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Does Mutual Effect of *Arcobacter butzleri* and Stress in Mice Lead to Gastric Ulcer?

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ABSTRACT

Stress has been suggested to play an important role in the pathogenesis of gastric damage by either acting as a predisposing factor or a primary factor. *Arcobacter* species have frequently been isolated from stomach ulcer cases of pigs, but the role of these agents in the pathogenesis of the disease remains unclear. The primary aim of the present study was, to reveal the role of *Arcobacter butzleri* and stress in the etiology of gastric damage by establishing an experimental mouse design. Infection was induced by intra-gastric gavage of *A. butzleri* in two experimental groups comprising five weeks old specific pathogen-free (SPF) Balb/c mice. At 1, 2, 3, 4, and 7th weeks of the experiment, the animals were euthanized and examined for lesions occurring in the stomach. Histopathology, culture, and Polymerase Chain Reaction (PCR) were employed to detect development and severity of lesions and pathogens. In addition, serum corticosterone levels indicating the presence of stress in the mice were investigated by an ELISA method. Microscopic examination showed that the stomach of the experimental group had inflammatory reactions to varying degrees, but ulcers were not observed in the gastric mucosa of the animals exposed to *A. butzleri* and stress groups. The results suggested that *A. butzleri* and stress were predisposing factors in the formation of gastric ulcer, but failed to provide evidence for their causative role.

1. Introduction

Humans and animals are constantly exposed to acute or chronic stress, which directly or indirectly affects numerous physiological events. All vertebrates give hormonal and physiological responses against stress. The hypothalamic-pituitary-adrenal (HPA) system is active when humans and animals exposed to stress factors such as inactivity, electrical shocks, ether, exercise, cold and food restriction. The activation of this axon

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causes an increase in corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH), endorphins and corticosterone (in rodents) or cortisol (in humans) (McEwen, 2007). These substances protect vertebrates against physiological and behavioral disorders that cause acute stress. For example, they play important roles in the regulation of the circulatory system and metabolic changes that activate glucose depolarizations and additional glucose storage to increase energy in muscle tissue as well as behavioral changes. Chronic elevated glucocorticoids have been associated with a number of physiological consequences that threaten survival, such as hyperglycemia, neuronal cell death, and suppression of the immune and reproductive systems (Wingfield and Romero, 2011). Experimentally, many methods have been used to create stress in animals, and cold stress, a form of stress caused by low environmental temperatures, has often been used (Koo et al., 1986; Aboubakr et al., 2013). Cold stress is among the environmental factors that is frequently encountered in many areas of human activity and has significant effects on the function of various physiological systems in the body. It is known that cold stress modulates the production of interleukin-1β, TNF-α, IL-6 and IL-10 and increasing glucocorticoids, catecholamines, β-endorphin and other stress hormone levels in peripheral blood, impairing motor and cognitive functions (Giagnoni et al. 1983; Ohno et al., 1987; Goundasheva et al., 1995).

Recent studies indicate that, in addition to non-infectious agents, some infectious agents apart from *Helicobacter pylori* may play a significant role in the etiology of gastric ulcers. *Arcobacter* species, the fourth most common bacteria in human gastrointestinal diseases, is one of the most important infectious agents (Vandenberg et al., 2004). *Arcobacter* species, particularly *Arcobacter butzleri*, but also *A. cryaerophilus* have been classified as a serious hazard to human health by the International Commission on Microbial Specifications for Foods. The genus *Arcobacter* currently contains 28 species. Among these, *A. butzleri* is the most common species isolated from humans, animals, and environmental samples [Ongör et al., 2004; Sekhar et al., 2017; Webb et al., 2017). *A. butzleri* can be found as commensals in the gut of humans and animals (Ongör et al., 2004; Sekhar et al., 2017). However, it is most commonly associated with clinical signs such as gastroenteritis, bacteremia, abortion, and mastitis (Collado and Figueras, 2011). Pathogenicity studies performed both in vitro and in vivo have reported that this agent carries many virulence genes and cause inflammatory and pathological disorders in various tissues such as liver, spleen, and stomach [Sekhar et al., 2017; Acik et al., 2016). There are limited studies reporting the presence of *Arcobacter* at high proportions in stomach ulcers and gastritis cases of pigs (Suarez et al., 1997; De Oliveira et al., 2010). The primary aim of this study was to reveal the possible role of *A. butzleri* in the etiology of gastric damage by establishing an experimental mouse model. In addition, serum corticosterone levels, as a stress indicator, were determined by ELISA method in the stress groups.

