Five Different Noise Intensities Robustly Affects Corticosterone, Gastrin and Endothelin-1 Responses and Initiated Gastric Tissue Damage in Wistar Rats

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Authors' contributions

Article Information

Original Research Article

ABSTRACT

Likewise other stress response noise stress is also affects the homeostasis of the biological systems and produce stress response in the form of Corticosterone to prevent the damage but if the exposure is longer with higher magnitude it may disrupt the robust ability of the homeostasis and could produce the damage to the biological systems. The goal of our study was to see how five different noise intensities affected stomach tissue damage. 42 healthy rats were divided into five different stress exposure group, normal control (NC) and sham control (SC) groups. Noise stress exposure was delivered for 1 hour per day continued for 30 days in all five noise exposed groups

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by specially designed noise chamber whereas sham control group of animals kept in noise chamber for 1 hour per day continued for 30 days without noise stress exposure and control group of animals neither exposed to noise stress of any intensities and nor kept in noise chamber without noise but remain in the same experimental room in their homecage for 30 days respectively. Results of the study showed that animals exposed to 60 and 80 dB noise give habituated and not significant Corticosterone, Gastrin and Endothelin-1 responses compared to NC and SC groups while animals exposed to 100, 120 and 140 dB had significantly higher Corticosterone, Gastrin and Endothelin-1 response and also chronic gastric damage was observed compared to later two noise exposed groups respectively. Study concluded that not only higher but also lower noise intensities also initiated the gastric damage even after the adaptation.

1. INTRODUCTION

The HPA axis is a major hormone system that is constantly activated in response to stress. When the HPA axis is activated, it secretes cortisol, which has receptors in almost all cell types and has broad impacts on immunity, metabolism and behavior, all of which aid in stress management [1]. We are now living in an environment in which we are surrounded by different types of stresses. As easy as modernity is, it is also the mother of many diseases. It is very difficult to know when the environment by which we are surrounded, initiates any disease because our surroundings now carried many triggering factors that may does possible pathogenesis of any disease. Not only mental stress, but many elements present in the environment which are the product of modernity, all of them have a bad effect on human and animal and so they all may considered as "stresses". One of the major and unavoidable stresses is "noise stress" that may affect the integrity of the living systems. There are many sources of noise from which it can be evolved and unfortunately, intentionally or unintentionally humans are in contact with this noise pollution. Noise pollution is the addition of many noise sources present in our surrounding environment in today's era. Noise pollution, whether it is from traffic or from a rock concert, whether we want it or not, it has an effect on biological systems. So, noise, which is defined as disruptive and intrusive sound, is regarded as a stressor and annoyance in the environment. Noise is a common feature of many modern communities and workplaces. Its harmful effects, including the formation of free radicals, are not restricted to the auditory system. The response to noise may be influenced by sound qualities such as loudness, frequency, sound complexity and duration. Noise exposure like any nature that surpasses 90 decibels has been identified as a stressor [2]. Noise exposure causes a variety of health issues, including deafness, poor sleep, and behavioral decline, as well as altering intellectual function. It also causes coronary heart disease, hypertension, higher death risk, significant psychiatric effects, headache, anxiousness and vomiting etc [3, 4]. Some research is being done to see if noise pollution near airports is linked to an increased risk of hypertension, cardiovascular disease, and cancer [5, 6]. Although hearing issues receive the majority of medical attention, non-auditory effects of noise, such as stomach symptoms and lesions, have long been documented. It has been documented that noise-exposed animals and humans both have gastric lesions [7, 8]. Additionally, gastrointestinal complications such as peptic ulcers, gastrointestinal motility disorder (GIMD) and gastritis are the major issues reported after the exposure with intense (high) levels of noise in populations [9, 10]. Hearing impairment, disturbed sleep, immunological function, hormone levels, psychological disorder, circulatory and respiratory systems are all well-known noise consequences [11, 12]. Noise exposure has been found to stimulate the hypothalamic-pituitary-adrenal (HPA) axis [13, 14, and 15] and the hypothalamus plays a important role in the pathophysiological effects of noise. Furthermore, the human autonomic nervous system, human auditory system and gastrointestinal tract are connected via enormous neuron and it has been reported that noise affects the gastrointestinal function through these connection [16, 17]. Some previous studies demonstrated the effects of noise on motilin, somatostatin (SS), substance P (Sp), gastrin, endothelin (ET), and nitric oxide (NO) plasma levels in the gastrointestinal system [11,18] and reports are also justify the fact that intestinal microvilli are destroyed by low-frequency noise, resulting in duodenal lesions. Furthermore, noise, particularly explosive noise, causes an increase in stomach acid secretion and

gastrointestinal transit [10, 19]. According to a study, road noise (traffic) increased both basal and induced stomach acid output [20].

Till now mostly research work that has been done so far has been utilized high level noise intensities, a previously reported study showed that noise-stress of 100 dBA (4hr/day) for thirty days could significant increment in working and reference memory error [21] but the environment in which we live contains many (low and high) levels of noise intensities and it is very difficult to find out that the effects that have happened in the human and animals body are completely results of which noise intensities and according to a report if the sound stressor is of sufficient duration, amount, and quality, the defensive response might become the stress that leads to the General Adaptation Syndrome, which was discussed more thoroughly by Hans Selye with its alarm, resistance, and exhaustion stages [22]. The hypothalamic-pituitary-adrenal axis is activated, resulting in an increase in adrenal cortisol and epinephrine secretion. In experimental animals, these endocrine alterations may cause gastroduodenal ulcers and renal abnormalities after extended exposure to loud sounds [23]. It may, however, have systemic effects, such as launching of HPA-axis that initiates the release of Corticosterone (in rodents), Gastrin and Endothelin-1 [24, 25]. As a result, the goal of this study was to see how some different level of noise intensities affected HPA-axis and results in gastric tissue damage. Our previous research investigated the 30 days noise exposure effects of five different noise intensities *viz*. 60, 80, 100, 120 and 140 dB onto the body weight, food and water consumption, and adrenal gland weight in wistar rats and concluded that the animals exposed to these five noise intensities initially showed reduction in body weight, food and water consumption after 15 days of exposure, two lower noise intensities likely 60 and 80 dB exposed group rats maintain the robust homeostasis towards the noise intensities delivered to them and improved in body weight gain, food and water consumption whereas two higher intensities noise stress exposed group (120 and 140 dB) did not adapted to the delivered noise stress and reduction in their body weight and food and water consumption recorded till 30th day respectively [26]. In connection with our previous study, we designed this study with an aim to analyzed the effects five noise intensities (60, 80, 100, 120 and 140 dB) on Corticosterone (CORT), Gastrin (GAST) and Endothelin-1 (ET-1) to determine

whether exposure to lower to higher (60-140 dB) noise intensities could produce adaptation to maintain robust the stress response or initiate the gastric damage of wistar rats. Thus, our study enlighting the path for the development of such noise induced stress animals model that robustly resembles the living organism's surrounding noise condition and developes understanding regarding noise induced stress health issues and may help in screening of futuristic anti-stress treatments.

