

**THE EFFECTS OF MEMANTINE ON RECOVERY FROM PROPOFOL
ANESTHESIA, COGNITIVE FUNCTION AND PAIN IN ELDERLY RATS**
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Abstract

Objective: Postoperative cognitive dysfunction (POCD) is a frequent complication after anesthesia and propofol is an intravenous anesthetic agent that has been proved to cause cognitive dysfunction. There are many risk factors such as age for POCD. Memantine has beneficial effects on memory deficits and learning process. Besides, it has neuroprotective effects and can be used in treatment of chronic pain syndromes. This study was designed to determine the effects of memantine on recovery, cognitive functions and pain in aged rats undergoing general anesthesia with propofol.

Materials and Methods: Thirty aged Wistar rats were divided into 5 groups randomly. For Group C 0,9% NaCl (1 mL i.p.) was administered at 21.day, for Group P propofol (100 mg/kg i.p.) was administered at 21st day, for Group (oral memantine+propofol) OMP propofol (100 mg/kg, i.p.) was administered after 20 days of treatment with 20 mg/kg/day oral memantine, for Group (oral memantine) OM memantine (20 mg/kg/day oral) was administered for 20 days, for Group (intraperitoneal memantine+propofol) IPMP propofol (100 mg/kg) was administered after 30 minutes memantine (1 mg/kg i.p. in 1 mL 0,9% NaCl) were applied. Recovery was evaluated by tail pinch test, cognitive functions were evaluated by radial arm maze (RAM), S100 β and neuron-specific enolase (NSE) and pain was evaluated by hot-plate.

Results: In this study recovery times were shorter in Group OMP and Group IPMP when compared to Group P ($p=0,011$, $p=0,034$, respectively). Cognitive functions of rats in Group OMP and Group IPMP were better than Group P for the first value of recovery ($p<0,05$). Hot-plate test values in all groups, except group C were longer at all time points when compared to control values ($p<0,05$). NSE and S100 β levels were higher in Group P when compared to Group C. The levels of S100 β and NSE levels were comparable in Groups C, OMP and IPMP.

Conclusion: This study showed that memantine has beneficial effects on negative effects of propofol on recovery, cognitive functions and pain.

Key words: Postoperative cognitive dysfunction, propofol, memantine, RAM, NSE, S100 β

Introduction

Postoperative cognitive dysfunction (POCD) is characterized by an impairment in the concentration, memory, language use and social communication of a person, and is especially common in elderly patients after major surgery (1,2). Although the risk factors are pain, underlying dementia, metabolic disorders; the most important risk factor is advanced age (3). In cognitive dysfunction studies performed on this elderly population who undergo surgery; serious side effects of propofol was detected but has been used frequently in recent years due to its many advantages (4,5). Although the mechanism of action of propofol is not fully known, it is thought that it acts by reducing the separation of gamma aminobutyric acid (GABA) from the receptor. At the same time, there is a common inhibitory effect on the central nervous system (CNS) by inhibiting glutamate receptors, slow calcium channels and voltage-gated sodium channels, which are subtypes of the excitatory N-methyl-D-aspartate (NMDA) receptors (6). The widespread inhibition effect of propofol on NMDA receptors contributes to the effects of the drug on the CNS (6).

Memantine is an NMDA receptor antagonist and has proven efficacy in the treatment of Alzheimer's disease. It is believed that the primary effect of memantine is to block the flow of NMDA receptors through high concentrations of glutamate and L-glutamate is the main excitatory neurotransmitter in the CNS. Neuronal transmission, however, has important roles in the plasticity and memory processes. It has also been shown to be a neuroprotective agent and to have positive effects on pain control (7-9).

In this study, we aimed to investigate the effects of memantine on the postoperative waking from propofol anesthesia, cognitive function and analgesia in elderly rats. To test this hypothesis, we used S-100 β and neuron-specific enolase (NSE) blood levels and radial arm maze (RAM) and pain measures previously associated with cognitive functions.

