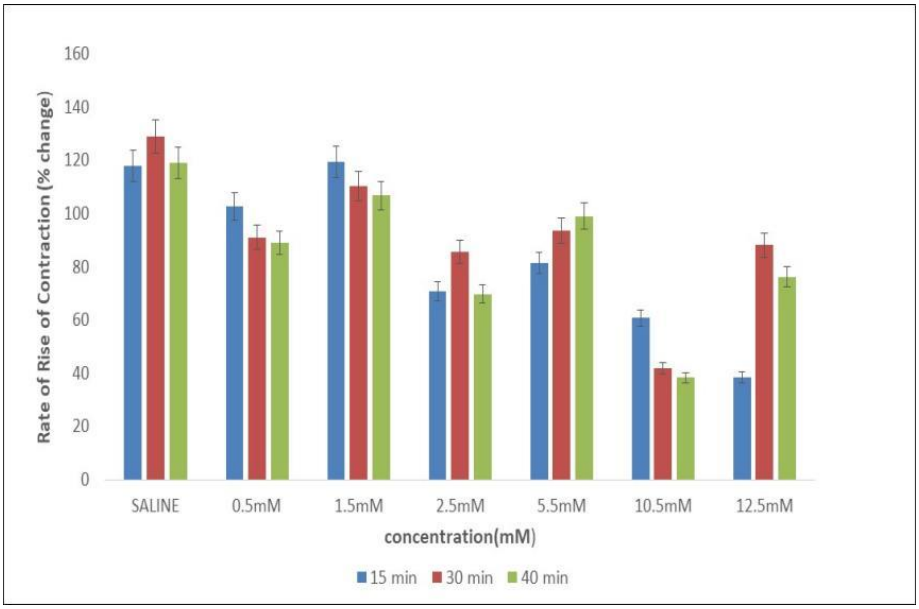


Figure 4: demonstrates the dose dependent effect of Sodium nitroprusside at 5 min intervals over 40 mins on rate of rise of contraction.

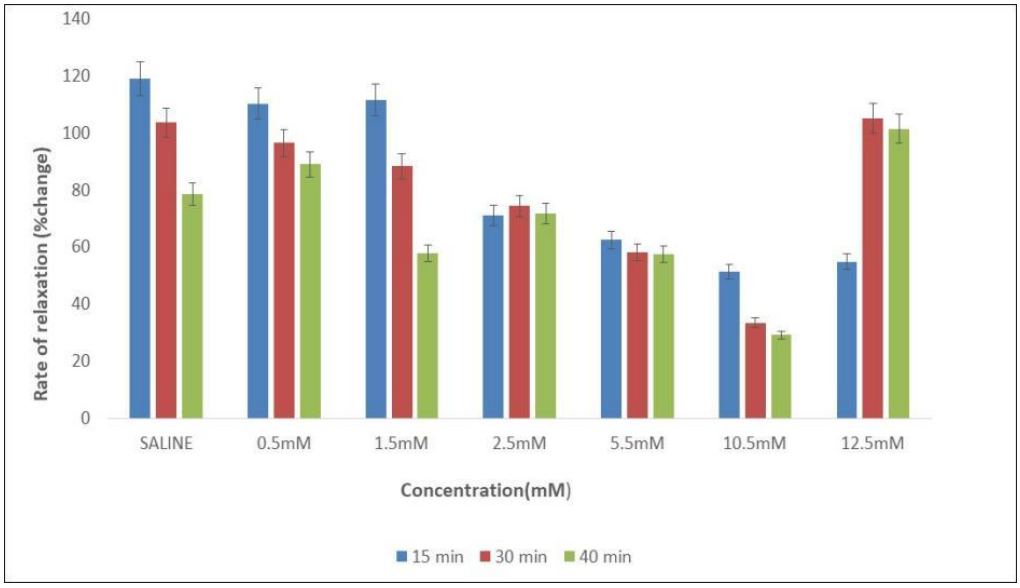


Figure 3: The dose dependent effect of Sodium nitroprusside at 5min intervals over 40 mins on rate of relaxation of isolated Rabbit Intestinal Nerve Muscle preparation.