2. MATERIALS AND METHODS

2.1 Experimental Animals

The rodents used were 42 Wistar male rats weighing 291-296 g. The rats were kept in prescribed room temperature with a 12 hour light and dark cycle in the groups of six rats per cage (lights on at 07:00 am). The animals were had freely accessed with prescribed feed and water except on the first, 14th, 29th and 14th day (post noise day) when they were deprived of food. The experimentation followed the CPCSEA rules for the care and use of laboratory animals and experimentation was initiated after the approval of IAEC (Approval number: KNIMT/PHAR/IAEC/18/03) of Deparment of Pharmaceutical Sciences, Kamla Nehru institute of Management and Technology, Sultanpur, Uttar Pradesh, India. The animals were divided into seven groups with 6 animals in each group. The control group was not exposed to noise similarly sham control group was not exposed to noise but kept in specially designed noise chamber for 1 hour per day continued to 30 days while the animals in the other five groups were subjected to 1hour per day (from 23:30 to 07:30) noise exposure of 60, 80, 100, 120 and 140 dB noise intensities for 30 days. After the exposure of noise stress for 30 days in noise exposed group and in sham control group, post noise stress and post habitat change effect was also evaluated on 7th and 14th day respectively. To established the statistical equality normal control group rats were also utilized till next 14th day after the 30 days of experimentation.

### 2.2 Designed Noise Chamber

To provide the noise stress, double-layered wooden rectangular box with dimensions of 28.8x18x18 inches was built. A hard-wearing material (ceramic dust) was imbedded and pasted between the two hardwood layers, and a black colour fabric sheet was used to cover the

entire plane of the rectangular box (inside and out). A riveted aluminium frame was used to cover the entire length of the noise box. For opening and closing the noise chamber, a door (with a handle) was supplied. The noise box's right-side plane had a magnet on the inside edge, so the door had to be closed carefully. With the help of a small exhaust fan, a ventilation system was installed in the noise chamber on the rectangular box's ceiling. For lighting, a five-watt LED lamp was installed within the noise chamber. A small wooden box was used to house an amplifier circuit, which was mounted to the noise chamber's upper roof surface. The noise chamber was powered by a 12 Volt 9 Amp battery.

The Gold Wave Digital Audio Editor Software was used to rewrite the noise and achieve a steady noise at 60, 80, 100, 120, and 140 dB levels. Bluetooth device used for noise input and it was connected to the amplifier. A sound controlling knob was fixed in the amplifier box and connected to the amplifier circuit. The 150 Watt speaker was linked to the amplifier circuit and utilized as an output source to generate the noise stress. The speaker source was situated 30 cm away from the rat cage within the noise chamber. A microphone was placed inside the noise chamber and connected to a mobile phone that has a noise level meter application installed to measure the noise level inside.
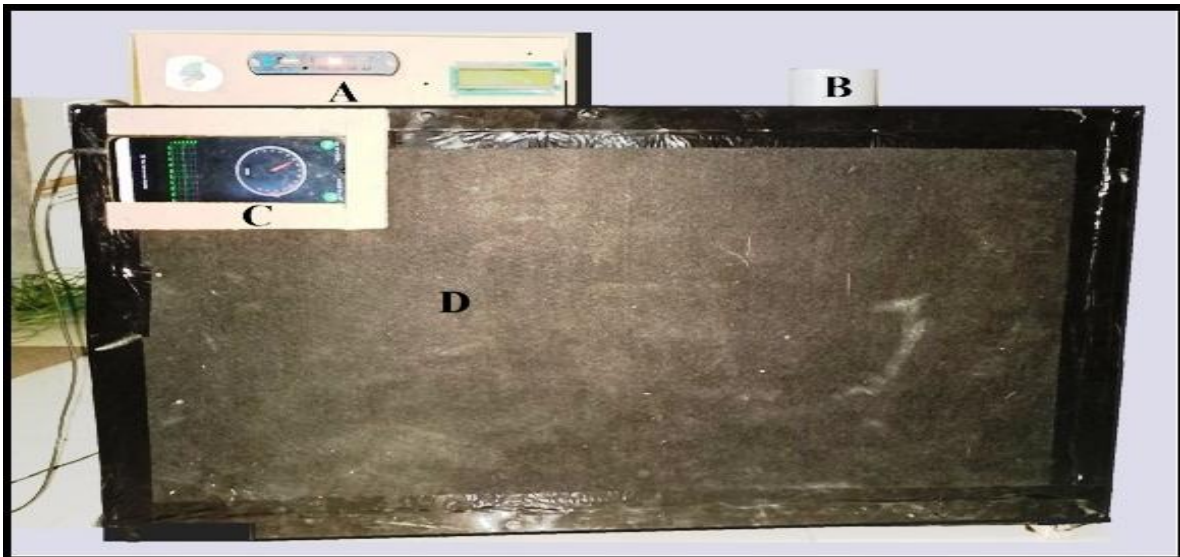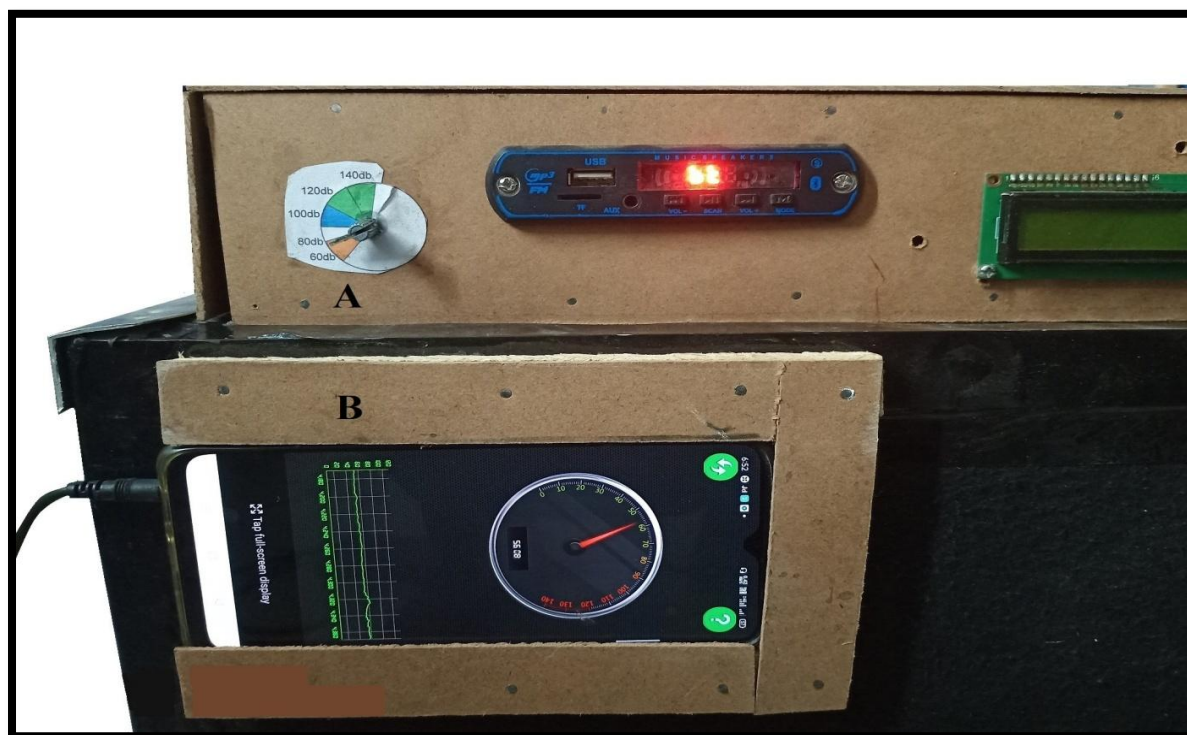

