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Intraperitoneal Infusion of Neutral-pH Superoxidized Solution in Rats: Evaluation of Toxicity and Complications on Peritoneal Surface and Liver

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Superoxidized water (SOW) is known to be a potent disinfectant. The aim of this study was to evaluate the toxicity and complications on the peritoneal surface and liver after infusion of pH-neutral SOW into the peritoneal cavity of rats.

Material/Methods: Thirty Wistar-Albino rats weighing 250–300 g were randomly divided into 3 groups (10 rats/group). Group1 (control group) rats received single dose of 10 mg/kg saline solution intraperitoneally. Group 2 (single-dose group) rats received a single dose of 10 mg/kg pH-neutral SOW intraperitoneally. Group 3 (multiple-doses group) rats received multiple doses of 10 mg/kg pH-neutral SOW intraperitoneally on days 1, 3, and 5. All animals were killed at 1 week after infusion. Blood specimens were taken to the laboratory and macroscopic and microscopic examinations were performed on each rat.

Results: All 30 rats survived after the infusion. The gross-macroscopic examinations revealed no pathologic findings in any of the 3 groups. The microscopic examination of peritoneum and liver showed no signs of toxicity or complications in any of the 3 groups. There were no statistically significant differences among the 3 groups with regards to the blood biochemistry, including hemoglobin, hematocrit, platelets, aspartate aminotransferase, alanine aminotransferase, urea, or creatinine levels ($p>0.05$). However, the leucocyte counts were lower in group 3 than in groups 1 and 2, but this was not statistically significant ($p=0.189$).

Conclusions: Intraperitoneal infusion of pH-neutral SOW does not result in any significant toxicity or complications on the liver and peritoneal surface. However, multiple infusions lead to low leucocyte counts and future studies with longer follow-up times are needed.

MeSH Keywords: **Disinfectants • Injections, Intraperitoneal • Toxicity Tests**

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Background

Superoxidized solution or water (SOW) is a recently developed broad-spectrum disinfectant that is non-toxic to human tissues, has a neutral pH, and is low cost [1]. SOW is produced by applying an electric current to salty water, followed by electrochemical processed in aqueous solutions from pure water and sodium chloride. Water is degraded into oxygen, ozone, and other unstable oxidized species, but the main active chemical compound generated during this process is hypochlorite and hypochlorous. There are many commercially available solutions with different concentration and pH values [2]. Many studies have proven its microbicidal activity against a variety of microorganisms, including bacteria, viruses, and fungi [3].

There are several published studies on use of superoxidized solutions in humans, such as in skin infections and ulcers [4], burns [5], diabetic foot [6], and peritonitis [7]. These products are also used widely for cleaning and disinfecting hospital surfaces and hemodialysis equipment [8]. However, there is a potential for these products to have toxic effects and waste problems due to their physicochemical properties in the human body [8]. *In vitro* studies on the antimicrobial activity of SOW showed its great broad-spectrum effect, and a rat burn model study reported beneficial effects in direct application of SOW to *Pseudomonas*-infected lesions [9].

Intraperitoneal abscess and infections are important health problems and cause mortality and morbidity. During the surgical operations, normal saline irrigation is used widely to clean the peritoneal surface which has not any antimicrobial activity. Therefore, it is rational to use an microbicidal agent like SOW for peritoneal irrigation which has a neutral pH and non-toxic compound during a surgery like perforated appendicitis or tubo-ovarian abscess rupture. The application of SOW on the body surface has been widely used and is well-known, but the intraperitoneal use and its toxicity and complications on the peritoneal surface and liver parenchyma has not been studied until now. The purpose of this study was to evaluate the gross and microscopic findings in the peritoneal surface and liver parenchyma following intraperitoneal infusion of SOW solution in rats, and also to determine the effect of SOW on blood parameters.

Material and Methods

This experimental study was approved by the Ethics Committee for Animal Experiments of Yuzuncu Yil University, reference number 19, dated 26/06/2015, and was performed in the Experimental Animal Research Laboratory of the university, located in Van, Turkey. Thirty Wistar-Albino rats (weight 250–300 gr) were used as a model. All the animals were treated humanely in accordance with the Declaration of Helsinki. All the animals were fed ad libitum with standard food approved by the Turkish Standards Institute. The rats were randomly divided equally into 3 groups (10 rats in each group).

