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Original Article

Effect of Adenosine on Purinergic receptors in the treatment of gastric ulcers in Wistar albino rats

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Introduction: Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (g.i.t) mucosa. It is believed that the Gastric ulcer develops due to an imbalance between aggressive factors (Helicobacter pylori, NSAIDs, Gastric acid) and protective factors (mucin, bicarbonate, prostaglandins), leading to the interruption in the mucosal integrity. Gastric ulcer, also known as peptic ulcer, is a localized area of erosion in the stomach lining, resulting in abdominal pain, possible bleeding and other gastrointestinal symptoms. The most common cause of gastric ulcer is a stomach infection associated with the Helicobacter pylori (H. pylori) bacteria. Current treatment and management of gastric ulcer disease include proton pump inhibitors, Ulcer protective agents, anti-histamines, anticholinergics, antacids, and anti-H.pylori drugs etc. **Aim:** The present study was designed to investigate the potential role of adenosine in indomethacin-induced gastric ulcers in Wistar rats. This study also aims to explore the role of purinergic receptors in gastric ulcers. **Materials and method:** Gastric ulcer was induced in Wistar rats (180-210g) of either sex by administration of indomethacin (5mg/kg, p.o., 14 days) and the animals were sacrificed, stomachs were removed for estimation of various parameters such as ulcer index, mucin percentage, percentage of inhibition of ulcer index, estimation of pH. Biochemical estimations such as MDA, GSH, SOD and MPO activity were performed. **Results:** Administration of indomethacin in Wistar rats leads to gastric ulcers by an increase in ulcer index, decrease in mucin percentage and decrease in pH. The biochemical parameters like MDA and MPO are found to increase in the indomethacin control group.GSH and SOD were found to decrease than in the normal control group. Now the administration of a test drug (adenosine) was found helpful in the treatment of gastric ulcers in Wistar rats by decreasing the ulcer index, increase in pH and increase in mucin percentage. The biochemical parameters MDA and MPO were found to decrease and increase in GSH and SOD when compared with the control group. Theophylline, an adenosine receptor antagonist, shows increased gastric ulceration compared to the control group. Thus, the present data demonstrated that adenosine benefits gastric ulcers. Also, the present data demonstrate purinergic receptors' possible role in adenosine's cytoprotective effect.

Keywords: Ulcers, Wistar rats, adenosine, indomethacin

Introduction

Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (g.i.t) mucosa. It is believed that the Gastric ulcer develops due to an imbalance between aggressive factors (Helicobacter pylori, NSAIDs, Gastric acid) and protective factors (mucin, bicarbonate, prostaglandins) leading to the interruption in the mucosal integrity. Gastric ulcer, also known as peptic ulcer, is a localised area of erosion in the stomach lining, resulting in abdominal pain, possible bleeding and other gastrointestinal symptoms [1-5]. The Helicobacter pylori (H. pylori) bacteria-related stomach infection is the most frequent cause of gastric ulcers. It is unclear how H pylori spread among people. However, it may do so by contaminating food and drink. According to some theories, the 19th century saw a marked increase in the prevalence of peptic ulcer disease in Western nations due to a shift in the epidemiology of H. pylori infections [5-10]. Another common cause of Gastric ulcers is the consumption of NSAIDs. NSAIDs cause inhibition of the enzyme cyclooxygenase, which further leads to the inhibition of prostaglandins (E2 and I2) and causes a decrease in mucosal secretion and leads to gastric ulcers. NSAIDs like Indomethacin act on mitochondria, inhibiting oxidative phosphorylation and forming free radicals like superoxides and hydrogen peroxide, ultimately leading to lipid peroxidation and apoptosis. There are also various behavioral factors that lead to the development of stomach ulcers. The various factors include smoking, Frequent use of steroids, Hypercalcemia, and Excessive consumption of alcohol [10-15].

According to the latest WHO data published in May 2014, Peptic ulcer disease deaths in India reached 85,487 or 0.96 per cent of total deaths. The age-adjusted death rate is 9.12 per cent of 100,000 of the population, ranking India 26 in the world. Adenosine, adenosine 5′-triphosphate (ATP), ADP, AMP, uridine 5′-triphosphate (UTP), UDP, and UDP-glucose are endogenous purines that activate P1, P2X, or P2Y purinoceptor families that are widely and differentially distributed in the ENS and non-neuronal cells in the git [15-20]. The tissue distribution and/or biological experiments suggest that up to 14 of 18 purinoceptors may be involved in secretomotor reflexes in the GI tract. The term Purinergic receptors were first introduced to describe classes of membrane receptors that, when activated by either neurally released ATP (P2 purinoceptor) or its breakdown product adenosine (P1 purinoceptor), mediated relaxation of gut smooth muscle [20-25]. P1 purinoceptor or adenosine (ADO) receptors mediate the biological effects of the endogenous nucleoside adenosine and its analogs. Adenosine acts on cell surface receptors that are coupled to intracellular signalling cascades and are mainly divided into 4 subtypes: A1, A2A, A2B, and A3 and all of these ADO receptor subtypes are G-protein coupled receptors (GPCRs). Adenosine is an important endogenous regulator of many physiological processes, including blood flow, seizure activity, airway resistance and neuronal activity. There is now increasing direct and indirect evidence that adenosine and its receptors are involved in the control of gastric acidity and modulation of gastric responses to histamine and acetylcholine [25-30]. Purinergic signalling occurs quickly during synaptic neurotransmission, a neuromuscular transmission that causes smooth muscle to contract or relaxes, and exocrine or endocrine secretion. However, the regulation of long-term events, including cell proliferation, differentiation, migration, and death by purinergic signalling, is now well documented in the contexts of development, regeneration, and wound healing [30-40]. P2X and P2Y receptors play prominent roles directly and by modulation of other signalling systems in embryonic development, including the nervous system, cartilage in limb buds, the mesonephros, retina, myotubes, and neuromuscular junctions. Adenosine inhibits gastric acid secretion, either directly by acting on acid-secreting parietal cells or indirectly by stimulating the release of the acid inhibitor, somatostatin [40-45]. Adenosine promotes tissue protection and repairs through four general modes of action: increased oxygen supply/demand ratio, preconditioning, anti-inflammatory effects and stimulation of angiogenesis. Thus, adenosine downregulation of inflammatory and immune responses in injured tissues plays a crucial role in the beneficial effects induced by this nucleoside [45-50]. Theophylline has been widely used in the treatment of airway diseases, although the mechanism of action of this drug remains unknown. Theophylline has well-documented effects on smooth muscle, possibly related to its ability to act as a non-selective phosphodiesterase (PDE) inhibitor. However, recent clinical observations have suggested that theophylline can also have significant effects on circulating inflammatory cells at plasma levels well below those required to affect airway smooth muscle. Thus the present study was designed to investigate the effect of Adenosint on purinergic receptors in the treatment of gastric ulcers [50-55].

Materials and Methods

Experimental Animals:

Wistar albino rats of either sex, weighing 200-250g were employed in the present study. They were fed on a standard chow diet (Ashirwad Industries Pvt Ltd, Ropar, Punjab, India). Food and water were provided *ad libitium* throughout the experimental period. They were housed in departmental animal house and were exposed to 12h light and 12h dark cycles. All animals were maintained as per the CPCSEA guidelines for the care and use of Laboratory Animals, the experimental protocol used in the present study was approved by Institutional Animal Ethics Committee [55-60].

Drugs And Chemicals:

Indomethacin (Octane Biotech Pvt. Ltd. Lucknow), Adenosine (central drug house Pvt. Ltd), Theophylline (central drug house Pvt. ltd), Lansoprazole (Casca Remedies Pvt. Ltd Kuldeep\ Nagar, Ambala), EDTA (Loba Chemie Pvt. Ltd Tarapur MIDC, Mumbai), Tris buffer (Finar chemicals Ltd. Ahmadabad), Acetic acid (Ozone International – Mumbai, India), DTNB (Chemika Reagents Ltd. Mumbai), Trichloroacetic acid (Research lab fine chem. Industries- Mumbai, India). The kits for TBARS, GSH and MPO were obtained from Transasia bio- medicals Ltd. Baddi (HP). All drug solutions were freshly prepared before use [60-65].

Indomethacin Induced Gastric Ulcer:

The gastric ulcers were induced by administering Indomethacin for 14 days. The rats were sacrificed on the 15^{th} day by cervical dislocation under anesthesia by urethane (1.25 gm/kg, i.p.). Stomachs were isolated and open along the greater curvature. The stomach was washed with ice-cold saline and the glandular portion was then exposed and examined for ulceration [65-70].

