INTRODUCTION
Postoperative adhesions are a major complication after abdominal surgical procedures and may cause bowel obstruction, chronic pelvic pain and/or infertility [1]. Adhesion formation can occur following any surgical procedure with an incidence ranging from 67 to 93% after general surgical abdominal operations and up to 97% after laparotomic gynecologic pelvic procedures [2, 3]. It also impacts negatively on clinical outcomes in patients undergoing a new surgery and increases the morbidity [4, 5]. Many treatment modalities have been evaluated to prevent or to reduce the formation of adhesions. The mechanism of adhesion formation is not elucidated clearly [3]. Therefore, preventive strategies have not been efficacious. Among the earliest factors identified as inducing peritoneal adhesion formation was ischemia [6]. Recent studies have shown that leukocyte dependent inflammatory reactions may cause adhesion formation through the actions of oxygen-derived free radicals and metabolites [7].

Trimetazidine is an antianginal drug that restores energy production in the ischaemic heart cell and reduces the generation or activity, or both, of oxygen–derived free radicals [8, 9]. Trimetazidine reduces intracellular acidosis, inhibits oxygen-derived free radicals and neutrophile infiltration in ischemia and hypoxia which are the primary steps of adhesion formation [10, 12]. Our aim is to study the anti-adhesion potential of trimetazidine in a rat uterine horn model.

MATERIALS AND METHODS
Before the study, approval of the experimental protocols was obtained from the local ethical committee at Suleyman Demirel University. Forty non-pregnant female Wistar-Albino rats weighing 170-240 g were used in this study. Rats were housed in a controlled environment at 23 -25º C on an illumination schedule of 12 h of light and 12 h of darkness each day. Rats were fed standard food and tap water ad libitum. The rats were randomly assigned, with ten in each group, to receive 2 ml saline, 5 mg/kg intraperitoneal trimetazidine postoperative, and as control and sham. In sham group, no cauterization and adjuvant therapy was administered; in control group, after cauterization no adjuvant therapy was administered; 2 ml SF was administered to SF group after operation intraperitoneally (i.p.). TMZ was administered to TMZ postoperative group 5 mg/kg i.p. daily for five days after the operation. TMZ was suspended in %0.9 SF to yield a concentration of 1 mg/mL. Doses of trimetazidine were based on previous studies [8, 13].

After acclimatization, all rats underwent midline laparotomy with intraperitoneal injection of 1:8 mixture of 2% xylazine (Alfamin, Alfasan IBV) and 2.5% ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) for anesthesia. A laparotomy was carried out through a 3–4 cm midline incision, after the lower abdomen was shaved and cleansed with povidone iodine solution. Both uterine horns were exposed, and than a 2 cm segment of the anti-mesenteric surface of the both uterine horns were traumatized in 10 spots with unipolar electrocautery for 2 seconds with a power of 50 Watts. All procedures were performed under aseptic, sterile conditions (Fig.1). The midline incision was closed in two layers. The musculo-peritoneum and fascia were closed with 4/0 polyglactin 910 (Ethicon). The skin was closed with simple interrupted sutures of 3/0 silk. Abdominal wall integrity and wound healing were checked during the first 2 days after surgery. No antibiotics were given during or after the procedure. All the animals were sacrificed by lethal dose of ether on postoperative 14th day. The abdominal cavity was entered carefully through the transverse subcostal incision and inspected for the presence of adhesions. The extent and severity of adhesions at the
operation side were evaluated by the same observer, who was blinded to the treatment regimen. Intraperitoneal adhesions were scored according to Leach et al.’s clinical adhesion scoring system [14]. Adhesions to the uterine horn defect were scored as follows: 0= no uterine adhesion; 1=1-25% involvement; 2=26-50%; 3=51-75%; and 4=76-100%. Adhesions were further characterized on gross examination for severity as follows: 0=no adhesions; 1=filmy avascular; 2=vascular or opaque; and 3=cohesive attachment of uterine horns to each other or other abdominal structure. The degree of adhesion formation was evaluated with the following adhesion scores: 0=no adhesions; 1=if the adhesion separated from tissue with gentle traction; 2=requiring moderate traction; and 3=requiring sharp dissection. Therefore, a total score of 10 was possible and for comparisons between groups, total scores were used. Adhesion-carrying tissues were excised en-bloc and fixed in 10% formalin and processed for parafin embedding.