4. DISCUSSION

The current study demonstrates the dose dependent effects of SNP on nerve muscle preparation of rabbit intestine. The results of this study show that SNP reduced the inotropic response in isolated ileum from rabbit in a time and concentration dependent manner as seen by a decrease in active tension after application of exogenous SNP to Ach and potassium chloride induced contractions. However, there was no significant effect of SNP on the rate of enteric muscle contraction.

Tepavcevic SN *et al.* 2008 studied the dose dependent effect of SNP on longitudinal muscle in the gut

immediately following drug administration at 3 mins time point while King BF 2021 studied the effect at 5 mins time point [13, 14]. Similar to our results, they reported that SNP inhibits contractions of the enteric musculature, thereby resulting in relaxation of the muscles.

SNP is an inorganic nitroso compound that exerts an inhibitory effect on the enteric nerves by releasing NO in a non-enzymatic fashion. NO is released from non-adrenergic and noncholinergic nerves of enteric nervous system mainly from mesenteric plexus and has shown previously to act as a primary inhibitory neurotransmitter in longitudinal muscles of ileum [13, 15, 16, 23].

This extrinsic source of NO has the same effect as that of intrinsically produced NO. NO is released intrinsically by nitrergic enteric neurons present in whole enteric system and its function is to regulate gut tone, peristalsis and various excitatory or inhibitory reflexes. It reduces the excitability of smooth muscles by two methods, hyperpolarization of membrane and largely by activation of soluble guanylate cyclase. Guanylate cyclase sequentially increases cGMP and protein kinase, thereby regulating the activity of proteins by phosphorylation in smooth muscle cells [17, 18].

The contraction of smooth muscle is reliant on calcium. When excitatory signals occur, Ca^{2+} enters the cell from the extracellular space and is released from intracellular stores. This calcium binds to calmodulin, which activates myosin light chain kinase (MLCK). MLCK then phosphorylates the regulatory myosin light chain (MLC), activating myosin ATPase and facilitating cross-bridge cycling, which leads to contraction. The dephosphorylation of MLC reduces cross-bridge cycling, resulting in the relaxation of the muscle [19]. In order to release NO from neuronal endings, extra-cellular Ca^{2+} ions are required [20]. In the gut, NO is mainly synthesized from NO synthase which is highly dependent on calcium calmodulin pathway [21]. This calcium calmodulin pathway regulates the activity of the phophatases which are involved in myosin cross bridge activity. Therefore, by acting on myosin cross bridges, Ca^{2+} activates the pacemaker cells of the gut, intestinal cells of Cajal [22].

This study is of pharmacological interest because it explores the dose-dependent effects of SNP on gut smooth muscle over a sustained period, whereas most prior research focused on short-term responses. Understanding these long-term effects improves insight into nitric oxide–mediated regulation of intestinal motility and may have implications for therapeutic modulation of gut function. However, the novelty is limited by the small sample size and absence of mechanistic exploration beyond descriptive analysis, highlighting the need for further studies.

All these mechanisms combined, result in the negative ionotropic effect demonstrated in this study.

5. CONCLUSION

Sodium Nitroprusside produces a dose-dependent negative inotropic effect on isolated rabbit intestinal smooth muscle, leading to reduced gut contractility. This relaxation is likely mediated through nitric oxide release and activation of the cGMP pathway, highlighting the physiological role of NO in regulating intestinal motility. The study provides pharmacological insight into the sustained effects of SNP on gut smooth muscle, which may have implications for therapeutic modulation of gastrointestinal function, although further mechanistic and larger-scale studies are needed.

ETHICAL APPROVAL AND ANIMAL WELFARE

Ethical approval was obtained from the Institutional Bioethical Committee, University of Karachi (ERC No. IBC KU-405/2024). The animal was cared for in accordance with the guidelines of the International Center for Chemical and Biological Sciences (ICCBS).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

SA: Conceptualization, methodology, data analysis and writing the original draft.

AS: Supervision of the study, methodology and critical revision of the manuscript.

MHR: Organization and setup of the organ bath assembly and experimental procedures.

HSK: Data collection, experimental observations and documentation of results.

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