Assessment Of Various Parameters [70-80]:

1. Estimation of ulcer score and ulcer index:

The animals were sacrificed by cervical dislocation, Then the stomachs were removed and dissected along its greater curvature and were finally fixed on a cork mat or transparent glass. The dissected stomachs were moistened with normal saline to prevent autolysis. The stomachs were examined by a Microscope and hand magnifying lens. Several methods have been designed to assess the extent of ulcerations and subsequently the calculation of an ulcer index as well as the protective and/or curative ratios for the ulcers. Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer,

0.5 = red colouration,

1 = superficial mucosal erosion,

1.5 = Hemorrhagic streak,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

The ulcer index was calculated using the following equation.

Ulcer index = Arithmetic mean of intensity in a group + number of ulcer-positive animals/Total numbers of animals ×2

% Inhibition = Ulcer index in the control group – Ulcer index in test group x 100/Ulcer index in the control group

2. Estimation of adherent mucin:

Adherent mucin was estimated by the method of Core et al., Alcian blue, which stains acidic mucin was used for quantitative estimation of adherent mucin:

Step 1. Isolated stomachs were socked separately in a solution of alcian blue (10mg/10ml), sodium acetate (200mg/50ml) and sucrose (2.73mg/50ml) for two hours.

Step 2. Dye removed by washing with sucrose (4.2mg/50ml).

Step 3. Mucus complexed with dye was diluted in 10ml of magnesium chloride (5.08mg/50ml) for two hours.

Step 4. The resulting blue solution is used for calculating the optical density of adherent mucin using a spectrophotometer at 605nm. The mean absorbance was calculated. Six additional animals were taken for the estimation of normal adherent mucin. Results were analysed by converting the mean absorbance of each group to the percentage of mucin content in comparison to the normal group.

Estimation of mucin % = Mean absorbance of test group × 100/Mean absorbance of normal group

Mean absorbance of normal animals = 0.6

3. Estimation of gastric pH:

The guts of the rats were removed after cervical dislocation was used to kill them. The volume of the supernatant was measured after the gastric juice had been collected, emptied into test tubes, and centrifuged at 1000 rpm for 10 min. A digital pH metre was used to record the pH of the stomach juice.

4. Macroscopic evaluation of the stomach:

The rats were sacrificed by cervical dislocation and the stomachs were dissected out, opened along the greater curvature, and rinsed with saline to remove gastric contents and blood clots. Then stomachs were examined by a 10X magnifier lens to assess the formation of ulcers. The number of ulcers was counted.

5. Collection of samples:

For biochemical estimation in the stomach tissue, the animals were sacrificed by cervical dislocation. The stomachs were removed and homogenised in phosphate buffer (pH 7.4) The clear supernatant, obtained after centrifugation at 3000 rpm for 15 min, was used to estimate (MDA) thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) level, Myeloperoxidase activity and superoxide dismutase.

6. Estimation of thiobarbituric acid reactive substances:

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in tissue, was performed according to the method of Nichans and Samuelson (1968).

In this method, malondialdehyde (MDA) and other TBARS were measured by their reactivity with thiobarbituric acid in an acidic condition to generate pink coloured chromophore which is measured spectrophotometrically at 535 nm. To 1.0 ml of supernatant of tissue homogenate, 2 ml of trichloroacetic acid-thiobarbituric acid-hydrochloric acid (TCA-TBA-HCl) reagent was added and mixed thoroughly. The mixture was kept in a boiling water bath for 15 min. After cooling, the tubes were centrifuged at 10000 g for 10 min. The colour developed in the supernatant was measured at 535 nm against a blank reagent. A series of standard solutions of tetra methoxy propane in the concentration of 1 to 10 nM was treated in a similar manner. Values were expressed as nanomoles per mg of protein.

Preparation of TCA-TBA-HCl reagent:

15% TCA, 0.25 N HCl and 0.375% TBA were freshly prepared and mixed in the ratio of 1:1:1 before use.

Preparation of 1, 1, 3, 3-tetramethoxy propane:

0.82 ml of 1, 1, 3, 3-tetramethoxy propane was diluted to 5 ml with distilled water to make 1 M solution. 1 ml of this dilution was further diluted to 10 ml with distilled water and this dilution process was further repeated for seven times to get 10 nM of 1, 1, 3, 3-tetramethoxy propane.

7. Estimation of reduced glutathione:

Reduced glutathione (GSH) content of gastric tissue was estimated using the method of Beutler et al. (1963). The supernatant of tissue homogenate was mixed with TCA (10% w/v) in a 1:1 ratio. The tubes were centrifuged at 1000 rpm for 10 min at 4°C. The supernatant obtained (0.5ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25 ml of 0.001 M freshly prepared DTNB dissolved in 1% w/v sodium citrate was added and absorbance was noted using a spectrophotometer at 412 nm. A standard curve was plotted using 10-100 µM of the reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

Preparation of 0.3 M disodium hydrogen phosphate

4.26 g of anhydrous disodium hydrogen phosphate was dissolved in 100 ml of distilled water.

Preparation of DTNB in 1% Sodium citrate

7.92 mg of DTNB was dissolved in 20 ml of 1% sodium citrate.

Preparation of 100 µM of GSH

12.28 mg of GSH was dissolved in 400 ml of distilled water.

8. Estimation of Myeloperoxidase Activity

The Myeloperoxidase (MPO) activity is measured as an index of neutrophil accumulation and was measured using the method of Krawisz et al. (1984) [81]. In the pellet obtained after tissue homogenisation, 10 ml of ice-cold potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB) and 10 mM ethylene diamine tetra acetic acid (EDTA) was added and subjected to one cycle of freezing and thawing and then Sonication for 15s was done. The contents were centrifuged at 15,000 g for 20 minutes. 0.1 ml of the supernatant obtained after centrifugation was mixed with 2.9 ml of phosphate buffer containing 0.16 mg/ml of o-dianisidine hydrochloride and 0.0005% hydrogen peroxide (H_2O_2). The change in absorbance was measured using a spectrophotometer at 532 nm. The MPO activity was expressed as a unit per gram of tissue weight where 1 unit is the quantity of enzyme able to convert 1 µM of H_2O_2 to water in 1 minute at

room temperature. The calculation of MPO activity was done using the formula:

MPO activity (U/g) = X / Weight of the tissue

Where X = 10 x change in absorbance per minute/volume of supernatant taken in ml

Preparation of potassium phosphate buffer containing HETAB and EDTA

Disodium hydrogen phosphate (60.5 g) and potassium dihydrogen phosphate (46 g) were dissolved in 1 L of distilled water to make a potassium phosphate buffer (pH 6.0). 5 g of HETAB and 3.72 g of EDTA were dissolved in 1 L of phosphate buffer (pH 6.0) to make a final solution.

Preparation of phosphate buffer containing o-dianisidine hydrochloride and H_2O_2

O-dianisidine hydrochloride (16.7 mg) was dissolved in 100 ml of potassium phosphate buffer (pH 6.0), followed by the addition of 1 µL of H_2O_2.

9. Estimation of SOD antioxidant enzyme concentration:

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund. The supernatant of tissue homogenate was mixed with 0.1 M phosphate saline buffer (1:4 (w/v), pH 7.4) and the homogenates were centrifuged at 2500 rpm for 10 min at 4°C. The reaction mixture consisted of 0.5 ml of TRIS-buffer (50 mM; pH 8.2), 0.5 ml pyragallol (0.5 mM), 0.5 ml EDTA (1 mM) and in different volumes, 0.025 ml, 0.05 ml, 0.75 ml and 0.1 ml of tissue homogenate. The change in absorbance was recorded at 420 nM. The activity was reported by its ability to inhibit a 50% reduction of pyragallol, which is expressed as a Unit/ml protein [82-85].

Preparation of Tris- EDTA buffer pH 8.2

A weight of 2.85 g of tris and 1.11 g of EDTA- Na 2 were dissolved in 1 liter of distilled water.

Preparation of Pyrogallol solution (0.2 mM)

A weight of 0.252 g of pyrogallol was dissolved in a solution of 0.6 ml of concentrated hydrochloric acid diluted in 1 liter of distilled water.

Calculation of SOD activity [86-90]

% Inhibition of pyrogallol autoxidation = ΔA test X 100%/ΔA test

SOD activity (U/ml) = % inhibition of pyrogallol autoxidation/50%

10. Experimental Design [90-100]

Six groups of Wistar rats were employed in the present study. All animals were randomly divided into these groups. Each group was comprised of six animals (n= 6).

Group I: [Normal Control]

Normal rats were maintained on a standard chow diet and water ad libitum for 14 days. No treatment was given to these rats.

Group II: [Indomethacin control 5mg/kg]

Indomethacin (5mg/kg/day p.o.) was administered to rats on standard chow at the end of fourteen days.

Group III: [Indomethacin-5mg/kg + Lansoprazole-50mg/kg]

Indomethacin (5mg/kg/day p.o.) was administered to rats for seven days and lansoprazole (50mg/kg/day p.o.) was administered for another seven days along with Indomethacin treatment.

Group IV: [Indomethacin-5mg/kg + Adenosine100mg/kg]

Indomethacin (5mg/kg/day p.o.) was administered to rats for seven days and Adenosine (100mg/kg

p.o.) was administered for another seven days along with Indomethacin treatment.