MATERIALS AND METHODS

Choice of subjects: Study was conducted after taking approval from Gazi University Animal experiments local ethics committee. In the study, 30 female Wistar albino elderly rats (> 12

months old) weighing between 200-320 g were used. The rats were housed in 12 hours light-12 hours dark and adaptation was achieved. Subjects were kept in standard light and temperature. While no liquid restriction was applied to animals receiving standard pellet feed, food restriction was applied during study days.

Methods: Rats in all groups were weighed, recorded, and rats were fed normal for 20 days and once a week, RAM and hot-plate tests were performed and the results were recorded. Rats were divided into 5 groups (n=6).

Group C: The rats in the control group (Group C) received 1 mL of 0.09% NaCl **intraperitoneal** (i.p.) and the rats were placed on the RAM after 30 minutes. The number and duration of entry and exit to the arms of RAM and hot-plate durations were measured and recorded at 0.1.2 hours of the rats.

Group P: Propofol 1% (Propofol 1%, Fresenius Kabi AB, Germany) i.p. at a dose of 100 mg/kg was administered to the rats in the propofol group (Group P) After the application, rats were expected to recover from anesthesia. Recovery from anesthesia was evaluated with tail pinch (compress the 3-4 cm distal of the tail's head with "Rubber dam" forceps for 30 sec) test and recovery time was recorded. After recovery; rats were placed on RAM. Recovery time was accepted as zero hour. The number and duration of entry and exit to the arms hours of the rats and hot-plate durations were measured at 0.1.2 hours.

Group OMP: Memantine (Ebixa 10 mg/g Merz + Co. GmbH & Co. Frankfurt / Main-Germany) was added to the water of the rats in propofol + oral memantine (Group OMP) for 20 days with a dose of 20 mg/kg/day. After administration of 100 mg/kg propofol i.p. at 21st day, recovery was evaluated with tail pinch test and recovery time was recorded. After recovery; rats were placed on RAM, The number and duration of entry and exit to the arms and hot-plate durations were measured at 0.1.2 hours.

Group OM: Memantine was added to the water of the rats in oral memantine group (Group OM) as 20 mg/kg/day for 20 days. These rats were put on RAM simultaneously with other group of rats which had 21st day recovery, the number and duration of entry and exit to the arms and hot-plate durations were measured at 0, 1 and 2nd hours.

Group IPMP: The rats in the intraperitoneal memantine + propofol group (Group IPMP) received 1 mg/kg/mL i.p. of memantine (Memantine hydrochloride Sigma-Aldrich Chemie St. Louis-USA) at 21th day and after 30 minutes of administration, propofol 1% at a dose of 100 mg/kg was give intraperitoneally. Drug application times were recorded. Rats are expected to be recovered from anesthesia. Rats were placed on RAM after recovery, the number and

duration of entry and exit to the arms and hot-plate durations were measured at 0, 1st and 2nd hours.

Radial Arm Maze measurement: All groups of rats were given training on RAM for 300 seconds once a week. Rats were fasted for two hours prior to this procedure and pellet feeds were placed at the end of each arm of RAM prior to commencement of work. The number of entries and exits of the rats into the arms and the duration of each stay were recorded.

Hot-plate Measurement: Baseline values were recorded before the initiation of 20-day oral memantine treatment and then hot-plate values were measured once a week for 20 days. The hot plate was heated to 55 °C and the movements of the rats such as licking and jumping were evaluated by the same person. The maximum duration of the rats on the plate was 25 seconds. (To avoid tissue damage, the time to remain in the hot plate was limited to 25 sec)

Neuron-specific enolase and S100 β measurement: After all these procedures were performed, intracardiac blood was taken from the rats and rats were euthanized. Blood samples were centrifuged at 4000 rpm for 10 minutes and serum samples were prepared and stored at -80 ° C in the Animal Experiment laboratory of Gazi University until the analysis period. NSE and S100 β levels were measured with elisa method by using “Enzyme-Linked Immunosorbent Assay Kit For Rat Enolase, NeuronSpecific (NSE)” (USCN Life Science Inc.) and “Enzyme-Linked Immunosorbent Assay Kit For S100 Calcium Binding Protein β (S100 β)” (USCN Life Science Inc.) kits.

Statistical analysis

Statistical evaluation was performed using the following tests in the SPSS 17.0 computer program. Statistical analysis data were presented as mean \pm standard deviation. The significance limit of all statistical analyzes was accepted as $p < 0.05$.