Experimental groups: The intraperitoneal injection of normal saline and SOW (Crystallin, Wound Care, NPS Biyosidal, İstanbul, TURKEY) was done by one of the authors (A.A.), who had experience and a certificate in performing intraperitoneal infusion in rats.

Group 1 (control group, intraperitoneal saline): An intraperitoneal single dose of 10 cc/kg 0.9 NaCL (normal saline) was injected into the peritoneal cavity using 22-French syringes.

Group 2 (single dose intraperitoneal SOW group): Rats received a single dose of 10 cc/kg SOW via injection into the peritoneal cavity using 22-French syringes.

Group 3 (Multiple-dose intraperitoneal SOW group): The intraperitoneal multiple dose of 10 cc/kg SOW was administered on the first, third and fifth day with 22-French syringes. After injections, the rats were returned to standard vivarium care.

After 1 week the animals were sacrificed by administration of intraperitoneal 75 mg/kg Ketamine. Immediately after that, intracardiac blood specimens were taken with 16-French syringes into 2-cc blood tubes. Then the blood specimens were centrifuged and stored in -80°C to be studied later. The studied hemogram parameters were hemoglobin, hematocrit, platelet and leucocyte count, and liver function tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) with urea and creatinine. All the laboratory parameters were studied in the Yuzuncu Yil University, Medical Faculty, Biochemistry Laboratory.

Each rat was placed on its back on the operating table and a vertical midline incision was made to open the peritoneal and pleural cavities. The peritoneal cavity, peritoneum, liver surface, and intestines were macroscopically evaluated for any adhesions or macroscopic changes by the pathologist, who is one of the authors (S.Y.) and was blinded to the 3 groups (Figure 1). The tissue specimens of liver, peritoneum, and kidneys were obtained. Following dehydration and paraffinization, the tissues were cut into sections (5-µm thick) and stained with hematoxylin and eosin. Samples were examined under a light microscope. Histopathologic examinations were done by the pathologist, who was blinded to the groups.

Statistical analysis

Statistical significance levels were considered as 5%. The SPSS statistical program (Version 20.0 – IBM Corp. Released 2011.

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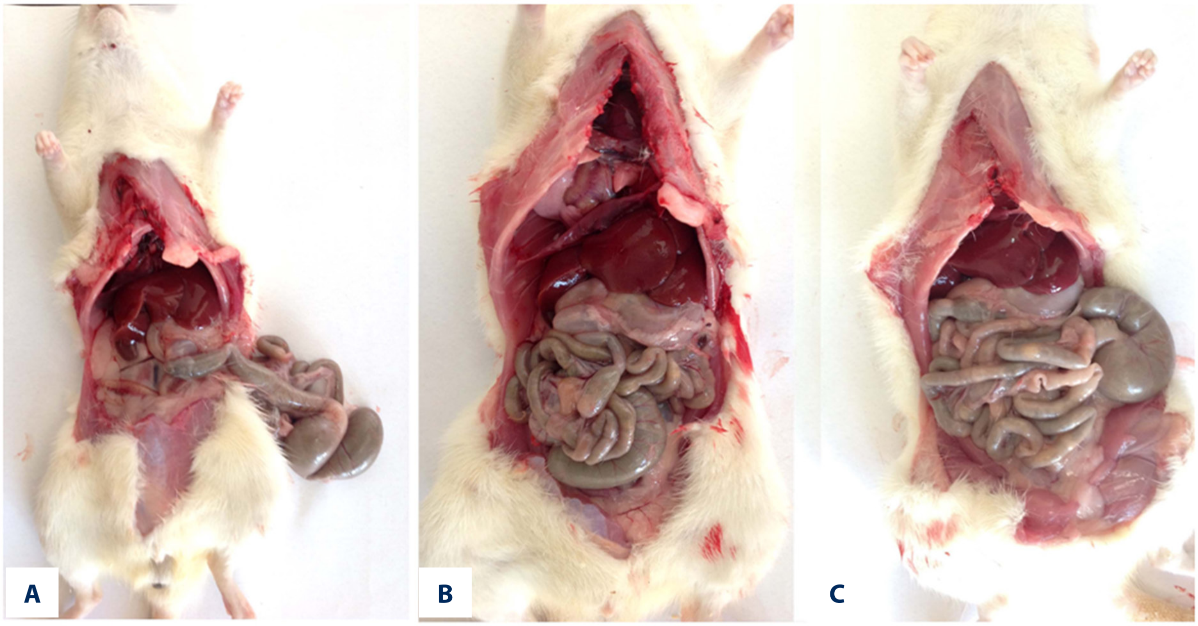

Figure 1. Macroscopic view of the 3 groups.