Image 1. Showing different parts of noise chamber (A) amplifier (B) Ventilation (C) Noise Level Meter (D) Body of noise chamber
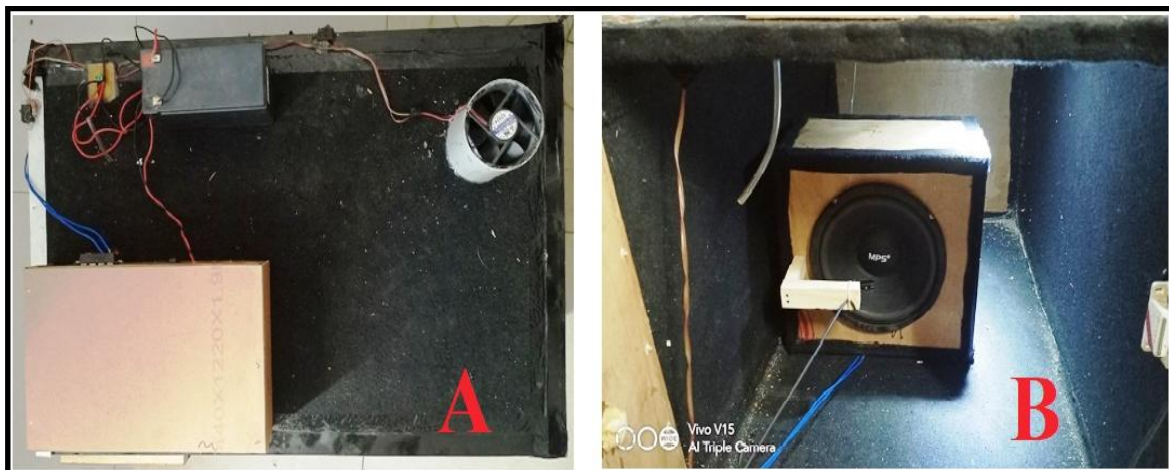
Image 2. Showing roof of noise chamber with amplifier, battery and ventilation system (A) and internal view of noise chamber with speaker (B)

Image 3. Showing noise controlling knob with marking (A) and mobile based noise level meter (B)

A mobile-based application was utilized to monitor and estimate noise levels. The software developer tested the correctness of the application (Sound Level Meter) with the help of another noise level meter (Bosch Pvt. Ltd). The construction and fabrication of noise chamber and development of mobile based noise level estimation (dB) was done by allproject.in firm in Meerut, Uttar Pradesh.

2.3 Schedule of Noise Exposure and Collection of Blood Serum

Noise exposure group exposed to five different noise intensities i.e. 60, 80, 100, 120 and 140 dB (1 hour/day for 30 days). 1 ml of blood sample was withdrawn from tail vein after mild anesthesia for the estimation of serum Corticosterone and serum Gastrin and Endothelin-1 concentration was estimated in blood collected retro-orbital plexuses respectively [27]. Blood samples were collected on 1st, 15th and 30th day during the noise exposure for the estimation of serum Corticosterone level and noise exposure was stopped after 30 days. Collection of blood samples were also done during a post noise days i.e. 7th and 14th day to estimate the post noise effect on serum

Corticosterone whereas, serum Gastrin and Endothelin-1 level were estimated on the 15th day of post noise day in all noise exposed groups respectively. Likewise, serum Corticosterone, Gastrin and Endothelin-1 level was estimated in normal control group and sham control group without exposed to noise but later one only kept in to the noise chamber for 1hour per day for 30 days respectively.

2.4 Estimation of Serum Corticosterone, Gastrin and Endothelin-1

Estimation of serum Corticosterone, Gastrin and Endothelin-1 was outsourced from SRL Diagnostic, Mumbai by Archita Diagonestic, Meerut by ELISA. Briefly, the concentration of the Corticosterone was detected by competitive enzyme-linked immunoassay (ELISA) with a CORT antibody (rabbit polyclonal). Each well was filled with samples (or standard) and conjugate, and the plate was incubated at room temperature for 1 hour without being blocked. The wells of ELISA plates were rinsed with buffer many times till a suitable colour production and then optical density was deliberated at 450 nm using an ELISA reader. In the same manner, a Gastrin 1 Rat in vitro competitive ELISA

(Enzyme-Linked Immunosorbent Assay) kit was used to detect Gastrin concentration using a goat anti-rabbit IgG antibody and an Endothelin-1 ELISA kit was used to determine the endothelin-1 levels (ET-1). 96-well plates were precoated with ET-1 specific antibody. The microplate was then incubated at room temperature with the standards and test samples, as well as an HRP-conjugated I Endothelin 1 detection antibody. TMB is utilized to visualize the HRP enzymatic activity after binding proteins have been removed by washing. TMB was catalyzed by HRP, resulting in a colourful product that changed colour when an acidic stop solution was added. The density of colouring was related to the amount of Endothelin 1 sample collected in the plate.

2.5 Histopathological Examination

The excised tissue were cut in to pieces and stored in freshly prepared formalin solution for interpretation of gastric damage. The histopathology of all the specimens was carried out by Pet Vet lab, Mumbai. Briefly, striped cut gastric tissue embedded into paraffin wax block and a fine section of 4-5 μm thickness were obtained and stained by Heamatoxylin and Eosin dye and fixed over a glass slide and observed under a light microscope with a magnification of 200X for interpretation of gastric tissue damage.

2.6 Statistical Interpretations

Graph Pad Prism version 5.03 was used to calculate the experimental data collected during the experiment. The mean±SEM was used to evaluate all experimental data. One-way ANOVA followed by Bonferroni multiple comparison tests was used to compare group data and $p < 0.05$ was considered the significant value in all cases.