Group V: [Indomethacin-5mg/kg + Adenosine 150mg/kg]

Indomethacin (5mg/kg/day p.o.) was administered to rats for seven days and Adenosine (150mg/kg/day p.o.) was administered for another seven days along with Indomethacin treatment.

Group VI: [Indomethacin-5mg/kg + Theophylline 5mg/kg]

Indomethacin (5mg/kg/day p.o) was administered to rats for seven days and theophylline (5mg/kg p.o) was administered for another seven days along with Indomethacin treatment.

11. Statistical Analysis

All values were expressed as mean ± S.D. The data obtained from various groups were statistically analysed using one-way ANOVA followed by Turkey's multiple comparison tests. The p-value of 0.05 was considered to be statistically significant.

Results

The experimental protocol comprises six groups. Gastric ulcers were produced by Indomethacin 5mg/kg/orally for 14 days of daily treatment and various parameters of gastric damage were assessed by comparing results obtained from different groups. Gastric ulcers were measured on their intensity; the ulcers were given scores as follows:

0 = no ulcer,

0.5 = red colouration,

1 = superficial mucosal erosion,

1.5 = Hemorrhagic streak,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

1: Effect of Adenosine on ulcer score, ulcer index and percentage of ulcer inhibition in Indomethacin-induced gastric ulcer in Wistar rats.

Administration of Indomethacin 5mg/kg orally for 14 days daily caused a significant ($p<0.005$) increase in ulcer score and ulcer index. Indomethacin administration showed ulceration in the glandular area of the stomach compared to normal control rats. Treatment with oral doses of Adenosine (100,150mg/kg) and lansoprazole (50mg/kg) which were test and standard drug respectively, in the present study, decrease the ulcer score, ulcer index and increases the

percentage of ulcer inhibition (Table 1 and Fig. 1, Fig. 2, Fig. 3) when compared to the indomethacin treated group.

2: Effect of Adenosine on pH and gastric mucus in Indomethacin-induced gastric ulcer in Wistar rats.

Administration of Indomethacin 5mg/kg/orally for 14 days daily caused ($p<0.005$) a decrease in gastric mucus and pH. Indomethacin administration showed significant ulceration with a decrease in gastric mucus and pH in the glandular area of the stomach compared to normal control rats. Treatment with oral dose of Adenosine (100,150mg/kg) and lansoprazole (50mg/kg) which were test and standard drug respectively in the present study, increases the gastric mucus and pH (Table 2 and Fig. 4, Fig. 5). when compared to the indomethacin treated group. The high dose of Adenosine (150mg/kg) was more effective than the lower dose of adenosine. (100mg/kg).

3: Effect of Adenosine on malondialdehyde and myeloperoxidase in Indomethacin induced gastric ulcer in Wistar rats.

Administration of Indomethacin 5mg/kg orally for 14 days daily caused a significant ($p<0.005$) increase in MPO and MDA. Administration of oral dose of Adenosine (100mg, 150mg /kg) and lansoprazole (50mg/kg) which were test and standard drugs in the present study produced a ($p<0.005$) dose-dependent decrease in malondialdehyde and myeloperoxidase, when compared with the indomethacin treated group (table 3, Fig. 6, Fig. 7). The high dose of Adenosine was significantly more effective than the low dose in decreasing the MDA and MPO.

4. **Effect of Adenosine on GSH and SOD in Indomethacin induced gastric ulcer in Wistar rats.**

Administration of Indomethacin 5mg/kg orally for 14 days daily caused ($p<0.005$) a decrease in GSH and SOD. Administration of oral doses of Adenosine (100, 150mg/kg) and lansoprazole (50mg/kg) which were test and standard drugs in the present study, produced a ($p<0.005$) dose-dependent increase in glutathione and superoxide dismutase, When compared to the indomethacin treated group (Table 4 and Fig. 8, Fig. 9). The high dose of Adenosine was more effective than the low dose in increasing the GSH and SOD.

Groups	Ulcer score	Ulcer index	% of ulcer inhibition
Normal control	00	00.00	100
Indomethacin control (5mg/kg)	1.833 ± 0.408 a	5.44	00a
Lansoprazole(50mg/kg)	0.416 ± 00.2041b	1.41	74.08 b
Adenosine (100mg/kg)	0.833a ± 0.258b	3.13	42.46 b
Adenosine (150mg/kg)	0.4166a ± 0.2041b	1.97	63.78 b
Theophylline(5mg/kg)	0.9166a ±0.2041b	5.79	- 6.43b

Table 1. *Effect of Adenosine on ulcer score, ulcer index and percentage of ulcer inhibition in Indomethacin induced gastric ulcer in Rats*

All values are expressed as Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control.

Groups	Mucin %	pH
Normal control	100	3.123±0.345
Indomethacin control (5mg/kg)	76.75	1.363 ±0.392
Lansoprazole(50mg/kg)	94.37	4.583±0.285
Adenosine (100mg/kg)	85.37	4.015±0.505
Adenosine (150mg/kg)	95.06	4.59±0.264
Theophylline (5mg/kg)	67.5	1.278±0.151

Table 2. *Effect of Adenosine on pH and gastric mucus in Indomethacin induced gastric ulcer*

All values are expressed Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control.

Groups	MDA (nmol/mg)	MPO (nmol/mg)
Normal control	2.237±0.529	10.89±0.79
Indomethacin control (5mg/kg)	4.626±0.16a	40.34±0.38a
Lansoprazole(50mg/kg)	1.588±0.23b	12.38±0.38b
Adenosine(100mg/kg)	2.556±0.248b	23.68±0.484b
Adenosine (150mg/kg)	1.764±0.291b	14.46±0.323b
Theophylline(5mg/kg)	5.195± 0.3869b	40.91 ± 0.263b

Table 3. *Effect of Adenosine on malondialdehyde and myeloperoxidase in Indomethacin induced gastric ulcers in Wistar rats*

All values are expressed Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control. Where Indo = Indomethacin; MPO= Myeloperoxidase; MDA= Malondialdehyde.

Groups	SOD (units/mg)	GSH (nmol/mg)
Normal control	46.98±2.86	6.73 ± 0.28
Indomethacin control (5mg/kg)	38.6 ±1.38a	1.54±0.26a
Lansoprazole(50mg/kg)	63.06 ± 1.02b	3.08±0.34b
Adenosine(100mg/kg)	65.45±1.03b	3.52±0.30b
Adenosine (150mg/kg)	71.48 ±2.89b	4.455±0.42b
Theophylline(5mg/kg)	34.45±3.21b	1.168 ± 0.12b

Table 4. *Effect of Adenosine on SOD and GSH in Indomethacin induced gastric ulcers in Wistar rats*

All values are expressed Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control. GSH = Glutathione; SOD= superoxide dismutase.