2. Materials and Methods

2.1. Reference strain

Arcobacter butzleri ATCC 49616 reference strain originating from human diarrheal stool sample (Kiehlbauch et al., 1991) was used in the study. The strain was subcultured using *Arcobacter* Broth media (CM0965, Oxoid, UK) under aerobic conditions at 25 °C for 24-48 h. The bacteria used in the experimental study were inoculated in Tryptic Soy Broth (Merck, 1.05459) and incubated at 30 °C for 48 h under aerobic conditions. Logarithmic phase cultures were obtained by diluting the culture overnight at 1:10 and incubating with shaking for 3 h at 30 °C. Following incubation, the suspension was centrifuged at 4 °C for 20 min at 5000 g. Pelleted bacteria were resuspended in physiological saline and bacterial growth was measured using spectrophotometer (OD 600 nm = 1) as a suspension of approximately 1 x 10^9 CFU/ml, confirming by a standard plate count method. The suspension was immediately administered to the animals under anesthesia by intra-gastric gavage.

2.2. Experimental Study

A total number of 160 five-week old Specific Pathogen Free (SPF) Balb/c mice at the weights ranging from 18 to 22 g were used in the experiments. The animals were housed in

chambers applied at 21-24 °C temperature, 55% ± 10% humidity and, 12 h light and 12 h darkness. All the mice were given sterile water and nutrients *ad libitum* and were housed in standard mouse cages. The animals were divided into 4 groups each containing a total of 40 animals. The first group was named sham and no action was taken. The CS group was exposed to cold stress (2 hours at +4 °C in the cold room every daily) and was also given a total volume of 0.1 ml via physiological saline intra-gastric gavage (Sesti-Costa et al., 2012). The Ab group was infected with bacterial suspension by intra-gastric gavage in a total volume 0.1 ml (10^9 CFU/ml). The CS + Ab group was exposed to cold stress (2 hours at +4 °C in the cold room daily) and was infected with bacterial suspension by intra-gastric gavage in a total volume 0.1 ml (10^9 CFU/ml). To minimize the effect of diurnal factors, the mice were stressed between 9:00 and 11:00. All the processes in each group were performed simultaneously under the same physical conditions. Bacterial suspensions and/or physiological saline were inoculated to animals by intra-gastric gavage using a 20 gauge metal feeding tube. At 1, 2, 3, 4 and 7^{th} weeks of the experimental study, eight animals from each group were euthanized and, half of the stomach specimens of each animal were examined for in terms of lesion development by hematoxylin & eosin staining. The remaining halves of the samples were used for culture and PCR analysis. All the experiments were performed in triplicate. This study was conducted with the approval of Bingol University Animal Experiments Local Ethics Commission, Turkey (20/02/2017 - 02-05).

2.2. Necropsy and Histopathological Examination

For obtaining blood in a comfortable and painless manner, xylazine at a dose of 10 mg/kg and ketamine at a dose of 100 mg/kg anesthesia was administered intraperitoneally with the aid of an insulin injector (Tranquilli et al., 2015). Blood samples collected from the hearts of the animals were transferred into 4 ml serum tubes. The mice were euthanized by cervical dislocation while still under anesthesia, and necropsy was carried out. At necropsy, longitudinally one-half of the stomach tissue samples which contain both regions were collected according to the Swiss-rolling technique and placed in 10% buffered formaldehyde (Bialkowska et al., 2016).

2.3. Processing of tissue samples

Tissue specimens were firstly fixed in 10% buffered formaldehyde solution for 48 h. According to the routine tissue processing protocol tissue samples were dehydrated in ascending grades of ethanol (50%, 70%, 96%, and 100%), cleared in xylene, and then embedded with paraffin. Sections at 5 µm thickness were obtained from paraffin-blocked tissues and stained with hematoxylin-eosin (Bancroft and Gamble, 2008). Finally, the slides were photographed using the imaging system adaptive light microscope (Leica, DM2500 / DFC295). Histopathologic lesions (inflammatory reactions) in the glandular stomach (gastric glands) were evaluated by light microscopy considering prevalence of inflammatory cell infiltration in 10x magnification objective area and were scored as negative = 0; <1% = 1; 1-9% = 2; 10-32% = 3; 33-65% = 4 and > 65% = 5 degrees according to blind analysis technique as indicated by small modifications of the current literatures by two researchers with the modifying of Updated Sydney System classification system. The prevalence of inflammatory reaction and scoring system was determined by microscopic examination of inflammatory cells (polymorphic and mononuclear cells) infiltration in the region of the tissue damage (Eaton et al., 2007; Rugge and Genta, 2005].