3. RESULTS

The noise stressed groups (60, 80, 100, 120 and 140 dB) had obviously highly significant ($p < 0.0001$; Fig. 1 A, B) CORT concentration on 1st and 15th day when compared to NC and SC group, however the CORT concentration among NC and SC groups was found not significant ($p > 0.05$; Fig. A, B, C, D, E) on 1st, 15th, 30th and post days i.e. 7th and 14th day respectively. The concentration of CORT was statistical not significant ($p > 0.05$; Fig. 1 A and B) between 60 and 80 dB on 1st and 15th day but the CORT concentration was significantly higher ($p < 0.0001$)

in 100, 120 and 140 dB when compared to 60 and 80 dB noise exposed groups on 1st and 15th day (Figs 1. A & B) , similarly the CORT concentration was significantly higher in 120 and 140 dB noise exposed groups when compared to 100 dB noise exposed group on 1st day (Fig. 1 A) while there was no any statistical differences observed in CORT concentration in between 100 and 120 dB noise exposed group and in contrast the CORT concentration was significantly higher in 140 dB noise exposed group when compared to 100 dB noise exposed group on 15th days (Fig. 1 B) respectively, however no any significant differences noted in CORT concentration in between 120 and 140 dB noise exposed groups on 1st day (Fig. 1 A) respectively. On 30th day, the CORT concentration observed was not significant ($p > 0.05$) in 60, 80, 120 and 140 dB noise exposed groups when compared to NC and SC, whereas 100 dB noise exposed group had significantly ($p < 0.05$) higher CORT concentration when compared to NC, SC and 140 dB noise exposed groups however there were no any significant differences ($p > 0.05$) in CORT concentration were observed between 60 versus 80, 100, 120 and 140 dB; 80 versus 100, 120 and 140 dB and 120 versus 140 dB noise exposed groups (Fig. 1 C) respectively.

The CORT concentration, after post noise days (Fig. 1 D & E) i.e. 7th and 14th was not found statistically significant ($p > 0.05$) in between all respective groups.

As shown in Fig. 2 A & B, the serum Gastrin and Endothelin-1 concentration were statistically not significant ($P > 0.05$) in between NC and SC; 60 and 80 dB noise exposed groups and 120 and 140 dB noise exposed groups, similarly the serum Gastrin and Endothelin-1 concentration of 60 and 80 dB noise exposed groups was not significant ($p > 0.05$) when compared to NC and SC in contrast the 100, 120 and 140 dB noise exposed group had significantly higher ($p < 0.0001$) serum Gastrin and Endothelin-1 concentration compared to NC & SC and 60 & 80 dB noise exposed group but there were no any significant differences ($p > 0.05$) found in serum Gastrin concentration when compare 100 dB noise exposed group with 120 & 140 dB noise exposed groups and 120 and 140 dB noise exposed groups (Fig. 2 A) but serum Endothelin-1 concentration was significantly higher ($p < 0.0001$) in 120 and 140 dB noise exposed group when compared to 100 dB noise exposed group (Fig. 2 B) on 15th (post noise day) respectively.
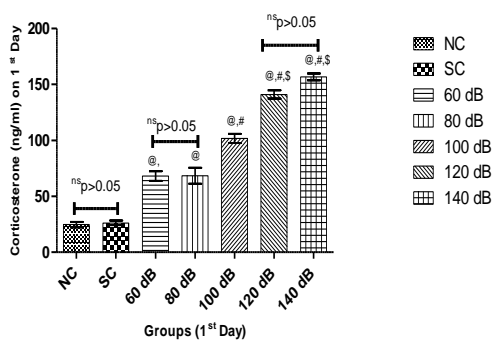
A: Corticosterone level between normal control (NC) versus Sham control (SC) not significant (nsp>0.05); 60 dB versus 80 dB not significant (nsp>0.05); 120 dB versus 140 dB not significant (nsp>0.05); whereas @p<0.0001 when compared with NC and SC; #p<0.0001 when compared with 60 and 80 dB; $p<0.0001 when comapred 100 dB with 120 and 140 dB on 1st day respectively
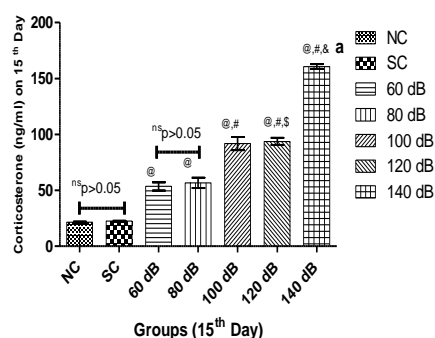
B: Corticosterone level between normal control (NC) versus Sham control (SC) not significant (nsp>0.05); 60 dB versus 80 dB not significant (nsp>0.05); whereas @p<0.0001 when compared with NC and SC; #p<0.0001 when compared with 60 and 80 dB; $p>0.05 when comapred 100 dB with 120 whereas &p<0.0001 between 100 dB and 140 dB and ap<0.0001 when compared 120 and 140 dB on 15th day respectively
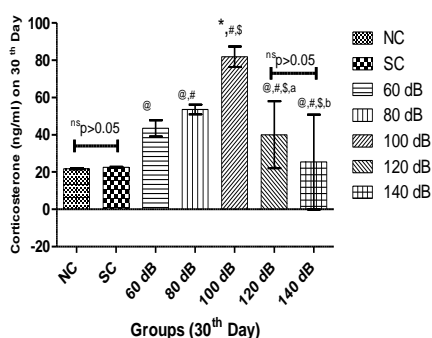
C: Corticosterone level between normal control (NC) versus Sham control (SC) not significant (nsp>0.05); whereas @p>0.05 when compared with NC and SC; *p<0.05 when compared 100 dB with NC and SC #p>0.05 when compared 60 dB versus 80, 100, 120 and 140 dB; $P>0.05 when compared 80 dB with 100, 120 and 140 dB; ap>0.05 when compared 100 dB and 120 dB; bp<0.05 when comapred 100 dB 140 dB; whereas nsp>0.05 between 120 dB and 140 dB on 30th day respectively
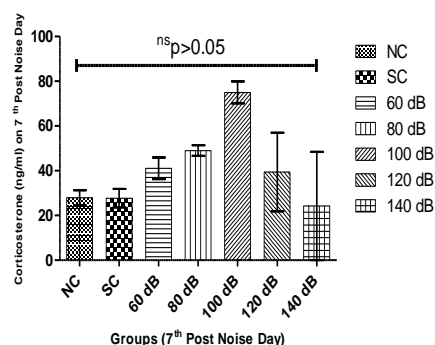
D: nsp>0.05 when compared all groups with each others on 7th post noise day
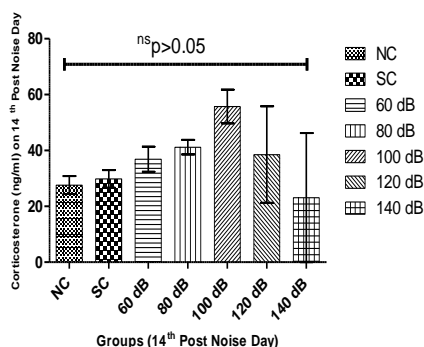
E: nsp>0.05 when compared all groups with each others on 14th post noise day