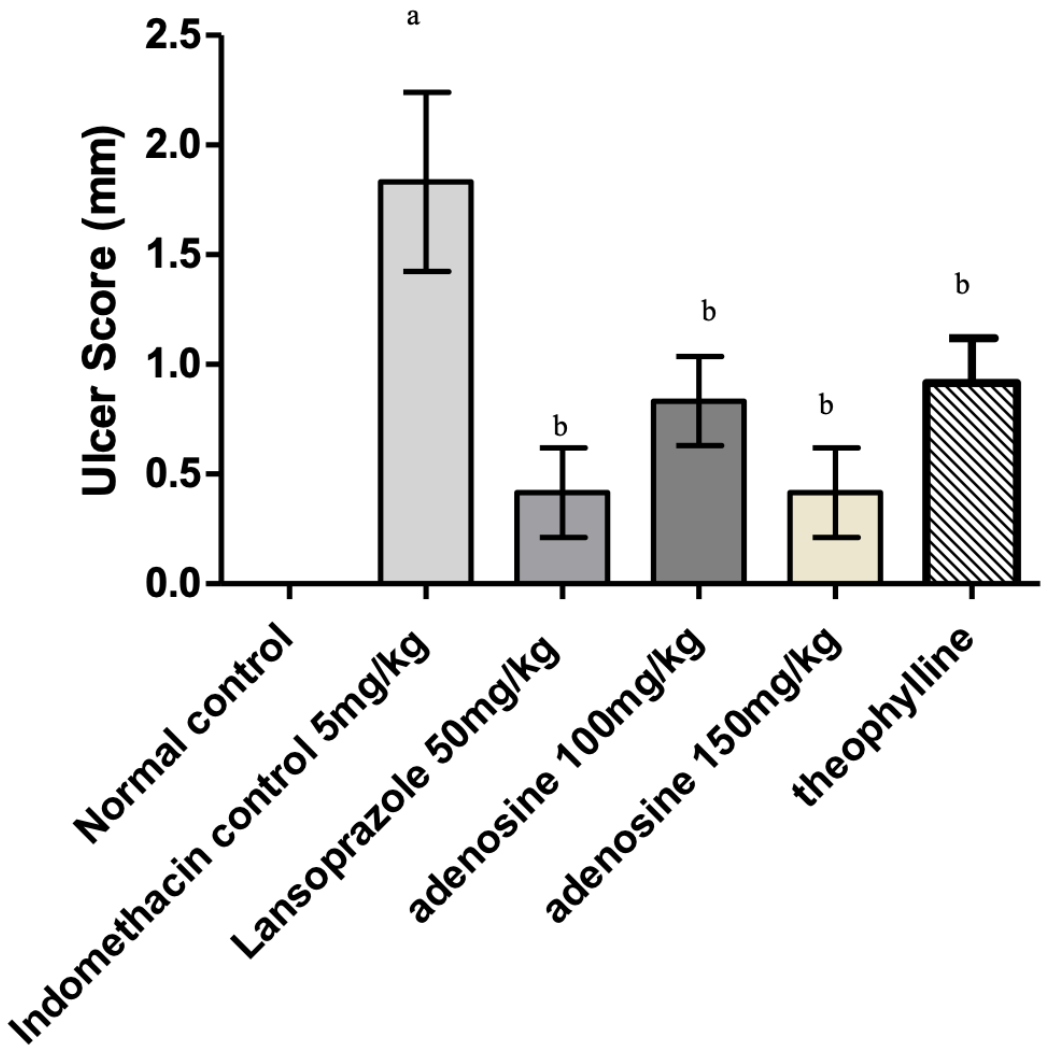

Figure 1. *Effect of Adenosine on ulcer score*

All values are expressed as Mean ± S.D; n = 6. [a] = $P<0.05$ Vs Normal control, [b] = $P<0.05$ Vs Indomethacin control.

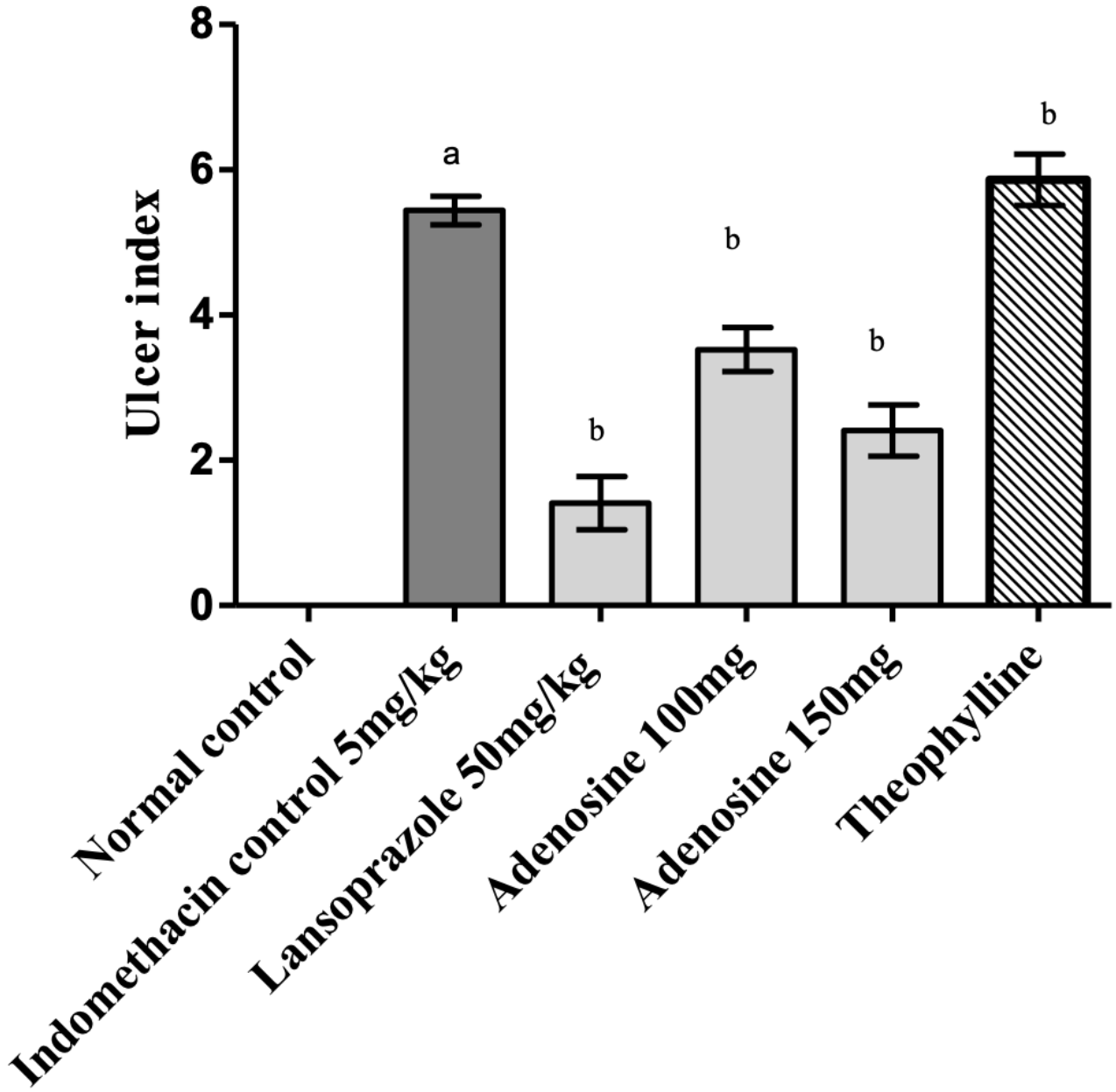

Figure 2. *Effect of Adenosine on ulcer index*

All values are expressed Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control. Where Indo = Indomethacin.

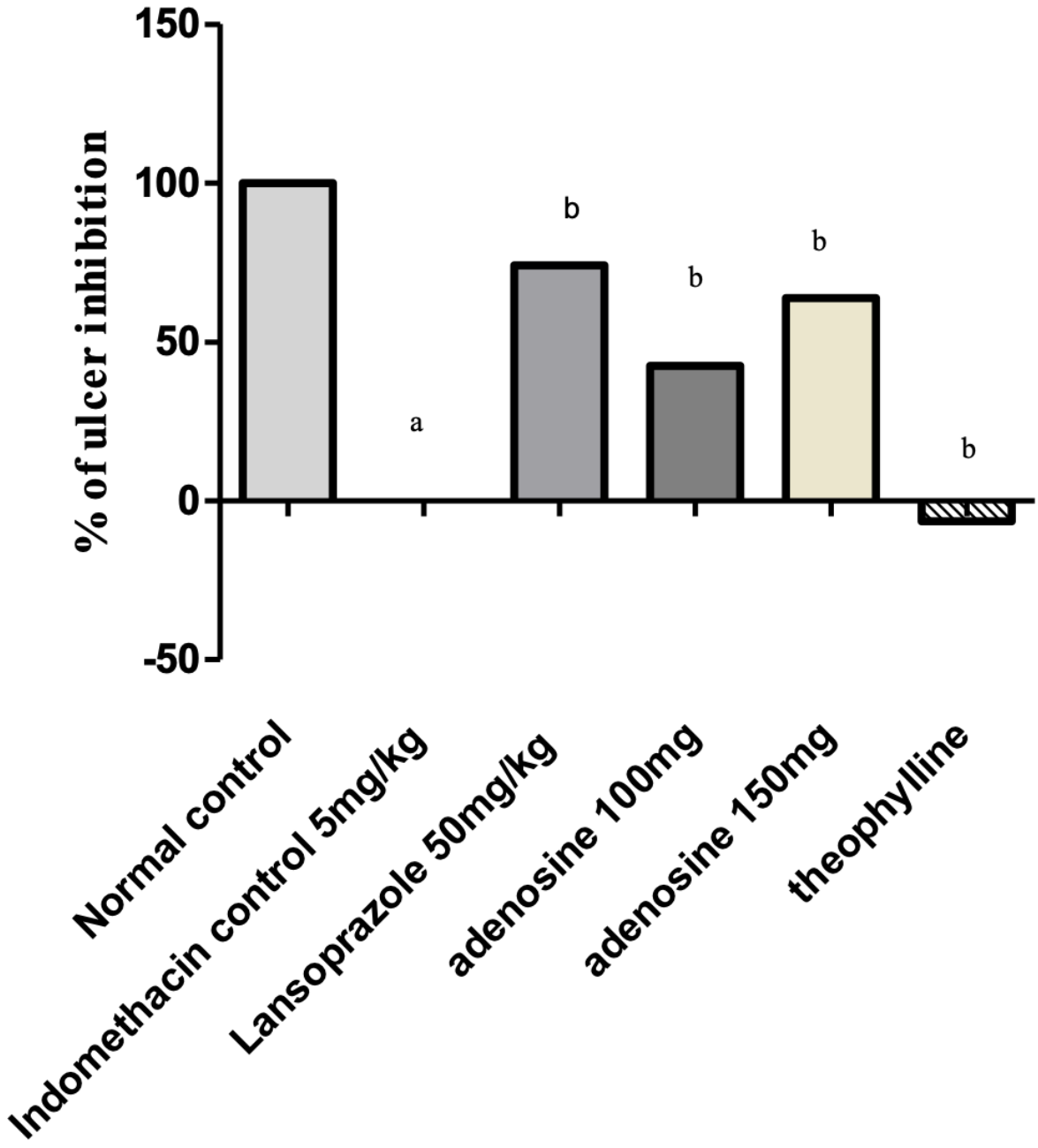

Figure 3. *Effect of Adenosine on percentage of ulcer inhibition*

All values are expressed as Mean ± S.D; n = 6. [a] = $P<0.05$ Vs Normal control, [b] = $P<0.05$ Vs Indomethacin control.