2.4. Re-isolation and Identification of A. butzleri

The stomach (both glandular and aglandular) and other internal organ samples (duedonum, ileum, liver, kidney, spleen) collected from each of the necropsied animals. Samples were cut into pieces using alcohol-sterilized scissors and were placed in sterile Stomacher bags, 10 ml of Brucella broth (Difco, Detroit, MI, USA) containing CAT (Cefoperazone, Amphotericin, and Teicoplanin, SR0174, Oxoid) supplement was added and the samples were homogenized using stomacher-bag mixer, for 30 s (Williams et al., 2012). Each tissue homogenate was cultured on Mueller Hinton agar (CM0337, Oxoid, UK) containing

sterile CAT supplement and 5% horse blood and incubated at 30 °C for 48-72 h (Acik et al., 2016]. Following the incubation, the growth bacteria were identified as *Arcobacter* spp. with the colony microscopic morphology and motility. The isolates were identified at species level using a pair of A. butzleri specific primers by PCR method (Houf et al., 2000).

2.5. Determination of Serum Corticosterone Levels by ELISA

Serum corticosterone levels in the samples were determined with a competitive assay using the Corticosterone ELISA kit (Abcam, ab108821, UK) described by Tintos et al. (2006) with some modifications (Cakan et al., 2016; Ozgecer et al. 2017). The ELISA kit protocol was followed according to the manual provided by the manufacture. Stop solution (sulfuric acid 10%, 50 µl/well) was added, and the absorbance was measured at 450 nm using a microplate reader (Biotek, Synergy HT, USA). The dynamic range of the assays was between 10-2000 ng/ml. Inter- and intra-assay coefficients of variations were below 10%.

2.6. Statistical Analyses

The results were presented as mean ± standard error of mean (SEM) and value of $p < 0.05$ was considered as significant. One-way analysis of variance (ANOVA) was used for time response measurements of corticosterone levels between the groups within the same week followed by post hoc Tukey's test comparisons. Shapiro–Wilk tests were used for checking normality of all the data. Homogeneity of variances was measured by Levene's test. IBM SPSS 22.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analyses of parametric data. Scores of inflammatory reactions were compared using Kruskal–Wallis variance analysis test followed by post hoc analysis using Mann–Whitney U test. The value of $p < 0.01$ was considered as significant in nonparametric tests. Besides, scores of inflammatory reactions were presented with medians and 95% confidence intervals. GraphPad Prism for Windows version 5.0 (GraphPad software Inc., SanDiego, CA, USA) was used to analyze nonparametric data and to create charts.

3. Results

3.1. Histopathological Findings

At necropsy, no macroscopic findings were found in any of the animals in the experimental and control groups, with the exception that the vessels in the serosal surfaces of the stomach tissues of the animals in Ab and CS + Ab groups were mildly congested.

In the microscopic examination no histopathological lesions were noticed in the stomach tissues of animals in sham group (Figure 1A, 2A, 3A). In the experimental groups, inflammatory reactions at various degrees were detected. In the CS (Figure 1B, 2B, 3B) and Ab (Figure 1C, 2C, 3C) groups, it was observed that degenerative and inflammatory reactions (edema formation, loss of normal mucosal glands and epithelium, infiltration of leukocytes) related to gastritis formation in the stomach of animals was encountered. The severity level of inflammatory reactions was determined to be higher in CS + Ab group than in either CS or Ab groups. All of the animals in CS + Ab group were determined to form atrophic chronic gastritis in the fundus region of the stomach at the 4^{th} and 7^{th} weeks. In these cases, the connective tissue fibers develop from the submucosa to the mucosa, limited neutrophilic and predominantly mononuclear inflammatory cells, and plasma cell infiltrations spreading to the basal cell layer, severe congestion in the vessels and complete destruction of the mucous layer were noted. It was detected that extensive disappearance of gastric glands, which were replaced by metaplastic glands and accompanied by different degrees of condensation of the fibrous components of the lamina propria. Also, it was observed that shrinkage and destruction of gastric glands accompanied by glandular atrophy in atrophic and chronic gastritis related samples (Figure 2D, 3D).