Fig. 1. Represents the outcome of noise stress on serum Corticosterone concentration of blood of Normal control (NC), Sham control (SC), 60, 80, 100, 120 and 140 dB noise on the rats on 1st (Fig. A), 15th (Fig. B), 30th (Fig. C) (noise stressed days) and 7th (Fig. D), 14th (Fig. D) (post noise day), whereas NC and SC did not exposed to noise respectively. Results in graphs are indicated as mean ± SEM. For n = 6, One-way ANOVA and Bonferroni Posttest were used to analyze significant differences

A

ns p>0.05: not significant in each represented case
a p>0.05: when compared with NC and SC; @ p<0.0001 when compared with NC and SC
c p<0.0001 when comapred 60 and 80 dB with 100, 120 and 140 dB;
p>0.05 when comapred 100 dB with 120 and 140 dB on 15 th (post noise day) respectively

B

ns p>0.05: not significant in each represented case
a p>0.05: when compared with NC and SC; @ p<0.0001 when compared with NC and SC
b p<0.0001 when comapred 60 and 80 dB with 100, 120 and 140 dB;
# p<0.0001 when comapred 100 dB with 120 and 140 dB on 15 th (post noise day) respectively
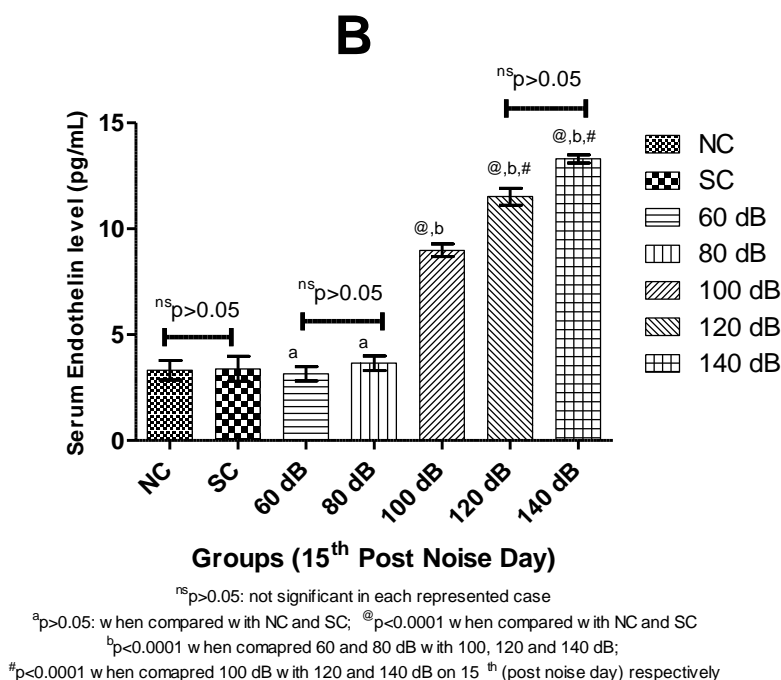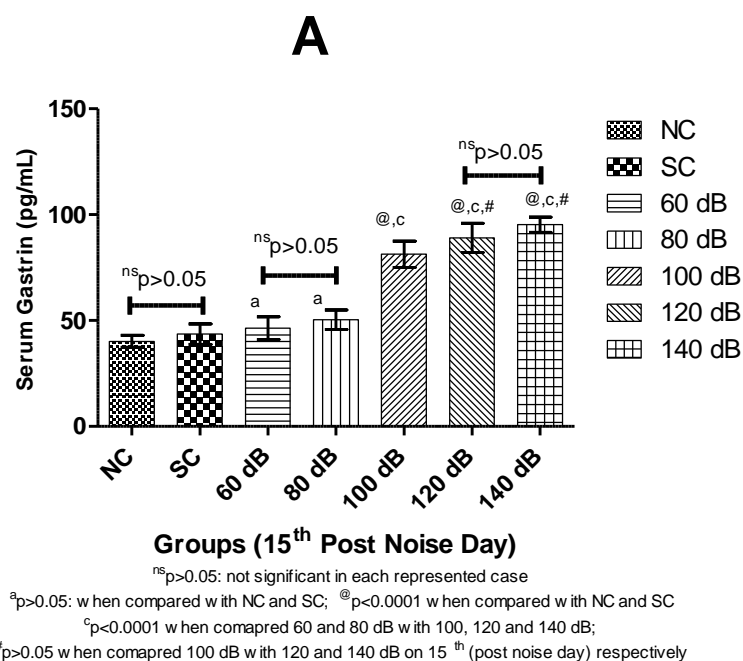
Fig. 2. Represents the concentration of serum Gastrin (Fig. A) and serum Endothelin-1 (Fig. B) of Normal control (NC), Sham control (SC), 60, 80, 100, 120 and 140 dB noise of the rats on 15th (post noise day) whereas NC and SC did not exposed to noise respectively. Results in graphs are indicated as mean ± SEM. For n = 6, One-way ANOVA and Bonferroni Posttest were used to analyze significant differences

The mucosa of gastric tissue of both (NC & SC) group rats appeared normal hence there were no any sign observed of hemorrhage, perforation, tissue damage and ulcerations, even the both groups (NC & SC) gastric tissues are pink in colour (Image 4; left hand side) and in similar manner pathology of gastric mucosa of both (NC & SC) groups rats showed that the epithelium linings are intact and normal gastric pits, glands and submucosa. Additionally mucosal histology