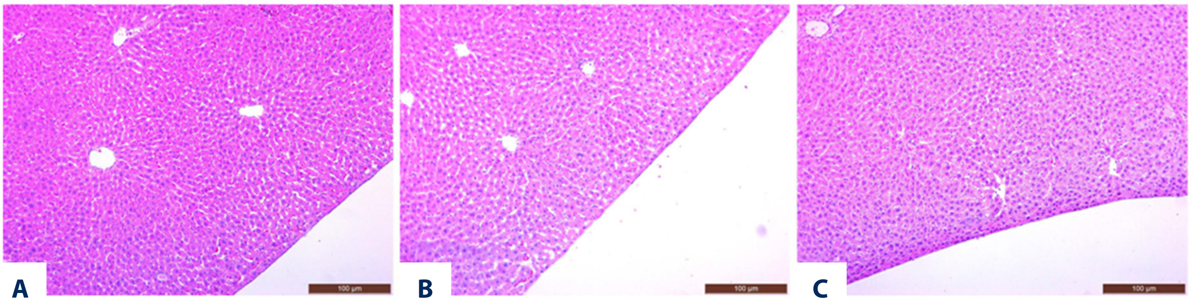

Figure 2. Microscopic picture of liver parenchyma of the 3 groups. (**A**) Shows group 1, (**B**) shows group 2, and (**C**) shows group 3.

IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.) was used for all statistical computations. Descriptive statistics for the studied variables (characteristics) are presented as median, mean, standard deviation, and minimum and maximum values. Kruskal-Wallis test was performed to compare group medians. Following the Kruskal-Wallis test, the Dunn multiple comparison test was used to determine different groups. In addition, for determination of linear relationships among the variables, Pearson correlation analysis was carried out in each group.

Results

All the animals remained healthy during the first week after injections. No incisional site reactions, including infection or allergy on the abdomen, were observed. On follow-up examination, no death of rats was observed. The macroscopic examination of the all 3 groups revealed no pathology or change in the peritoneum, small intestines, and liver, as shown in Figure 1. No gross findings regarding fibrosis, adhesions, ischemia, or bleeding on the surface of intraabdominal organs were observed. When microscopically analyzing the peritoneum specimens for any findings suggesting toxic effects of SOW, including cellular degeneration, inflammatory cell content, and hyperemia or fibrotic reactions, we found no difference between the 3 groups (Figure 2). The peritoneal surface of the 3 groups showed no signs of inflammation or fibrosis reaction. The liver surface and parenchyma were examined microscopically for any findings of toxicology, revealing no change or difference between the 3 groups (Figure 3).

The toxic effects of SOW on the hematological parameters, liver, and kidney function tests were analyzed. The hemoglobin, hematocrit, platelet, AST, ALT, GGT, urea, and creatinine

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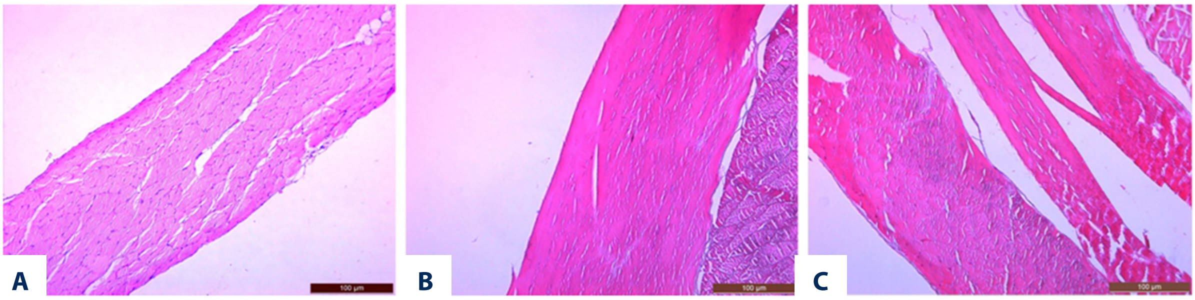

Figure 3. Microscopic picture of peritoneal surfaces of the 3 groups. (**A**) Shows group 1, (**B**) shows group 2, and (**C**) shows group 3.