The inflammatory reaction scores obtained by histopathologic examination of the stomach tissues of the animals in the experimental groups were provided in Figure 4. According to the results, the highest inflammatory reaction score were detected in CS + Ab group at the 7^{th} week. It was found that the inflammatory reaction score in the experimental groups increased in a direct proportion with time.

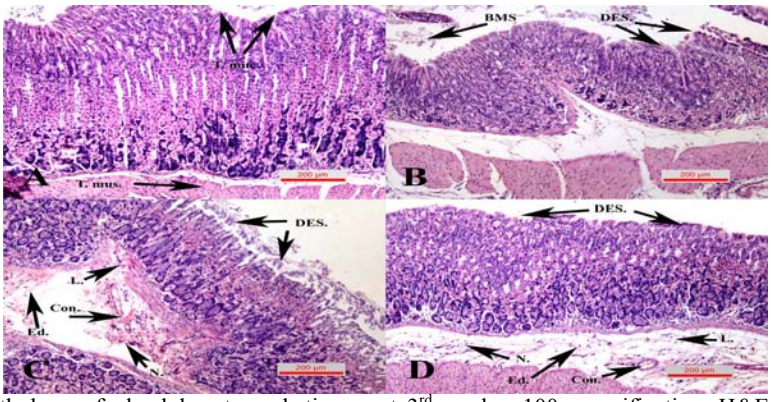

Figure 1. Histopathology of glandular stomach tissues at 3rd week; x100 magnification, H&E staining. Normal histological structure of stomach in the sham group (A). Degenerative and inflammatory changes showed with arrows in the stomach tissues of the CS group (B), Ab group (C), CS + Ab group (D), respectively. T. muc.: Tunica mucosa of gaster; T. mus.: Tunica muscularis of gaster; BMS: Basofilic mucous substance; DES: Desquamation of epithelium cells; L.: Lymphocytes; N.: Neutrophils; Ed.: Edema; Con.: Congestion of blood vessels.

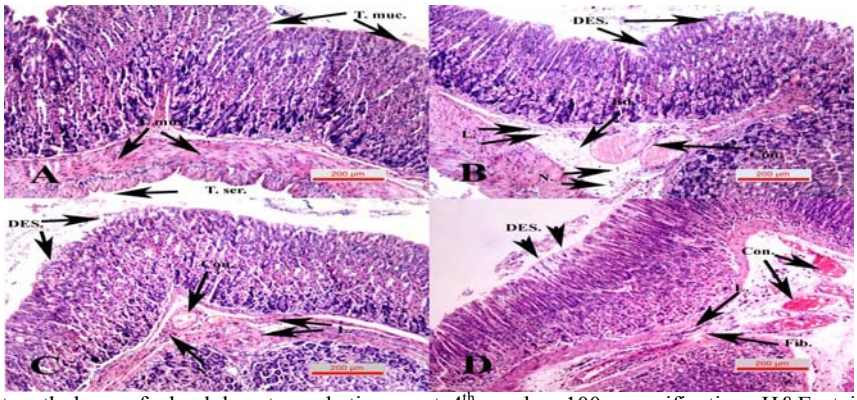

Figure 2. Histopathology of glandular stomach tissues at 4th week; x100 magnification, H&E staining. Normal histological structure of stomach in the sham group (A). Degenerative and inflammatory changes showed with arrows in the stomach tissues of the CS group (B), Ab group (C), CS + Ab group (D), respectively. T. muc.: Tunica mucosa of gaster; T. mus.: Tunica muscularis of gaster; BMS: Basofilic mucous substance; T. ser.: Tunica serosa of gaster; DES: Desquamation of epithelium cells; L.: Lymphocytes; N.: Neutrophils; Ed.: Edema; Con.: Congestion of blood vessels. Fib.: Fibrosis.