was showed normal microscopic architecture that shows no any signs of damages. However, some neutrophillic infiltration were seen in gastric mucosa and submucosa in both the rats tissues (Image 4; right hand side) respectively.
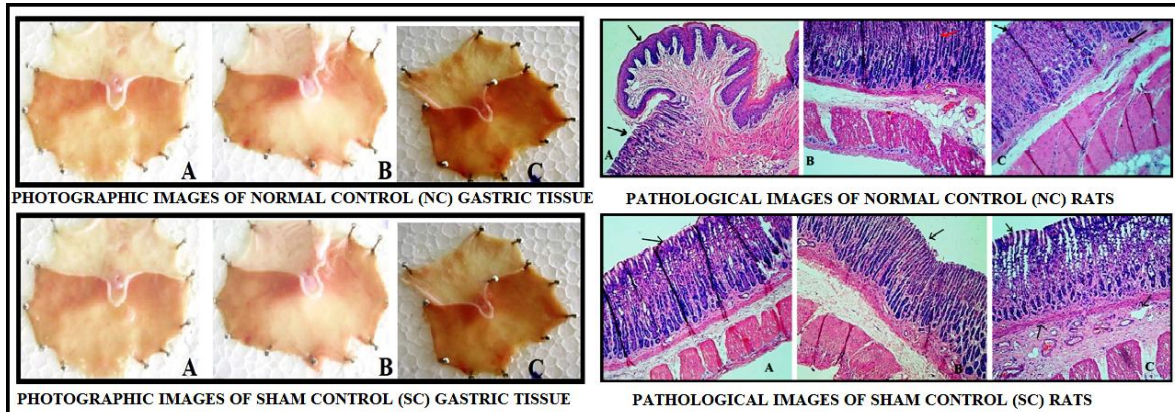
Image 4. Showing photo of NC & SC rats (Left hand side) and pathological examination (H & E stained) of gastric mucosa of NC & SC rats (Right hand side)
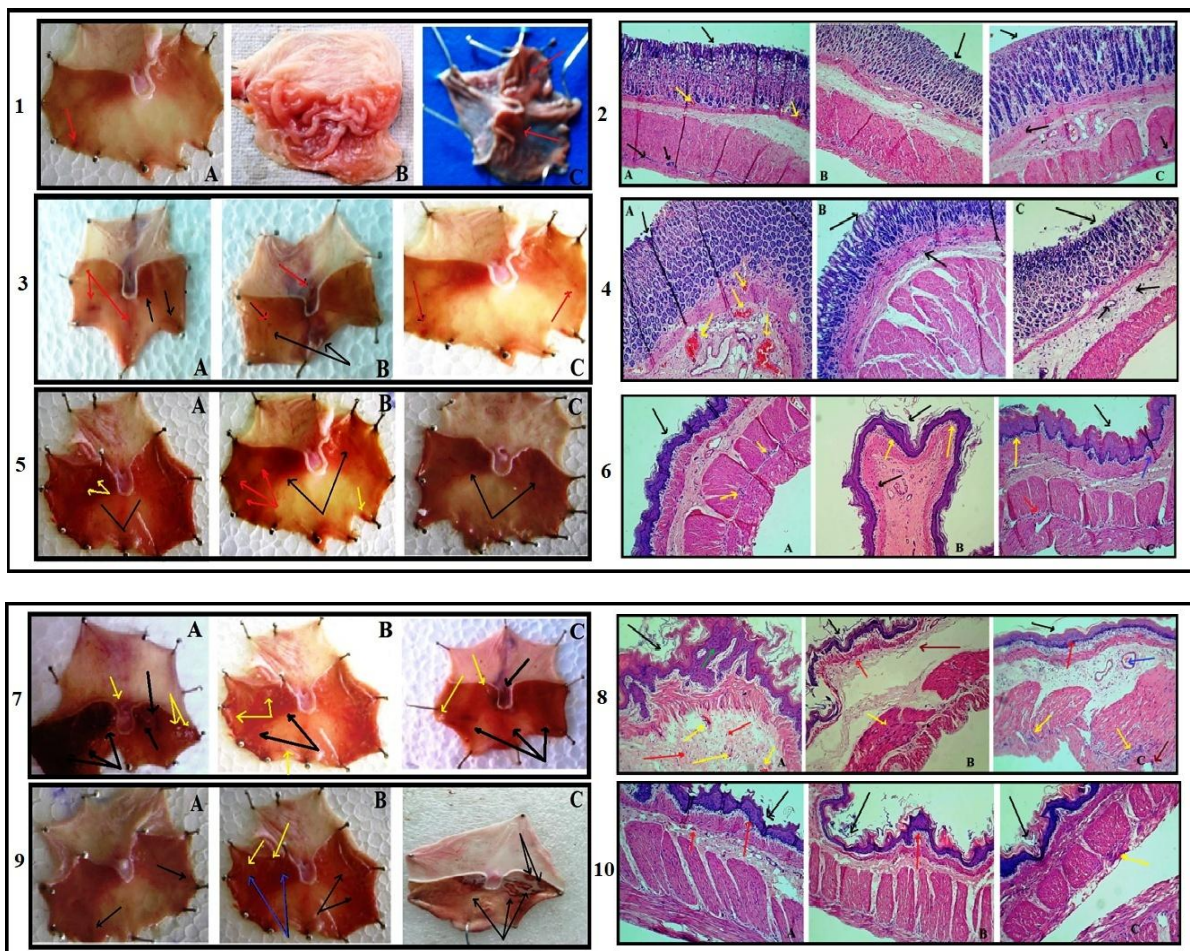
Image 5. Showing photo of 60, 80, 100, 120, 140 dB noise exposed rats (1, 3, 5, 7, 9; left hand side) and pathological examination (H & E stained) of gastric mucosa of 60, 80, 100, 120, 140 dB noise exposed rats (2, 4, 6, 8,10; right hand side)

The Image 5 (1, 3, 5, 7, 9; left hand side) represents the photographic pictures of gastric mucosa of 60, 80, 100, 120 and 140 dB noise exposed groups for 1h/day for 30 days respectively. The 60 and 80 dB noise exposed groups were showed some spot ulcers but not of perforated grade but multiple spot ulcers of perforated grade were evident on gastric mucosal tissue of 120 and 140 dB noise exposed group (Image 5 of 7 and 9; left hand side) additionally, multiple gastric ulcers but not perforated grade were seen on the gastric mucosa of 100 dB noise exposed rats (Image 5 of 5; left hand side). The gross examination confirm that the colour of gastric mucosa was appeared reddish brown and some mild to moderate hemorrhagic cluster was seen on the mucosa of 60 and 80 dB noise exposed rats (Image 5 of 1 & 3; left hand side) whereas, the colour of gastric mucosa was deeply reddish brown and intense haemorrhagic clusters were evident on the mucosal layer of 100, 120 and 140 dB noise exposed groups (Image 5 of 5, 7, 9; left hand side). Some mild to moderate edema pouch were seen on the gastric mucosal layer of 100 dB and 140 dB noise exposed rats (Image 5 of 5 & 9; left hand side) respectively.

The histology of 60 and 80 dB noise exposed rats showed normal epithelium lining and gastric mucosa (Image 5 of 2 & 4; right hand side), only a single rat histology (Image 5 of 4C; right hand side) showed mild damaged to the mucosal thickness whereas, microscopic examination of 100, 120 and 140 dB noise exposed rats evident the severe disruption of mucosa and surface epithelium layer, mild focal edema and MNC infiltration were seen at sub mucosal layer (Image 5 of 6, 8,10; right hand side). Mild neutrophilic infiltration were observed with MNC in 60 and 80 dB noise exposed rats (Image 5 of 2 & 4; right hand side) whereas severe neutrophilic infiltration with MNC were observed on mucosal thickness, hypertrophied and congested blood vessels, coagulative necrosis of entire mucosal thickness was seen in 100, 120 and 140 dB noise exposed rat of 100, 120 and 140 dB noise exposed rat (Image 5 of 6, 8, 10; right hand side). The histology of gastric tissue revealed that the 60 and 80 dB noise exposed rats initiated the inflammatory phase (Image 5 of 2 & 4; right hand side) whereas formation of ulcers, severe inflammatory reaction and the morphological changes in 100, 120 and 140 dB noise exposed rats leading to mild ulcerative-gastritis (Image 5 of 6, 8, 10; right hand side) respectively.