Shapiro-Wilk test was applied to the measured parameters to determine whether the distribution was normal or abnormal. One-way ANOVA was used for independent groups in determining whether there was a difference between groups for normal distributions. In case of discrepancy, comparison between groups was made by Bonferroni test.

In data such as hot plate and RAM entries and exits; presence of a statistically significant difference during repeated measurements in groups was investigated with Repeated Measurements Analysis of variance. When there is statistical significance as a result of repeated measurements analysis of variance, the Bonferroni Corrected comparison test was used to determine the measurement time that caused the difference.

Results

Tail pinch test, in which recovery from anesthesia was evaluated, was significantly shorter in Group OMP and Group IPMP in which memantine was applied, when compared with Group P ($p=0,011$, $p=0,034$, respectively), (Table 1).

When measured hot plate 1st week values were compared, there was no difference between groups (Table 1). When the hot plate values measured during the period when oral memantine was given were compared with Group C, Group P and Group IPMP at the 2nd week and 3rd week measurement time; a significant increase was found in Group OM (2nd week values $p=0,017$, $p=0,007$, $p=0,047$) $P <0,0001$) and Group OMP ($p=0,011$, $p=0,004$, $p=0,029$) ($p <0,0001$ for 3rd week respectively). The values of hot plate measured after the administration of propofol and IPMP were significantly increased (Table 1). The number of RAM entries and exits were similar between the groups before the application of anesthesia (Table 2). According to Group C in Group P, the number of entries and exits decreased significantly at 0, 1th and 2nd hour measurements ($p=0,005$, $p<0,0001$, $p<0,0001$, respectively). In Group OMP, the number of entries and exits decreased significantly at the 1st and 2nd hour measurements according to Group C ($p=0,001$, $p=0,008$). Similarly; the number of entries and exits decreased significantly at the 1st and 2nd hour measurements in Group IPMP when compared with Group C ($p<0,0001$, $p<0,0001$). In group P; the number of entries and exits decreased significantly at the 1st and 2nd hour measurements when compared with Group OM ($p=0,003$, $p<0,0001$, $p<0,0001$, respectively). According to Group P in group OMP, the number of entries and exits significantly increased at the 1st hour measurement time ($p=0,026$). In group OMP, the number of entries and exits decreased significantly at the 1st and 2nd hour measurements when compared with Group OM ($p=0,022$, $p<0,0001$, $p<0,0001$, respectively). In group IPMP, the number of entries and exits decreased significantly at the 1st and 2nd hour measurements when compared with Group OM ($p<0,0001$, $p<0,0001$) (Table 2).

In-group evaluation; the number of entries and exits were similar when 1st day measurement was compared with other measurements in Group C and Group M. On the other hand the number of entries and exits in Group P significantly decreased at 0 and 1st hour measurements ($p=0,011$, $p=0,011$, respectively). In Group PM only zero hour entries / exits were significantly reduced ($p=0,006$) (Table 2).

There was a significant difference between the groups when a comparison was made between groups in terms of NSE enzyme activity ($p=0,030$). NSE enzyme activity was found to be significantly higher in group P than in group C ($p=0,009$). NSE enzyme activity was found to be significantly lower in group OM than in group P ($p=0,006$) (Table 3).

When the groups were compared among themselves in terms of S-100 β activity, There was a significant difference between the groups ($p=0,044$). S-100 β activity was found to be significantly higher in group P than in group C ($p=0,024$). S-100 β activity was significantly lower in OM and IPMP groups when compared with P group ($p=0,024$, $p=0,026$, respectively) (Table 3).

Discussion

In our study; in which we investigated the effect of memantine, an NMDA receptor antagonist, on recovery, POCD and acute pain after propofol anesthesia in older rats; we observed that administration of memantine accelerated anesthetic recovery, improved cognitive functions, and had positive effects on acute pain with NSE and S-100 β levels.