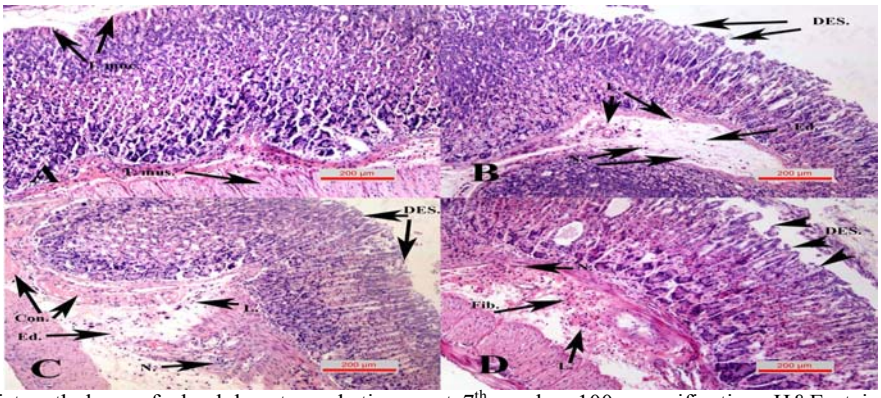

Figure 3. Histopathology of glandular stomach tissues at 7th week; x100 magnification, H&E staining. Normal histological structure of stomach in the sham group (A). Degenerative and inflammatory changes showed with arrows in the stomach tissues of the CS group (B), Ab group (C), CS + Ab group (D), respectively. T. muc.: Tunica mucosa of gaster; T. mus.: Tunica muscularis of gaster; DES: Desquamation of epithelium cells; L.: Lymphocytes; N.: Neutrophils; Ed.: Edema; Con.: Congestion of blood vessels. Fib.: Fibrosis.