2.1. Histology and morphometric analysis
Paraffin embedding, 5 mm sectioning, mounting, and hematoxylin and eosin staining were completed by the Department of Pathology, Faculty of Medicine, Suleyman Demirel University, Isparta according to standard techniques. The slides were visualized and the images were captured digitally by using a photo-light microscope. Inflammation and fibrosis were scored as 0,1,2,3 subjectively [3].

2.2. Statistical analysis
Statistical analysis was accomplished on a personel computer, using statistical program for social sciences version 15.0 (SPSS 15.0). The data were expressed as the medians (minimum-maximum). Kruskal-Wallis test was used for comparisons of the groups. A P-value of < 0.05 was assumed to be significant. When a significant result was found, Mann-Whitney u test was used in order to determine which groups were differing.

RESULTS
The standardized surgical procedures and the administration of the protocols were well tolerated by the animals. There was no mortality in the study groups. All laparotomy sites were intact and none of the rats had an incisional hernia. Table I summarizes the extent, severity, degree and total adhesion scores (TAS) as well as histopathological results among the groups. TAS of control group was 10 while the sham group’s was found to be 0. TAS of the placebo group that was given saline (TAS: 5), and postoperative TMZ applied group (TAS: 3.5) were significantly lower that the control group (p < 0.05). In Table 1 the distribution of the average adhesion total score was shown according to the groups.

When the groups were compared in terms of horn adhesion extent, the average score of the treatment group significantly decreased (p<0.05) according to the control group (score:4). Table 1. When the groups were compared in terms of horn adhesion severity, the control group’s score was 3, while the score of the group that was given saline was 2 (p> 0.05). The score of the group which was given postoperative TMZ was 1 and the sham group’s score was 0. The severity scores of postoperative TMZ and sham groups were significantly smaller than the other groups (p<0.05).

When the groups were compared in terms of horn adhesion inflammation and fibrosis scores, the control group, saline treated and postoperative TMZ applied groups have similar inflammation and fibrosis scores (p> 0.05)

Discussion
In order to prevent adhesion formation, several agents and approaches have been
used to date. In animal models to minimize or prevent formation of postoperative adhesions, Several approaches and adjuvants have been suggested, however the agents have not been sufficient to warrant their clinical use [14, 17]. In the present study our aim is to study the anti-adhesion potential of trimetazidine in a rat uterine horn model. This is the first study investigating the effect of intraperitoneal TMZ on peritoneal adhesions. Trimetazidine inhibits the formation of reactive oxygen species and protects cells from ROS-induced injuries [10].

Ellis revealed that tissue ischemia is one of the most important factor inducing intra-peritoneal adhesion formation [8, 18, 19]. Peritoneal ischemia causes inhibition of fibrinolitic activity [20]. Recent studies have showed that reactive oxygen species formed during ischemia and reperfusion after ischemia played important role in inflammation reaction [8, 19]. During the first 5 min of ischaemia, there is already a significant production of ROS, which are able to react rapidly with oxygen, exacerbating the oxygen deficit [21]. Locally generated free radicals such as superoxides, peroxides and hydroxyl radicals are potential oxidizers of polyunsaturated fatty acids [22]. Lipid peroxidation of cellular membranes occurs and increased vascular permeability, leads to the formation of serosanguineous exuda, which in turn initiates adhesion formation. These adhesions are generally lysed within 72 h after formation by the endogenous fibrinolytic activity and much of the healing is complete within 5 days [23]. If there is an imbalance between fibrin deposition and dissolution, deposited fibrin may persist and fibrinous adhesions may develop [24]. The first week after the trauma is the most critical period and methods to prevent adhesion formation should clearly inhibit series of reaction created in this period.

Tsimoyiannis et al. studied the effect of i.v. TMZ in adhesions due to ileal ischemia and reperfusion [8]. They reported that preoperative intravenous trimetazidine administration reduces the incidence and severity of peritoneal adhesion formation induced by ileal ischaemia and reperfusion. We found that postoperative administration of intraperitoneal TMZ is locally effective to prevent and decrease the severity of adhesions in cautery induced peritoneal damages at rat horn. The sidewall model has been used for general abdominal surgery, but the uterus horn model is better suited to simulate gynaecological procedures [25]. The promising efficacy demonstrated by the intraperitoneal TMZ in this model warrants further investigation in clinical trials focused on gynaecological procedures.