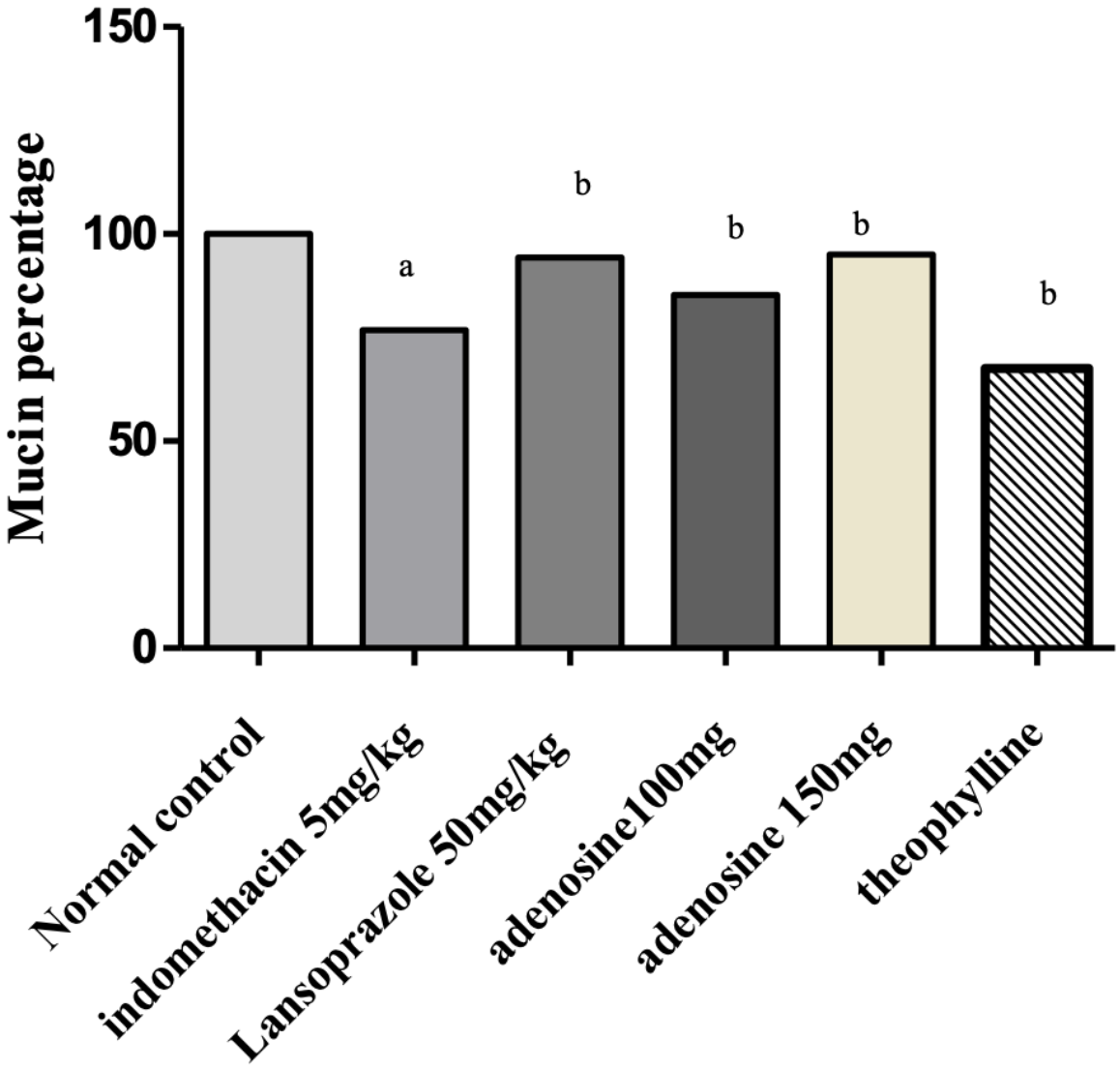

Figure 4. *Effect of Adenosine on rat gastric mucin*

All values are expressed as Mean ± S.D; n = 6. [a] = $P<0.05$ Vs Normal control, [b] = $P<0.05$ Vs Indomethacin control. Where Indo = indomethacin.

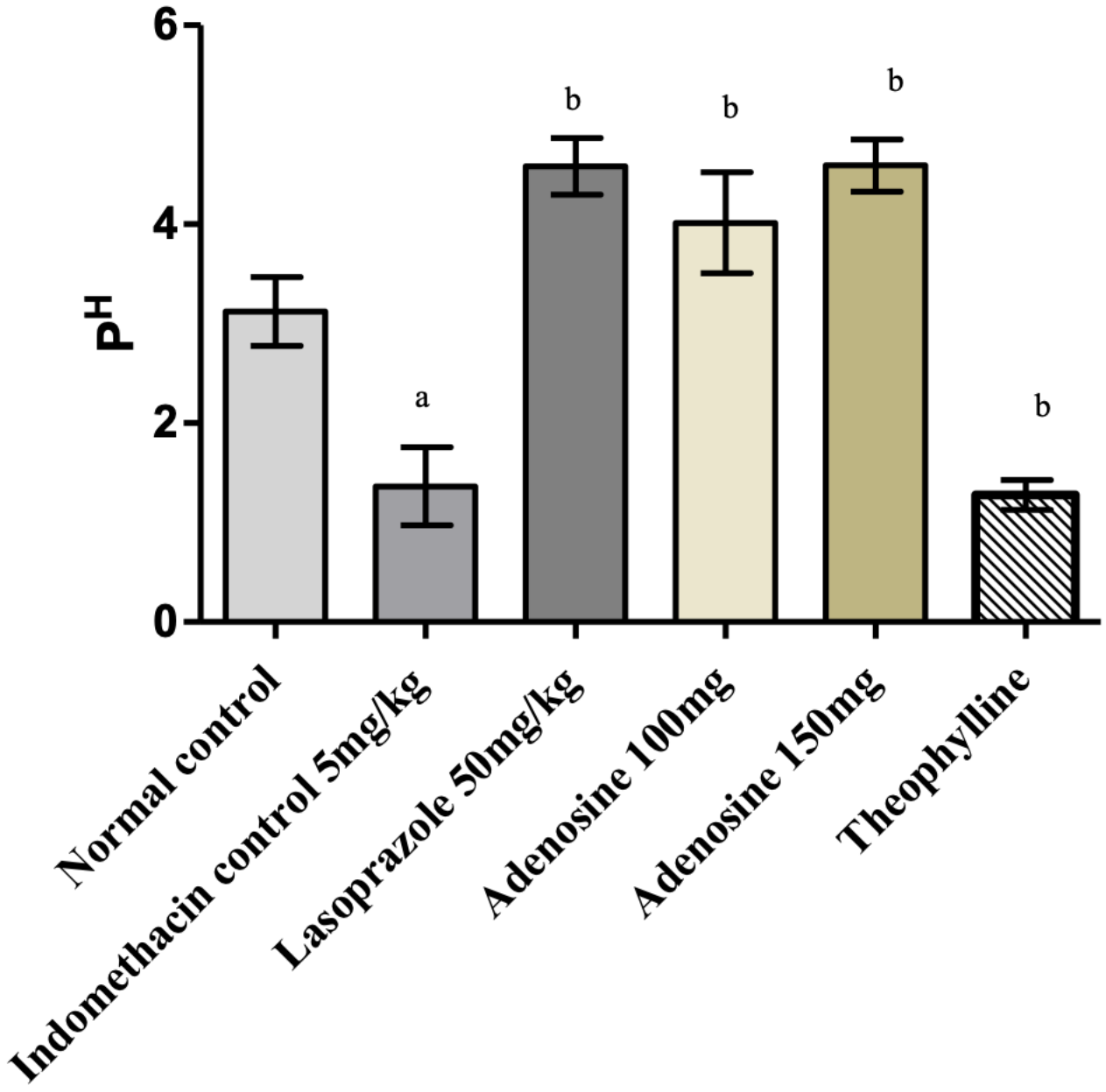

Figure 5. *Effect of Adenosine on pH in Indomethacin induced gastric ulcer*

All values are expressed Mean ± S.D; n = 6. [a] = $P<0.05$ Vs normal control, [b]= $P<0.05$ Vs Indomethacin control. Where indo= Indomethacin.