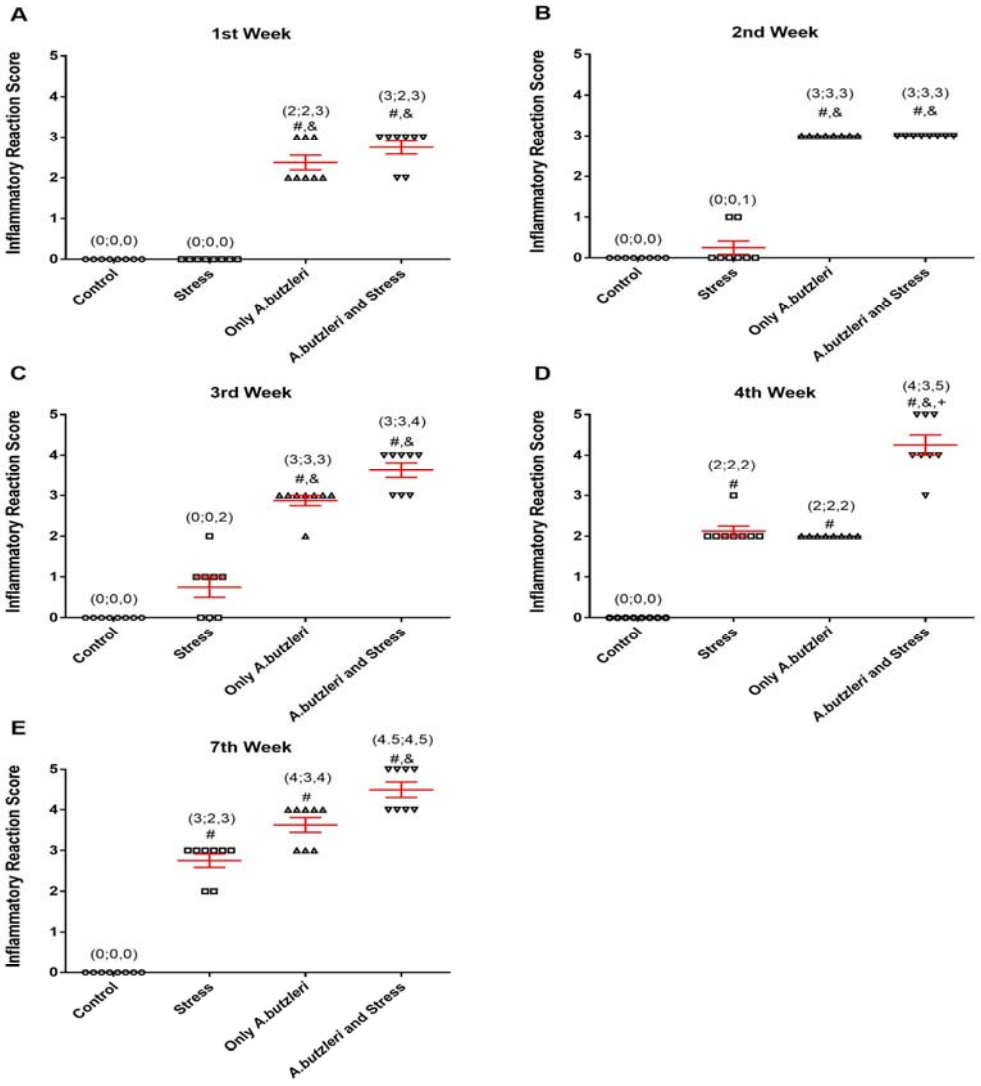

Figure 4. The graphs represent mean inflammatory reaction scores at 1, 2, 3, 4 and 7th weeks (A, B, C, D, E) weeks of the experiment in mice, respectively. The error bars indicate the standard error of mean. 95% confidence intervals for the medians are shown above of the groups (median; lower bound, upper bound). Small symbols on top of each group indicate significant difference among groups ($p < 0.01$). # indicates significant difference compared to the sham group. & indicates significant difference compared to the CS group. + indicates significant difference compared to the Ab group.

3.2. Serum Corticosterone Levels

Corticosterone levels in blood sera collected from mice were presented in Figure 5. According to the results, corticosterone levels in CS group which was exposed to stress for 1 and 4 weeks were significantly higher than those obtained for the sham group and, the differences were statistically significant ($p < 0.01$). On the other hand, there was no statistically significant difference in the serum corticosterone levels between CS group and the sham group at the 7th week of the experiment. While serum corticosterone levels in the CS group were significantly high in the first week, a partial decrease was observed as the time elapsed. In contrast, it was determined that serum corticosterone levels in Ab group increased as the time elapsed.

3.3. Re-isolation and Identification of A. butzleri

A. butzleri was isolated and identified from stomach and small intestine samples of all animals in the experimental groups exposed to the agent following the euthanasia at the 1, 2, 3, 4 and 7th weeks of the study. Although bacterial isolation from liver, spleen and kidneys was failed at the first week of the experiment, it was determined that *A. butzleri* administered by intra-gastric gavage caused bacteremia and was recovered from various tissues and organs of the body from the second week onwards. All the samples found positive for *A. butzleri* by culture and biochemical tests were also confirmed by species-specific PCR assays. The agent was not detected from stomach and other internal organs of the animals in the CS and sham groups.

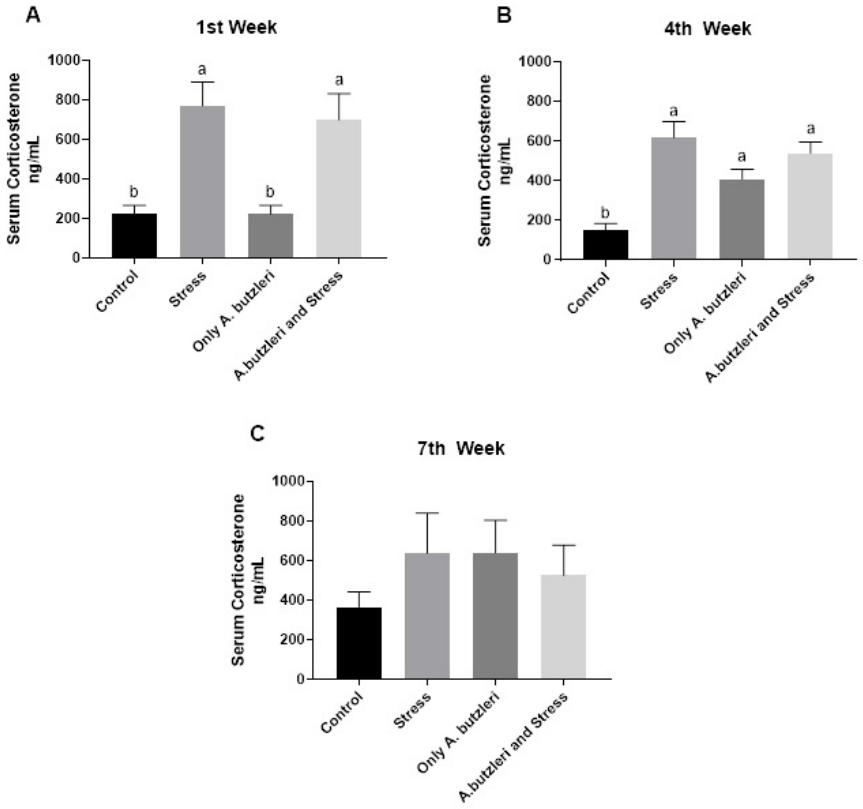

Figure 5. Bar graphs represent mean serum corticosterone (ng/mL) levels at (A) 1, (B) 4, and (C) 7th weeks of the experiment in mice. The error bars indicate the standard error of mean. Small alphabet on top of each bar indicates significant difference; $p < 0.05$ by Tukey Posthoc test.