Propofol produces widespread inhibition of NMDA, a subtype of glutamate receptors, through modulation of the door mechanism of sodium channels (6). It is known that the modulation of these receptors leads to POCD. Kunimatsu et al. used propofol as a component of **total intravenous anesthesia** (TIVA) and neuroleptic anesthesia and they observed POCD in the early period. They retrospectively analyzed patients who had undergone oral malignancy surgery for more than 10 hours in their study and they found the ratio of POD as 36%. They found most POCD in patients to whom TIVA was applied with propofol, patients over 60 years old, patients with preoperative mental dysfunction and patients with excessive bleeding during operation (10). Nishikawa et al. investigated the effects of propofol and sevoflurane anesthesia, which were used in addition to epidural anesthesia, on postoperative recovery and delirium in patients aged 65 years and older who underwent laparoscopic surgery for 3 hours or longer. As a result, although the recovery was faster in the sevoflurane group, it was shown that the delirium scores on the 2nd and 3rd days were higher in the propofol group (11). From this data, anesthesia and POCD model were performed with propofol in this study which was proved to cause POCD and provide early recovery and elderly rats were preferred due to age which is one of the most important risk factors in the development of POCD.

Memantine, a uncompetitive NMDA antagonist; is a medication that can be used for cognitive dysfunction, moderate Alzheimer's with behavioral impairment and severe Alzheimer's disease and got **Food and Drug Administration** (FDA) approval due to this indication (12). There are many pharmacological models showing that memantine increases learning and memory. **Minkeviciene** et al. treated 8 month old male rats carrying APP and PS1 gene mutations with oral memantine 30 mg/kg/day for 3 weeks. In the learning model made by morris water tank with rats; An increase in learning function was detected (7). Wise and Lichtman examined the effects of memantine on memory in 12-16 month old male rats.

They performed learning and memory tests with RAM model in rats given low dose memantine with 0.3 and 0.56 mg/kg i.p. and high dose memantine of 3 and 10 mg/kg i.p. In rat group which received 0.3 and 0.56 mg/kg low dose of memantine; In RAM, they found that they reduced the number of entries and exits back to the arms in RAM and extended the memory. On the other hand in group which received high dose of memantine (3 and 10 mg/kg); they found that the RAM run was impaired. None of the rats entered the arms to whom 10 mg/kg dose was applied. For 3 mg/kg dose; only one rat entered eight-arms and only one rat entered four arms and none of the remaining entered any arms. As a result of this study; Wise and Lichtman found that the effect of memantine is dose-dependent (13). [Zajackowski et al.](#) used memantine at a dose of 20 mg/kg/day and in rats with cortex lesions, memantine reverse memory deficits induced by lesions. As a result of the study, it was concluded that the causes of pathological damage such as cortical lesion could be reversed by memantine treatment and this could correct cognitive functions (14). The positive effects of memantine on such studies and the conflicting effect of propofol and neurotransmitter levels are also a cause of using propofol anesthesia in this study. Considering the studies that are ranked; a standard dose for memantine in different indication studies has not yet been identified and propofol anesthesia with an indication for its effect on cognitive function has not been found in the literature. For this reason, an oral dose of 20 mg/kg/day was selected in this study and this dose was tried to be used for 20 days to obtain a stable plasma level. On the other hand, lack of plasma level is a limitation of this study. However another group was added into study in which i.p form can be applied. Thus, oral administration was comparable by administering 1 mg/kg intraperitoneally half an hour before propofol anesthesia. When we evaluated to recover from anesthesia with tail squeezing only in propofol-treated rats, we obtained response after $87,50 \pm 18,45$ minutes and recovery from propofol anesthesia was observed after $51,33 \pm 14,51$ minutes ($p=0.016$) in rats where we administered memantine orally and $38,33 \pm 23,6$ minutes ($p=0.016$) in rats where we administered intraperitoneal memantine. This period was statistically significant, but there was no difference between oral and i.p. implementation. In a similar study, Emik et al. observed that recovery from propofol anesthesia was shorter after administering 1 mg/kg i.p. memantine (15).