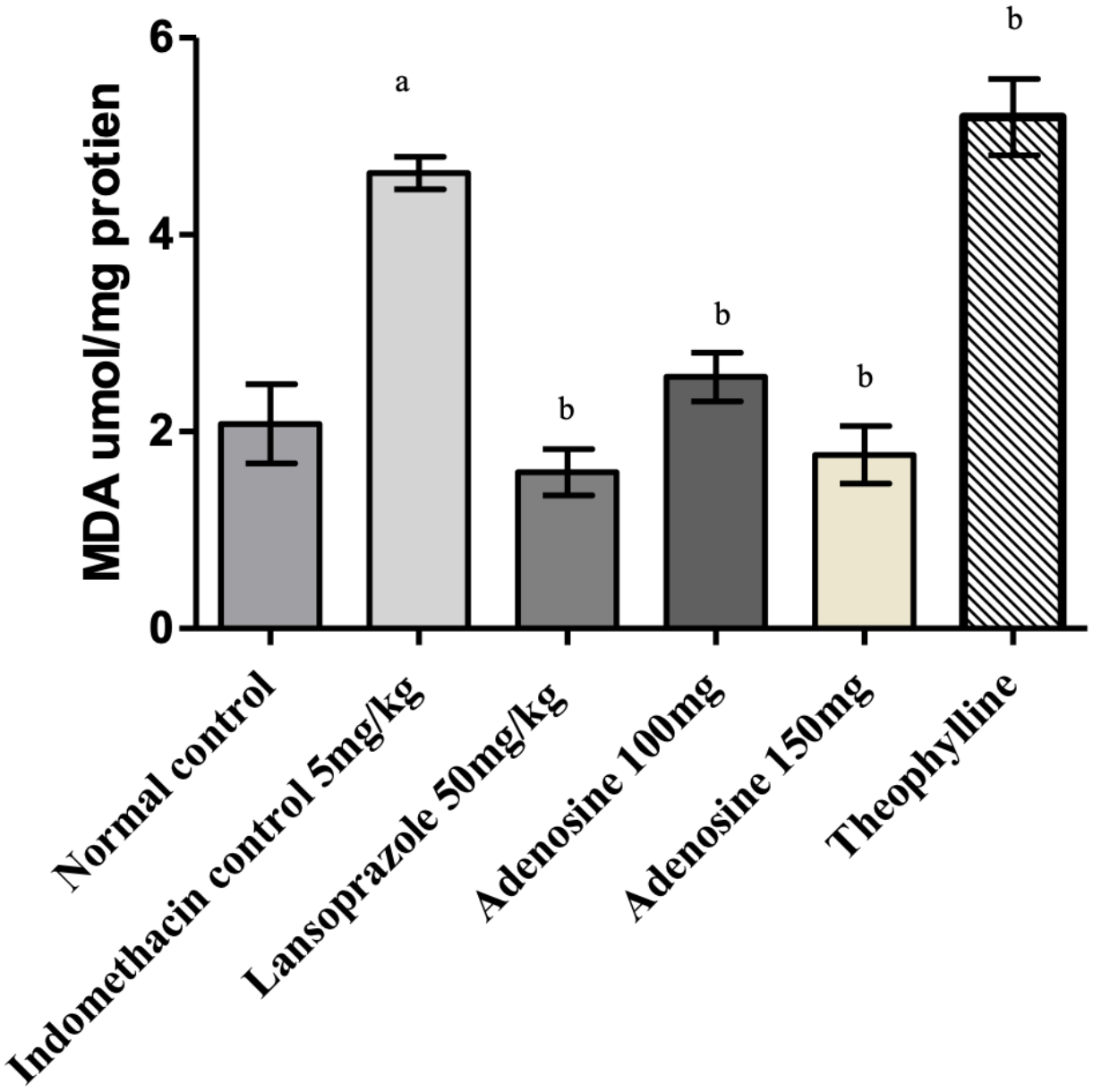

Figure 6. *Effect of Adenosine on malondialdehyde, in Indomethacin induced gastric ulcers*

All values are expressed as (Mean ± S.E.M), n=6, *p<0.05 when compared with control group. Where Indo= Indomethacin; MDA= Malondialdehyde.

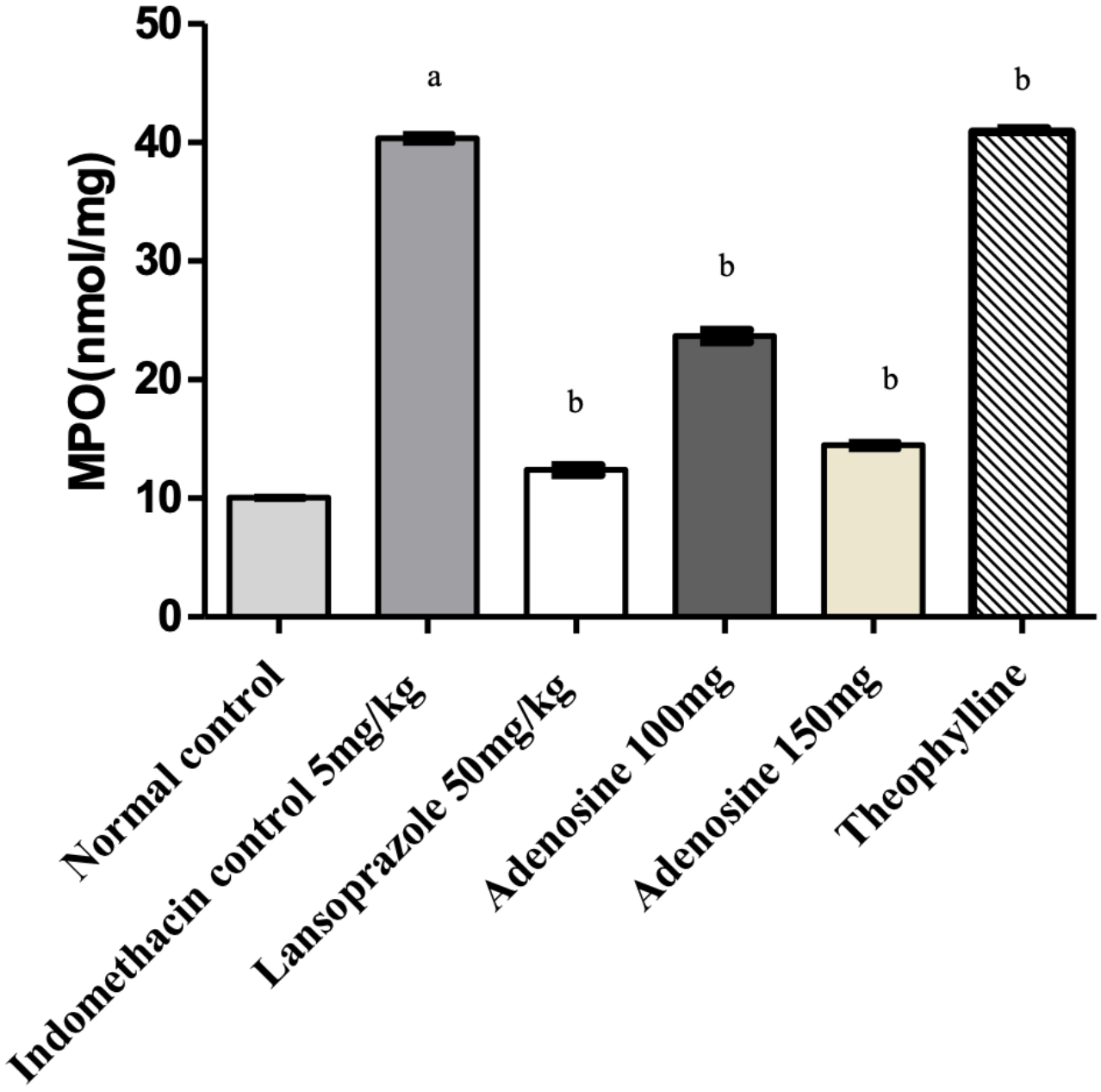

Figure 7. *Effect of Adenosine on myeloperoxidase, in Indomethacin induced gastric ulcers*

All values are expressed as (Mean ± S.E.M), n=6, *p<0.05 when compared with control group. Where Indo= Indomethacin; MPO= Myeloperoxidase.