4. DISSCUSSION

The HPA-axis is a regulatory and the living organism's central control system that joint central nervous system to the hormonal system. This stress-responsive neuroendocrine system helps living organisms adapt and maintain or restore homeostasis after a stimulus. It is also critical for maintaining the physiological system's normal functioning. The end product, Cortisol (in human) or Corticosterone (in rodents), has a wide range of physiological impacts on the body system. This end product (cortisol or corticosterone) is important in metabolism because it provides energy, which aids in overcoming the demanding host's high metabolic demand. It can also control other vital physiological systems like the SAM (Sympathetic-Adrenal-Medullary Axis), the immunological system, the cardiovascular system, and the cognitive process. Corticotropin-releasing hormone (CRH) is released by the hypothalamus in response to stress, prompting the pituitary to release adreno-corticotropic hormone (ACTH). The adrenal cortex is forced to secrete glucocorticoids by ACTH. Cortisol is the most common glucocorticoid in humans [28]. In general terms, noise affected the living systems via its auditory and non- auditory approaches. The activation of HPA-axis is in connection with the auditory and non auditory response. Noise is one of the most important variables that cause stress in the human body as well as a variety of non-auditory consequences on the GI tract [29, 30]. The alerting response of non-auditory approach activates the HPA-axis. A shunting of blood away from vegetative regions like the stomach, an increase in adrenalin, noradrenalin, and 17-hydroxycorticosteroids, and a release of glucose into the blood stream are all examples of these reflexes [31, 32]. These hormones stimulate the activity of the HPA-axis and the production of Corticosterone from the adrenal cortex (mostly in rodents) [33]. As a result, noise may alter the HPA-axis via the auditory system, leading to elevated Corticosterone levels in rodents [34] which were also confirmed in our present study. Our findings reveals that noise stress of 60, 80, 100, 120 and 140 dB significantly elevates the level of serum Corticosterone in the stressed groups than that of normal and sham control groups on 1st day and that may be due to activation of HPA axis. The higher intensity noise stress could activates HPA-axis more aggressively and it was established in our study that elevated level of Corticosterone in noise exposed groups was

directly proportional to the noise intensities applied because 120 and 140 dB noise exposed rat had significantly higher Corticosterone level among all other noise exposed rats on 1st day (Fig. 1 of A) respectively. Constraint to the later consequences, our results are in line with the documented fact that the activation of HPA-axis is depends upon the form, length, and intensity of the stressor [35, 36]. Here, in our results it may noted that there were no significant differences observed in between NC and SC, 60 and 80 dB noise exposed group and 120 and 140 dB noise exposed group on 1st day respectively. In case of SC rats it may be suggested that the change in habitat of rats of SC group from their homecage to designed equipment for 1 hour per day till 30 days does not impose any elevation in stress response and in case of noise exposed rat the 80 and 140 dB noise were give similar effects on Corticosterone level as like 60 and 120 dB because the mean differences of Corticosterone were significantly not differ when analyzed on 1st day respectively (Fig. 1 A) however the Corticosterone concentration of all noise exposed groups was still significantly higher than that of NC and SC rats but based upon the noise intensities used, the level of Corticosterone was significantly higher in 100, 120 and 140 dB noise exposed rats compared to NC, SC, 60 and 80 dB on 1st day respectively. Results of our study shows that the mean Corticosterone was reduced on 15th day compared to 1st day in 60, 80, 100 and 120 dB noise exposed rats but not in 140 dB noise stressed rats but on 15th day there was no any significant difference in Corticosterone level observed between 100 and 120 dB noise exposed rats and on 15th day again, 60 and 80 dB noise exposed rats had no significant differences in their Corticosterone concentration respectively. This reduction in Corticosterone concentration indicated that may the animals gets habituated to the exposed noise stress in fifteen days and give habituated Corticosterone response. This event may be because that adaptation of the HPA axis to changes in physiological and behavioral responses to acute and chronic stress. Rodents routinely subjected to restraint stress showed a habituated Corticosterone response when challenged with acute restraint stress, demonstrating that the HPA response becomes desensitized or stable when the same stressor is repeated [37].

On 30th day, surprisingly, there were no any significant differences in the Corticosterone concentration observed in between 60, 80, 120 &

140 dB noise exposed rats when compared to each others. The ELISA did not detected the Corticosterone concentration in serum sample of two and five rats of 120 and 140 dB noise exposed group so the mean value of Corticosterone reduced of later two groups respectively. These consequences may suggest that CRF and the glucocorticoid receptor are both located in the cochlea. Furthermore, CRF expression in the cochlea has been described, showing that the cochlea expresses both the start (CRF) and the end (glucocorticoid receptors) of systemic HPA axis signalling [38]. The decrease in Corticosterone level is assumed to have occurred as a result of the loss of the auditory system in those rats (120 and 140 dB exposed), which resulted in a diminished stress response. There is broad agreement that prolonged exposure to sound levels less than 70 decibels (dB) does not cause hearing damage but sound levels over 85 decibels for longer than 8 hours can be detrimental to the auditory system [39]. The mean Corticosterone of 60 and 80 dB noise exposed rats was reduced compared to 1st and 15th day and similarly the mean Corticosterone concentration of 100 dB noise exposed rats was also reduced compared to 1st and 15th day but still 100 dB noise exposed rat had significant higher Corticosterone level when compared to NC & SC rats and from 60, 80, 120 and 140 dB noise exposed rats on 30th day respectively. The post noise effects were also evaluated after 30 days of continues noise exposure (1 hr/day) further on 7th and 14th day in all noise exposed groups in our study and results obtained reveled that there were no any significant differences observed in Corticosterone concentration in all noise exposed groups when compared to NC & SC and each other on 7th and 14th post noise days (Fig. 1 D and E) respectively. Our study reveals that, repeated single noise intensities induced adaptation called syndrome of adaptation in 60 and 80 dB noise exposed rats and following exposure to 100, 120 and 140 dB noise intensity showed exhaustion results gastric tissue damaged in rats. These events of our study are in line with the "General Adaptation Syndrome" (GAS) theory given by the Hans Selye, according to his theory, biological systems follows three stages during the any stress; Alarm, Resistance and Exhaustion stage. Initially when a body exposed to the stress (s), activation of sympathetic nervous system is occurred and Cortisol (in human) or Corticosterone (in rodents), adrenaline and nor adrenaline hormones are come in to force to release in systemic circulation and exhibits "fight