Table 1. Comparison of laboratory analysis in the 3 groups.

Variables	Group 1 (n: 10)	Group 2 (n: 10)	Group 3 (n: 10)	P value
Hb, gr/100 ml (mean ±SD)	15.03±2.37	15.28±1.15	15.01±1.64	0.868
Hct, % (mean ±SD)	50.53±8.45	50.76±4.33	49.73±6.31	0.884
Plt, (×10^3/ml)	483±218.62	536.80±119.8	638.56±89.36	0.296
Leucocyte count, (×1000/mm^3)	5.43±1.68	5.12±1.54	3.44±1.18	0.189
AST, (mean ±SD)	158.6±49.8	170.2±56.7	191.1±69.91	0.473
ALT, (mean ±SD)	52.0±24.14	41.80±14.46	53.60±17.84	0.345
Urea, (mean ±SD)	48.90±10.02	41.40±9.43	42.60±5.44	0.236
Creatinine, (mean ±SD)	0.66±0.5	0.69±0.10	0.64±0.3	0.254

HB – hemoglobin; Hct – hematocrit; Plt – platelet; AST – aspartate aminotransferase; ALT – alanine aminotransferase; SD – standard deviation. Group 1 – intraperitoneal saline infusion; Group 2 – intraperitoneal single dose SOW infusion; Group 3 – intraperitoneal multiple-dose SOW infusion.

level did not show any statistically significant differences between the groups (Table 1). The only significant difference was found in the leucocyte count for group 3, which had repeated doses of SOW (at 1, 3, and 5 days). Leucocyte count was lower in group 3 than in groups 1 and 2, but this was not statistically significant (p=0.189). However, the leucocyte counts in groups 1 and 2 were similar 1 (Table 1).

Discussion

Superoxidized water (SOW) is a relatively newer disinfectant and it is a well-validated solution used as an antiseptic for hospital floors, hand-cleaning, and sanitizing hospital equipment [10,11]. These solutions have also been used for a variety of human conditions, including skin ulcers, infections, mediastinal irrigations, open heart surgery, and in the treatment of peritonitis [12]. However, the first superoxide solutions had low pH values and were corrosive, with toxic potential to human tissues because its contains strong acids. The new-generation SOW solutions are pH-neutral, with a longer shelf life (>12 months) than the former superoxide solutions. These new-generation SOWs are intended for the topical treatment of infective chronic and acute wounds, like diabetic ulcers. Most importantly, it is non-irritating and non-sensitizing [10]. Although the external use of these products are well-known, the intraperitoneal use of these new-generation SOWs has not yet been investigated clinically or experimentally. So based on these data, we conducted an experimental rat study to evaluate the complications and toxic effects of the intraperitoneal infusion of SOW on the peritoneum and liver.

Our experimental study showed that the intraperitoneal use of superoxidized solution (Crystalin©) has no toxic effect on the peritoneum and liver and does not lead to any complications such as inflammatory response, fibrosis, adhesion, or bleeding. The important finding from our study is that the repeated use of intraperitoneal SOW infusion lead to leucopenia, which was significantly different from the single dose, which did not show such an effect.

Superoxidized solutions are widely used, cheap, and do not increase economic burden. It has been reported that neutral-pH superoxidized solutions have bactericidal-fungicidal,

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virucidal, and sporicidal activity [13]. Landa-Solis et al. reported that Microcyn (another pH-neutral superoxidized water solution) has a wide antimicrobial spectrum, with major advantages over acidic SOWs, including neutral pH, lower free active chlorine (51–85 ppm), and long shelf life (1 year) [13]. A rat study that evaluated the local tolerability and the effects on histopathology of wound beds revealed that neutral-pH superoxidized solutions were not only non-toxic to wounds, they could even induce wound healing [14]. A study on the effect of superoxidized solutions on diabetic wounds found that pH-neutral SOW had more favorable results than Povidine iodine, which is another widely used disinfectant [6].