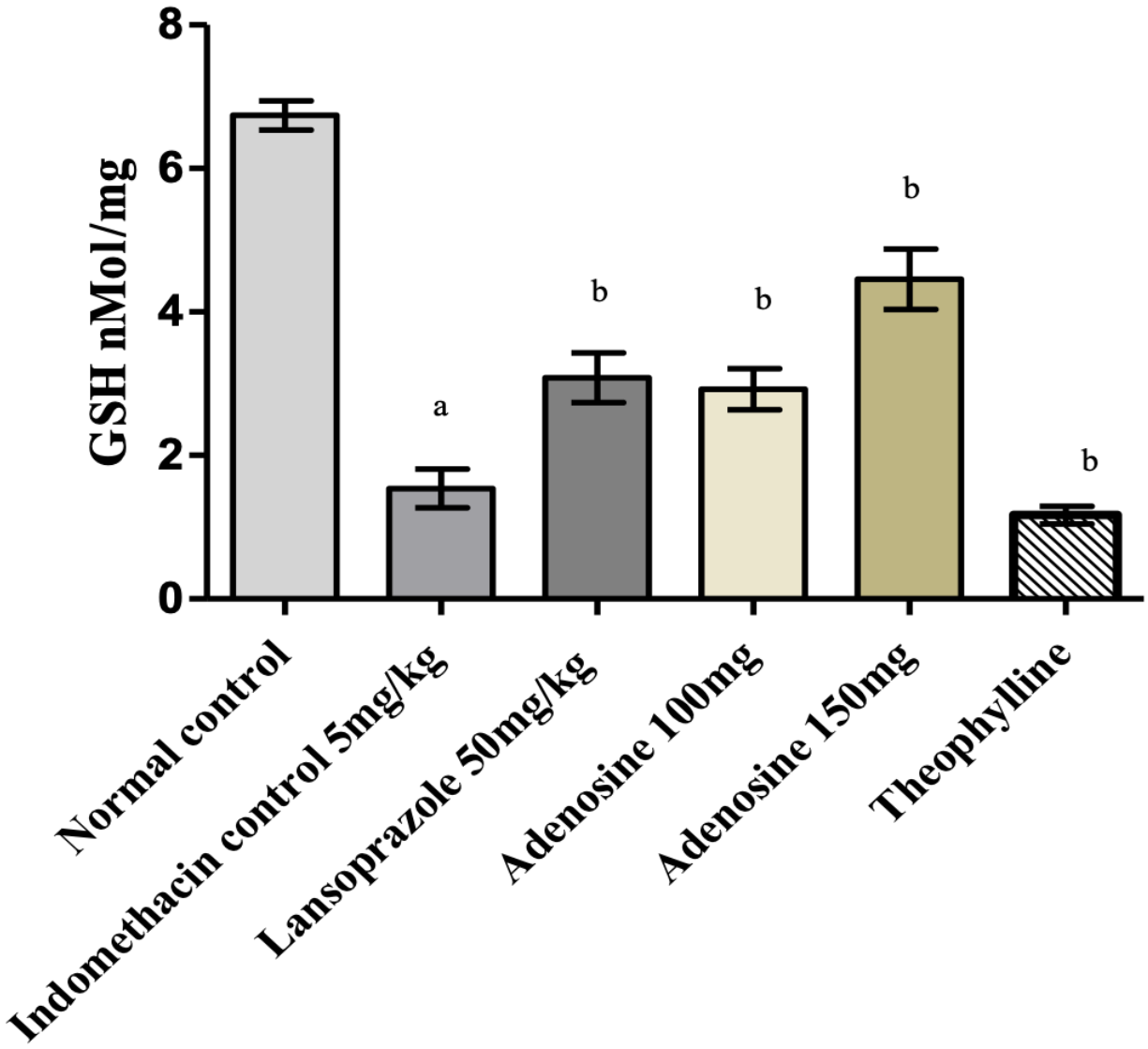

Figure 8. *Effect of Adenosine on Glutathione, in Indomethacin induced gastric ulcers*

All values are expressed as (Mean ± S.E.M), n=6, *p<0.05 when compared with control group. Where Indo= Indomethacin; GSH= Glutathione.

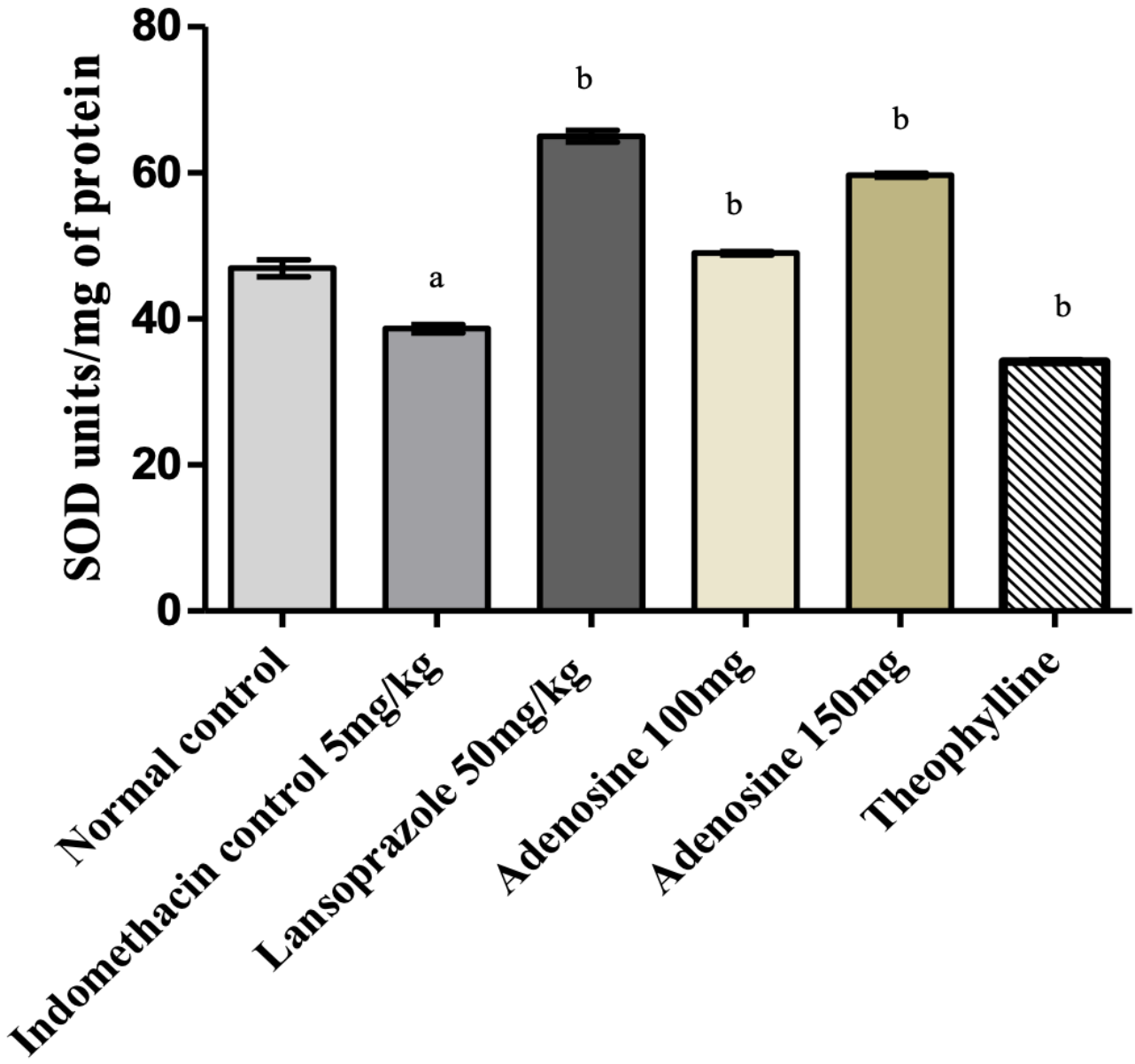

Figure 9. *Effect of Adenosine on superoxide dismutase in Indomethacin induced gastric ulcers*

All values are expressed as (Mean ± S.E.M), n=6, *p<0.05 when compared with a control group. Where Indo= Indomethacin; SOD= superoxide dismutase.

Discussion

The present study was undertaken to determine the effect of Adenosine on Indomethacin treated rats and the effect was compared with the standard drug lansoprazole used in gastric ulcers. We observed that Adenosine positively affects the alteration of various parameters of gastric ulcers. Gastric ulcer is an important contributor to the genesis of ulceration in experimentally induced animal models. Current gastric ulcer therapies show moderate efficacy against mucosal lesions/ ulceration but are often associated with several side effects [90-95].