4. Discussion

Stress is an important risk factor for gastric ulcer formation. Physiological stress has been showed to play an important role in the stomach colonization of *H. pylori* in Balb/c mice (Guo et al., 2019). Stress has been suggested to contribute to the formation of peptic ulcers at the ratios ranging from 30 to 65% and a synergistic relationship between stress and primary disease agent has been noted (Levenstein, 2000). Also, it has been reported that stress as a primary determinant in the etiology of the gastric ulcers and 15.6% of ulcer cases, where *H. pylori* were not detected and non-steroidal anti-inflammatory drugs were not used (Chen and Chang, 2008). Severe hemorrhagic ulceration has been reported in 80% of the rats that were experimentally exposed to cold stress (Das and Banerjee, 1993).

In contrast to the latter study, no macroscopic or microscopic findings indicating gastric ulcers were detected in mice exposed to cold stress in the present study. The animals in this group were observed to develop gastritis from the 2nd week onwards and the severity of inflammation increased with time. Therefore, the data obtained here supported previous studies reporting stress as a predisposing factor in the etiology of gastric ulcers.

Although there is a paucity of information about the role of *Arcobacter* in the etiology of gastric ulcer, the agent has been reported to be associated with gastritis in a limited number of experimental and clinical studies. (Vandamme et al., 1992; Skirrow, 1994; Acik et al., 2016; Ata and Bayram, 2016). The peroral administration of *A. butzleri* to Balb/c mice might be producing inflammation in various organs of the body, especially in the stomach and intestines (Ata and Bayram, 2016). Likewise, gastritis with increased severity depending on time was observed in the Ab group in the present study. Similar findings were also obtained in the CS group.

Stress has been reported to cause gastritis and gastric ulcer by increasing gastric motility, gastric acid secretion, mast cell degranulation and mucosal blood flow, and by decreasing prostaglandin levels (Miller, 1987). Although the cold-related stress may trigger some of these pathological defects, the main factors in the formation of these defects are hormones that are released due to stress. Corticosterone, which is activated by acute stress, plays an important and facilitating role in gastric mucosal defense, and one of the most important hormones released during stress (Laine, 2008). Acute elevation of corticosterone during stress is a strong gastric protective component of the hormonal response against stress. It is assumed that the protective effect of glucocorticoids actualizes due to glucose homeostasis, gastric blood flow, and mucus secretion maintenance, increased gastric motility, and decreased microvascular permeability (Laine, 2008). However, studies have reported that long-term release of corticosterone due to chronic stress facilitates the release of histamine, leukotrienes, and other chemical mediators from several cells. The released chemical mediators predispose individuals to gastric ulcers by triggering gastric acid secretion, gastric contraction, and vascular occlusion (Kitagawa et al., 1979; Oktedalen et al., 1984). The finding of the current study that no evidence of gastritis was observed in animals exposed to cold stress for one week despite high corticosterone levels supported this view. As the duration of stress was increased, the severity of gastritis increased in parallel with high corticosterone levels.

Different results have been reported in previous studies with respect to corticosterone levels independently from the stress period in animals exposed to the same type of stress. Although a few studies reported that the level of corticosterone increased due to the stress application, most of the studies indicated that the corticosterone level decreases with time and those animals develop adaptation to stress (Marti and Armario, 1998; Radahmadi et al., 2015). In our, the corticosterone levels were determined to be significantly higher in the experimental groups when compared to the control group in the first (except for Ab group) and 4th weeks, whereas the difference was not statistically significant in the 7th week (Figure 5). This indicated the development of adaptation in animals exposed to long-term stress. This adaptation is also related to energy reserves. The stress response is weakened due to the use of energy reserves for heat production (Wang et al., 2015). However, the increase in energy consumption during stress usually increases glycolysis induced by the corticosterone hormone. This hormone inhibits phospholipase A2, the nonexistence of arachidonic acid triggers the absence of prostaglandin and decreases secretion of gastric juice, which can cause ulcers. The histopathological finding obtained in the current study that mucous substance on the surface of the gastric mucosa of animals in CS + Ab group disappeared over time can be explained by this mechanism. This probably triggers ulcer formation and the exacerbation of the inflammatory reaction due to stress and *A. butzleri* (Gyires and Fehér, 2017).