Peritoneal infections and residual abscess formation remain the important causes of morbidity and mortality after perforated appendicitis, tubo-ovarian abscess rupture, and other intraabdominal conditions, resulting in prolonged hospital stays and higher costs [15]. The most frequently used material in the peritoneal lavage during these surgeries is saline irrigation, but saline has no antimicrobial effect. Thanks to the broad spectrum of SOW against microorganisms, it is used in burn wounds, mediastinitis, and open heart surgery [16]. However, the previously used superoxidized solutions are all pH-acidic solutions and have potential mucosal irritant effect. The best study in the literature (Kubato et al. [17]) on the efficacy of superoxidized solutions with low pH value was in patients with perforated appendicitis. They found that peritoneal lavage and wound washing with strong acid electrolyzed water have no adverse effects and are effective in preventing surgical site infection [17]. However, it is obvious that strong acid electrolyzed water may have corrosive effect is not used widely for intraperitoneal conditions. Our search of the literature found no study that evaluated the effect of intraperitoneal use of SOW on rats or humans, so it was rational to conduct such a study and use microbicidal SOW with neutral pH in a nonirritating solution.

In our hospital we use SOW (crystallin) in for hand washing and disinfecting floors and external surfaces of contaminated wounds; however, we do not use it during peritoneal abscess surgery because of the data on the irritant effect of strong acid electrolyzed water on the mucosal surface and lack of microbicidal effect of saline infusion in peritoneal infections, as well as the lack of no data on use of pH-neutral SOW in the peritoneum. Therefore, we tried to find if there is any toxic effect of SOW on the peritoneal surface or liver in an experimental rat study. We found no adverse toxic effects or complications of intraperitoneal infusion of SOW.

The non-toxic effect of pH-neutral SOW can be explained by the study of Medina-Tamayo et al., who studied the effect of pH-neutral superoxidized solution on murine bone marrow-derived mast cells. They found that neutral pH superoxidized solution acts like a mast cell-membrane stabilizer, inhibiting the cell machinery for granule secretion without altering the signal transduction pathways induced by IgE-antigen receptor crosslinking [18]. These results based on this animal study indicate that an intraperitoneal use trial of pH-neutral SOW is safe with respect to the possibility of any toxic reactions or complications in the peritoneal surface and liver. In our study, we observed no deaths in the rat group receiving SOW, which shows the lack of toxicity of superoxidized solution. A study is needed to investigate the antimicrobial activity of SOW in a peritonitis model in rats.

The most important finding of the current study was lower white blood cell counts in the rats receiving multiple doses of SOW. However, the leucocyte counts were in normal range in all rats administered a single dose of intraperitoneal SOW infusion. Our literature search revealed a review by Goldstein reporting that formaldehyde as a disinfectant can be a potential cause of human leucemia. The review proposed several mechanisms for the leukemogenesis of formaldehyde, stating that the evidence suggests an apparent discrepancy between studies in laboratory animals, which generally fail to show evidence of penetration of formaldehyde into the blood or evidence of blood or bone marrow genotoxicity, and studies of exposed humans in which there tends to be evidence of genotoxicity in circulating blood cells [19]. However, there is no specific data in the literature about the effect of new-generation superoxidized water solution on the hematological system. It is clear that our finding of low leucocyte count with multiple doses of SOW should be investigated further.

This study has several limitations. First, no long-term follow-up of longer than 1 week has been done to observe the toxic reaction or complications. However, we think that this disinfectant solution shows its effect immediately when it comes into contact with the tissue, and 1 week will be enough to see any toxic effects, but the hematopoietic effect may take more time to develop. The abnormal finding in the hematopoietic system may be due to bone marrow inhibition; therefore, to produce comprehensive data, bone marrow biopsies should be examined. Second, we focused on peritoneal and liver complications occurring after infusion of SOW, and did not assess the other systemic complications such as fever or involvement of other organs. The main strength of our study was that all histopathologic examinations were performed by the same experienced pathologist, and we performed blood count analysis and other laboratory evaluations for liver and kidney functions.

Conclusions

We performed intraperitoneal infusion of new-generation pH-neutral superoxidized solution in rats and we found no evidence of adverse toxic effects or complication in the peritoneum and liver. We think that single-dose intraperitoneal infusion of SOW can be used in intraperitoneal abscess surgery due to its microbicidal-disinfectant activity, without resulting in significant complications. However, repeated doses of SOW infusion lead to leucopenia and this should be studied further in experimental toxicology research.

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Disclosure

No financial disclosure.

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