Non-steroidal anti-inflammatory drugs (NSAIDs) such as Indomethacin induce an injury to gastrointestinal mucosa in experimental animals and humans, and their use is associated with a significant risk of hemorrhage, erosions and perforation of both gastric and intestinal ulcers. The molecular basis is their inhibitory activity against mucosal blood flow, disturbance of microcirculation, decrease in mucus secretion, lipid peroxidation and neutrophils activation, which are involved in the pathogenesis of the gastric mucosal disorder. According to previous studies, in most cases, the etiology of the ulcers was due to an increase in the activity of aggressive factors and a decrease in the activity of protective endogenous defence mechanisms. Agents that increase the activity of factors like HCl, bile acids, and drugs like indomethacin, aspirin and decrease the activity of factors like mucous formation, bicarbonate and prostaglandin synthesis lead to ulcer formation. The defensive actions are gastroprotective in nature and involve the anti-oxidative enzymes: superoxide dismutase (SOD), which causes the dismutation of superoxide radical's anion (O¬¬2-) into less noxious hydrogen peroxide (H_2O_2) and glutathione peroxide H_2O_2 to water. Indomethacin has been known to cause lipid peroxidation with depletion of endogenous antioxidants [95-100].

Cells and tissue are in a stable state if the rate of free radical formation and scavenging capacity are essentially constant and in equilibrium. However, an imbalance between them results in oxidative stress, which further deregulates cellular functions leading to different pathological conditions. In the present study, the increased concentration of MDA and reduced activity of SOD and GSH in the stomach of indomethacin-ulcerated rats are a manifestation of facilitated lipid peroxidation and overproduction of free radicals resulting in mucosal damage. NSAID administration was reported to cause suppression of antioxidant defence and to initiate lipid peroxidation in the stomach. A well-known mechanism responsible for gastric damage induced by indomethacin is the inhibition of cyclooxygenase, a rate-limiting enzyme in the synthesis of PGs [100-110]. However, recent studies have suggested that NSAIDs such as indomethacin give pro-oxidant activity and initiate lipid peroxidation by generating reactive oxygen species, thereby interfering with an endogenous antioxidant system of the mucosa cells. Our results showed that Indomethacin administration significantly increased gastric tissue histamine content. These results are in harmony with previous investigations concluding that PGs are extremely potent inhibitory of mast cell degranulation, and mast cells are capable of releasing a variety of mediators, like leukotriene C4 and platelet-activating factor, that can contribute to mucosal injury. NSAIDs could reduce gastric mucosal blood flow and thus contribute to the pathogenesis of ulcer disease [110-130].

On the other hand, mechanisms for such actions of NSAIDs seem to be complex and multifactorial, including the inhibition of PG synthesis, induction of apoptosis and necrosis of gastric mucosal cells, neutrophil penetration, dysfunction of microvessels, reduced secretion of bicarbonates and mucus, and increased gastric motility. Indomethacin produced more severe gastric disturbance associated with gastric mucosal injury. Robert introduced the concept of gastric cytoprotective, a property by which prior administration of a non-secretory dose of a prostaglandin would protect the rat stomach against the damaging effect of various agents. There are so many models to induce ulcers that the indomethacin model is very commonly used to induce experimental ulcers in animals. In this study, we studied the effect of adenosine on indomethacin induced gastric ulcers. The results obtained from this study showed that adenosine has great mucosal protective action. Treatment with adenosine reduced the ulcer index level compared with the indomethacin treated group. In this study, 150mg of adenosine showed the most gastroprotective effect than the 100mg adenosine treated group. The efficacy of adenosine was compared with the Lansoprazole group, 150mg of adenosine treated group showed very near results to the standard Lansoprazole group. According to the old hypothesis, acid secretion was thought to be the single cause of ulcer formation and reduction in acid secretion was thought to be the major approach towards therapy. The treatment of ulcers mainly targets increasing the defensive system and lowering acid secretion. In the present study also, adenosine had proved its cytoprotective action in the gastric mucosa of rats against indomethacin induced gastric ulcers [130-140].

The induction of the gastric ulcer model employed in the present study is proven and well-reported

to mimic the clinical situation of ulcers in the stomach due to inhibition of mucus formation and finally causing injury in the stomach of the Wistar rats. The model has the advantages of precision of the ulcer site, production of constant ulcer size and easier performance. NSAIDs have been reported to impair the mucus layer in the stomach and the mucus layer protects the stomach tissue from the injury caused by excessive acid. Therefore, Indomethacin was employed to induce gastric ulcer and the parameters like ulcer index, mucin percentage, pH and percentage of ulcer inhibition were obtained to check the severity of ulcers and the improvement after giving the test drug. In the present study, gastric ulcers were produced by giving Indomethacin (5 mg\kg) orally to the rats for 14 days and the rats were sacrificed by cervical dislocation, stomachs were analysed by microscope and ulcer index was obtained. The pH of the gastric content was checked by the digital pH meter. After analysis, the stomachs ulcer index was found to be higher in the control and theophylline groups and lesser in the test group (adenosine treated group). Mucin content in the control and theophylline groups was very less than in the test group animals. The percentage of inhibition of ulcers in the test group was found higher than in the control and theophylline groups. In gastric ulcers, there occurs alteration in various biochemical parameters in the body, so the various biochemical parameters like MDA, MPO, GSH and SOD were checked in the present study. The level of MDA and MPO in the control group was higher than the normal control group, but the level of MDA and MPO in the treated groups was lower than in the control group. The level of GSH and SOD was found less in the control group, but in the treated groups, their level increased. In gastric ulcers, lipid peroxidation occurs, and MDA is the end product of lipid peroxidation. Thus, the concentration of MDA in the control group (Indomethacin treated group) contains a higher amount of MDA than the normal and treated groups. In test groups (adenosine treated), the MDA level was decreased than the control group, another parameter MPO. It is an enzyme that catalyses the production of ROS and other reactive substances and increases the severity of injury. The level of MPO was found to be high in the control group and decreased in the test group (adenosine treated).

In theophylline group (adenosine receptor antagonist), the level of MDA and MPO was found very higher than the level of MDA and MPO in the control group (Indomethacin treated). The level of GSH and SOD in the Theophylline group was found very lesser than in the control group and the level of GSH and SOD was found to increase in the test group (adenosine treated group). Theophylline blocks the adenosine receptors, decreases the effect of adenosine and leads to an influx of gastric ulcers in the Wistar rats. Purinergic receptors are divided into P1 receptors: A1, A2A, A2B and A3 differing by pharmacological and functional properties and P2 receptors that are further subdivided into two distinct families (P2X & P2Y) based on their molecular structure, transduction mechanisms and pharmacological properties. Currently, seven P2X receptors (P2X1-7) and eight P2Y receptors (P2Y1, 2, 4, 6, 11, 12, 13 & 14) subtypes have been recognised. The treatment of theophylline, which is a Purinergic receptor antagonist, has abolished the cytoprotective effect of adenosine in a significant dose-dependent manner, thereby clearly implicating the potential role of purinoceptors in gastric ulcers. Therefore, with support from the literature and data in hand, it is concluded that the cytoprotective effect of adenosine probably involves purinergic receptor channel activity. However, further in-depth studies are needed to substantiate these findings. Therefore, the present data demonstrated that adenosine has a beneficial effect on gastric ulcers. Also, the present data demonstrated the possible role of purinergic receptors in the cytoprotective effect of indomethacin induced gastric ulcer [140-146].

Conclusion

A stomach ulcer is a deep lesion that extends through the whole mucosa of the gastrointestinal tract. The disruption of the mucosal integrity is thought to be the result of an imbalance between aggressive factors—Helicobacter pylori, NSAIDs, gastric acid—and defensive factors—mucin, bicarbonate, and prostaglandins. A gastric ulcer, also called a peptic ulcer, is a small section of the stomach's lining that has eroded. It causes stomach pain, potential bleeding, and other gastrointestinal symptoms. The Helicobacter pylori (H. pylori) bacteria-related stomach infection is the most frequent cause of gastric ulcers. By reducing mucus production in the stomach,

indomethacin (5mg/kg p.o.) administration for 14 days produces gastric ulcers in Wistar rats. In both people and experimental animals, non-steroidal anti-inflammatory medicines (NSAIDs) like indomethacin caused damage to the gastrointestinal mucosa, and their usage is linked to a high risk of haemorrhage, erosions and perforation of both stomach and intestinal ulcers. The molecular foundation for these effects is thought to be their inhibition of mucosal blood flow, disruption of the microcirculation, reduction in mucus secretion, lipid peroxidation, and activation of neutrophils. The current study aimed to evaluate the effects of adenosine with the widely used treatment for stomach ulcers, lansoprazole, on rats treated with indomethacin. We found that adenosine had a good impact on changing a number of gastric ulcer-related parameters, including pH, mucin percentage, ulcer score, ulcer index, and ulcer inhibition %. Additionally, adenosine exhibits beneficial effects on biochemical indicators like MDA, MPO, GSH, and SOD. The treatment of theophylline, a Purinergic receptor antagonist, has abolished the cytoprotective effect of adenosine in a significant dose-dependent manner, thereby clearly implicating the potential role of purinoceptors in gastric ulcers. Therefore, the present data demonstrated that adenosine has beneficial effects on Gastric ulcers. Also, the present data demonstrated the possible role of purinergic receptors in the cytoprotective effect of indomethacin induced gastric ulcers.

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