Recovery from anesthesia is mainly dependent on the reduction of the concentration of the anesthetic agent in the brain tissues, which is, the rate of elimination of the agent (5). We think that early recovery from propofol anesthesia in rats to whom memantine was administered might be due to common interaction of both agents to NMDA receptors rather than increase of elimination rate. In a study supporting the effect of this interaction on the

level of anesthesia; Brosnan et al. picrotoxin (GABA-A receptor antagonist) was used in rats under isoflurane anesthesia and then MK-801 (NMDA receptor antagonist) was administered to these rats. When looking at isoflurane **minimum alveolar concentration** (MAC) with standard tail clamp, While MAC value due to intravenous administration of MK-801 decreased when picrotoxin increased the isoflurane MAC value. Studies have shown that NMDA receptor inhibition is a major effect on anesthetic immobilization and that the use of NMDA receptor antagonists is also influenced MAC value (16). Kuroda et al. reported in their study, in which they investigated effects of NMDA receptor antagonist MK-801 on isoflurane MAC value, that MK-801 decreased isoflurane MAC levels and that this effect was due to receptor interaction on GABA (17). However, we believe that these claims should be supported by further studies.

In our study, in which we evaluated cognitive functions with RAM, we took a weekly measurement for all the groups. There was no difference between oral memantine treatment and control group in these measures. After confirming recovery from propofol anesthesia with tail squeezing, we accepted this measurement as zero hour. At hour zero measurement; rats in the normal propofol group enter and exit with an average number of $1,17 \pm 1,60$ times in the maze arm and rats in oral memantine ($3,67 \pm 1,37$ times) group and in i.p memantine group ($3,67 \pm 3,20$ times) enter and exit maze arm. These values were high in memantine groups but this increase was not statistically significant. When we repeated this maze measurement at intervals of one hour, we observed that the statistical difference disappeared, although the rats that did not receive memantine at the second hour still moved slowly. Since we limit our measurements to 2 hours after recovery, we can not comment on late-period effects.

There are many methods and laboratory analyzes to identify the cognitive dysfunction that occurs in the postoperative period. It is noted that, in recent years, the serum markers of cerebral damage including S-100 β protein and NSE may be indicative of the cognitive dysfunction (18). In our study, these enzyme values were also evaluated within the scope of the study. An increase in NSE and S-100 β values together was observed only in propofol applied group when compared with control values and on the other hand values of S-100 β and NSE in rats to whom memantine was applied both oral and i.p when compared with control group. The increase in the values of these enzymes and higher values of search in the RAM in memantine-administered rats suggests positive effects on the recovery and cognitive functions after propofol anesthesia. In contrast to our study, the effects of propofol on NSE were investigated by Gan et al, and they investigated NSE changes in cases of isoflurane and propofol anesthesia due to acute cranioserebral trauma and measured enzyme levels at the 2nd

hour of operation and at the end of operation. As a result, the increase in serum NSE concentration was found to be in line with the severity of brain damage and the propofol infusion could reduce the serum NSE level and reduce the cerebral damage (19). Linstedt et al. found that there was an increase in S-100 β protein levels in cases with POCD (20). Liu et al. evaluated S-100 β and NSE levels in pediatric patients with cardiac disease. Both biochemical marker levels were found to be high in patients receiving surgery (21).

It has also been observed that the results of neuropsychiatric tests used to determine cognitive dysfunction in the postoperative period and the results of serum markers such as NSE and S-100 β protein may not always comply with each other. One of the most important reasons for the inconsistency between the results is to choose the appropriate time for the test and serum values in the postoperative period (22-24). In this study, blood samples were collected at the earliest 3 hours after anesthesia recovery and hourly follow-up, and the measurement times were tried to keep close to each other in all the groups.

Another aspect of our study was the assessment of the effect of memantine on acute pain. NMDA receptor antagonists have created new promise in clinical use for pain therapy with demonstrating the role of NMDA receptors in neuropathic pain. Despite the most common use of ketamine, its use is limited due to psychomimetic side effects, sleeping effect and causing hallucination. Memantine is a new hope for relieving pain with good tolerance to ketamine in patients (25). In our study, baseline pain levels in all Group were measured by hot plate test before use of memantine and ($p=0,972$) was found similar. In the group using oral memantine, no effect was observed on week 1 and duration of resistance against painful stimulus prolonged at 2nd and 3rd weeks. Our result is compatible with the study of Chen et al. in which they reported that administration of memantine (20 mg/kg/day) in the form of s.c infusion to diabetic rats for 2 weeks was effective for the control of neuropathic pain (26).