or flight" responses. If a stressful event persists, the body reacts to the stressor and adjusts in such a way that the body reaction begins to return to equilibrium and the person remains excited. In response to the stressor, the parasympathetic nervous system in the body returns numerous physiological processes or functions to a normal or average level and cortisol are in continuation to circulate at a prominent level and that indicated the entry of biological system into the resistance stage and it is also called as "Adaptation stage" and in the Exhaustion stage, if the stressor's activity continues to exceed the body's capability, the body's physical and psychological energy utilised to combat the stressor is drained or depleted, and the body begins to create sickness and death. A serious sickness can emerge when an individual's energy for adaptability to combat a stressor is depleted. Short-term stress (acute stress) is unlikely to deplete a person's energy, but long-term stress (chronic stress) can generate a continual state of alert, resistance, or adaptation, which, in turn, can deplete energy and lead to mortality [22]. Constraint with this, our histopathological findings confirmed that 140, 120, and 100 dB noise-exposed groups exhibits glandular damage of gastric tissue (Fig. 2; right hand side), whereas mild inflammation and some hemorrhagic pinpoint ulcers were visible in the 80 and 60 dB noise-exposed groups (Fig. 2 of 2; right hand side) respectively. The gastric damage could also may directly linked to the significant elevation of serum Gastrin and Endothelin- 1 level (Fig. 2 A and B). Altarion of Gastrin and Endothelin-1 in tissue-specific and circulation-specific levels is similarly influenced by hyperactivation of the HPA-axis and elevated CORT levels. The increased level of Corticosterone could play a role in the control of Gastrin and Endothelin-1 secretions [40]. In previously reported study traffic noise stimulates the HPA-axis, resulting in Corticosterone secretion, as well as an increase in gastic pepsin secretion in the 14 and 21-day noise-exposed groups. The vagus nerve in the medulla may be to blame for this impact. The hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated to be stimulated by noise [41, 42 and 43] and it is assumed that the hypothalamus plays a significant role in noise pathology. Additionally, there are numerous neural connections that connect the human auditory system, the autonomic nervous system and the gastrointestinal tract, and it appears that noise and gastrointestinal function interact [44, 45]. Noise has previously been shown to affect

motilin, somatostatin (SS), substance P (Sp), gastrin (GAST), endothelin (ET), and nitric oxide (NO) in the gastrointestinal system [11, 24]. In our study, noise-exposed rats, particularly those exposed to 100, 120, and 140 dB noise, had significantly higher serum Gastrin and Endothelin-1 levels than NC & SC and 60 & 80 dB noise-exposed rats (Fig. 2 A & B), whereas serum Gastrin and Endothelin-1 levels were not significant in NC & SC, 60 & 80 dB noise-exposed group, and 120 & 140 dB noise-exposed group (Fig. 2 A & B) respectively.

Noise stress increases motilin and decreases CGRP (Calcitonin-gene related peptide) in blood plasma, according to the study [46]. As a result, it has an impact on Ca2+ concentrations both inside and outside the cell, which is an important element in relaxation-contraction processes. In an earlier reported study it was proved that water immersion restraint stress decreases the CGRP level and induced the gastric damaged to the experimental rat [47, 48 and 49]. CGRP and motilin are known to be important contributors in the development of gastric and duodenal ulcers. CGRP regulates intracellular Ca2+ homeostasis and decreases the permeability of the cell membrane to Ca2+, in addition to controlling blood current and GI motility. Gastrointestinal stress ulcers are linked to aberrant gut hormone excretion as a result of HPA axis activation. However, in our study we did not evaluated the CGRP and motilin but it thought to be that this may also occurred in our study and could initiated the pathogenesis of gastic damage in noise exposed rats. However, we measured serum Gastrin and Endothelin-1 levels in all experimental groups on the 15th day after the 14th day of post-noise day. It's possible that serum Gastrin and Endothelin-1 levels increased on the first day of noise exposure in 60 and 80 dB because serum Corticosterone levels were significantly higher on that day in these groups, and these groups may have adapted to the respective noise exposure (Fig. 2 A & B). Furthermore, it is possible that during the 30 days of noise exposure at 100, 120, and 140 dB, serum Gastrin and Endothelin-1 levels were higher because 15th day (post noise day) results showed that, serum Gastrin and Endothelin-1 levels were still higher compared to NC & SC and 60 & 80 dB noise exposed rats and even noise exposure was not carried out during post noise days respectively (Fig. 2 A & B).

According to Liu et al. 1998 [50] the possible mechanism causing an increase in Gastrin level

after noise exposure (Explosive noise) is that after explosive noise, the sympathetic nerve system becomes overexcited, inhibition of the vagus nerve occurs, and the level of Cortisol rises, causing dysregulation in the peptidergic system, causing G-cells to stimulate uncontrolled Gastrin that leads to excess of acidic secretion, lowered pH and this results in edema, congestion and finally inflammation in gastric mucosa. Our histopathology findings may confirm these occurrences in 100 (Image 5 of 6), 120 (Image 5 of 8) and 140 dB (Image 5 of 10) noise exposed rats. Although we did not use explosive noise in our study but it is quite possible that 30 days continued exposure to 100, 120, and 140 dB noise (1 hour per day) causes deregulation in the negative mechanism of Gastrin secretion, resulting in an increase in fasting serum Gastrin levels in these subjects. Furthermore, the Endothelin-1 suggested to be play a crucial role in stressed-induced damaged because of its vasoconstrictive properties [51]. Plasma ET levels have been found to rise under a range of stressful events, including constant noise exposure [52], labour anxiety [53], hypovolemic, osmotic pressures and dynamic exercise [54, 55]. Controlling the flow of oxygenated blood to the gastric mucosa is critical for maintaining gastric mucosal integrity, and several vasoconstrictors like Endothelin-1, have been found to have pro-ulcerogenic effects in the stomach [56]. In a previous noise stress study, serum ET levels in the test group were considerably greater than in the control group following exposure to explosive noise, implying that ET had a role in stress ulcer growth. It is most likely because the explosive noise overexcited sympathetic nerves, causing submucosal capillaries to constrict, causing endodermic cells to produce excessive ET, intensifying vasoconstriction and reducing blood flow to the gastric mucosa, eventually leading to ulcer erosion and development [50].

5. CONCLUSION

Our study demonstrated that not only high level noise intensities but lower one could also initiate the pathogenesis of gastric damage even after the adaptation to the noise. We exposed our animals continuously (1hour/day) with different noise intensities for 30 days. In which 100, 120 and 140 dB noise exposed robustly exhibits the severe gastric damage while 60 and 80 dB noise exposed groups were evident mild damage to gastric tissue when analyzed after 15 days of 30 days continuous noise exposure respectively. It

may be proposed on the basis of our study that, of course, the damage is not limited to the high noise intensity level (100, 120 and 140 dB), even a low noise intensities (60 and 80 dB) could start the pathogenesis of the disease even after the adaptation, if an organism stays in it for a long time. Currently in this modern era, where industrial traffic, crowding and some concert noise confer a mixture of noise intensities and a result biological system intentionally or un-intentionally meet with them regularly. Present study also demonstrated that adaptation, of course, helps the body to cope with stress for some time, but if the stress continues, and then biological and molecular damage is sure to happen. Our study insight the futuristic development of such animal models which uses the noise intensities as a stress stimulus to mimics the environmental noise pollution and evaluates the other harmful effects and their treatments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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