It was determined that the corticosterone level was significantly higher in the CS group than the Ab group. It is thought that the increase in corticosterone levels may be the result of stress caused by the infection or the agent itself leads to corticosterone release. Also, bacterial liposaccharides have been reported to increase corticosterone levels by causing inflammatory reactions in the host (Shini et al., 2008). Sharma

et al. (2017) reported that fecal corticosterone levels in chickens infected with *Salmonella* Typhimurium were higher than those of the control group from the first day to the 6th week of the study. Although there is no study showing the relationship between *A. butzleri* and serum corticosterone levels, *A. butzleri* carrying lipopolysaccharides (LPS) on the cell wall has been suggested to have the potential to contribute to an increase in serum corticosterone level following experimental infection (Johnson et al., 1996). On the other hand, the increase in stress hormones makes living beings more susceptible to several diseases and stimulates bacterial growth related to colonization in the intestines (Sharma et al., 2017; Verbrugghe et al., 2016). These data demonstrated that there was a mutually stimulating effect between bacterial infection and corticosterone (Tzeng et al., 2018).

Serum corticosterone levels in rodents vary depending on circadian rhythm throughout the day. Serum corticosterone levels reach the highest levels in the early evening in rodents that are active at night (Koch et al., 2017). Gong et al. (2015) reported that serum corticosterone levels peaked in the evening hours and decreased to the lowest level in the morning hours in a study in mice. In this study, by applying cold stress during the morning hours when naturally low corticosterone release, changes related to circadian and ultradian rhythm were minimized. The circulating corticosterone levels may increase above normal values due to psychological and physiological stressors, as well as an increase due to other stressors (such as inflammation) (Koch et al., 2017). Corticosterone release occurs more rapidly by stress-induced HPA. In this study, it was thought that the level of corticosterone in the group who applied cold stress for the first week was higher than the stress-free groups, and that the HPA axis was caused by stress. On the other hand, while the corticosterone level in the Ab group is low in the first week, this rate increases dramatically as time goes on. It may be the reason for the low level of corticosterone in the first week because *A. butzleri* colonizes the gastrointestinal tract after administration and the occurrence of fire develops more slowly.

The anatomical and physiological structure of pig stomach has similar characteristics to the human stomach. For this reason, following the isolation of *H. pylori* in the human stomach, the first experimental studies aimed at revealing the infection mechanism and virulence properties of this agent have been carried out in gnotobiotic pigs (Krakowka et al., 1991). Therefore, the isolation of *Arcobacter* species from pig stomach supports the hypothesis that these agents may be involved in the etiology of gastric ulcers in humans. Due to the lack of infrastructure, using the pig model was beyond the scope of this study. Instead, mice which have often been used as model animals to form gastric ulcer were preferred for the experimental study. In fact, previous studies showed that pigs and gerbils were the most suitable models for in vivo experiments in the formation of gastric ulcer by *H. pylori* (Krakowka et al., 1995; Burkitt et al., 2017). On the other hand, while mice have successfully been used as an ideal animal model in the formation of gastritis [(Burkitt et al., 2017), some studies conducted in mice put forward that colonization of *H. pylori* was usually temporary and in case of the persistence of infection, it was only associated with mild gastritis (Lachman et al., 1997). On the other hand, when gnotobiotic mice were experimentally infected with *A. butzleri* orally, bacteria were easily colonized in the intestines, but no clinical and histopathological findings showing enteritis were observed. In the same studies, it was found that bacteria induced both intestinal and extra intestinal immune responses depending on the duration of infection and strain difference (Gölz et al., 2015; Heimesaat et al., 2015). The inability to establish gastric ulcers may, therefore, be related to the animal species used in the experiment. However, bearing in mind that the etiology and pathology of gastric ulcer in humans are rather different than those in experimental animals, the role of *Arcobacter* in gastric ulcer cases of humans should be investigated in detail.

Conclusions

In conclusion, no evidence was obtained about the causative role of *A. butzleri* in gastric ulcer in the present study. In addition, it was shown that either *A. butzleri* or stress alone was not sufficient to induce gastric ulcers in mice, and *A. butzleri* or stress may be involved as a predisposing factor in the induction of ulcer. It

was also demonstrated that stress and *A. butzleri* increased serum corticosterone levels significantly, but due to time-dependent adaptation, a decrease was observed in the level of this hormone after a certain time. Finally, in order to have a better understanding of the role of *A. butzleri* in the etiology of gastric ulcer, biopsy specimens taken from clinic patients are required to be examined for the presence of *Arcobacter* as well.

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Refereces

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