Another result is the prolongation of analgesia durations of the rats in all memantine-administered groups, including the propofol group, when compared with the control group. Interestingly, there was no difference in terms of analgesia durations between propofol, propofol + memantine and i.p. propofol + memantine groups. This can be attributed to some analgesic properties of propofol (6). Limiting the duration of stay on the hot plate is recommended to avoid thermal damage to the animals in analgesia studies. We also limited this duration to 25 seconds in our study. This short duration may have prevented a significant difference due to both residual sedative effect and analgesic feature. Since we limit our study to 2 hours after recovery, we can not comment on the late period analgesic activity. However, the increase in hot plate values after 2 weeks is considered to be the effect of memantine on

acute pain to continue for 2 hours after propofol anesthesia. As a result of this experimental study; Memantine has been shown to shorten the period of follow-up after propofol anesthesia, improve neurocognitive functions, and have positive effects on pain. In the light of the data we obtained as a result of the study; we believe that the administration of memantine to the patients in the risk group may reduce the POCD problem. We believe that clinical trials with memantine should further investigate the efficacy of memantine on POCD and anesthesia.

Table 1. Values of tail flick and hot plate values of the groups [Mean \pm SD].

	Group C (n=6)	Group P (n=6)	Group OMP (n=6)	Group OM (n=6)	Group IPMP (n=6)	P
Tail flick (min)	-	87,50 \pm 18,45	51,33 \pm 14,51+	-	38,33 \pm 23,63+	0,016
Hot plate 1. week (second)	4,10 \pm 1,35	4,35 \pm 2,46	4,45 \pm 1,49	4,55 \pm 2,02	4,42 \pm 1,43	0,972
Hot plate 2. week (second)	5,72 \pm 1,26	5,42 \pm 0,82	8,65 \pm 1,89*,+,#	8,50 \pm 1,05*,+	6,03 \pm 1,59&,.?	0,003
Hot plate 3. week (second)	5,43 \pm 1,29	6,15 \pm 1,05	12,90 \pm 2,45*,+,#	13,13 \pm 4,23*,+,#	6,12 \pm 1,44&,.?	<0,0001
Hot plate 0.hour (second)	5,60 \pm 1,87	20,31 \pm 7,06*,#	23,13 \pm 4,57*,#	14,25 \pm 2,41*,&,#	22,98 \pm 4,94*,?,&,#	<0,0001
Hot plate 1. hour (second)	5,48 \pm 1,73	22,63 \pm 3,00*,#	23,28 \pm 4,20*,#	12,50 \pm 3,57*,+,&,#	20,17 \pm 5,49*,?,&,#	<0,0001
Hot plate 2. hour (second)	5,49 \pm 1,66	21,70 \pm 3,73*,#	21,33 \pm 6,83*,#	10,70 \pm 2,70*,+,&	19,38 \pm 6,18*,?,&,#	0,001

*: p<0,05 (When compared with Group C), +: p<0,05 (When compared with Group P), &: p<0,05 (When compared with Group OMP),?:p<0,05 (When compared with Group OM), #: p<0,05 (When compared with 1st week measurements)

Table 2. Radial arm maze entry and exit data of the groups [Mean ± SD].

	Group C (n=6)	Group P (n=6)	Group OMP (n=6)	Group OM (n=6)	Group IPMP (n=6)	P
Week 1 (entry-exit)	9,17±1,94	7,00±2,10	7,33±2,07	7,33±2,16	6,83±2,32	0,434
Week 2 (entry-exit)	5,83±3,06	7,17±1,33	6,33±1,63	7,50±3,73	6,33±1,97	0,774
Week 3 (entry-exit)	7,33±1,21	6,50±1,52	5,33±2,07	6,33±2,66	6,17±1,17	0,319
Hour 0 (entry-exit)	5,33±3,20	1,17±1,60*,≠	3,67±1,37+	7,00±1,79+,&	3,67±3,20	0,009
Hour 1 (entry-exit)	6,17±1,47	1,83±1,47*,≠	2,50±1,87*	7,17±1,17+,&	1,83±1,60 *,?,≠	<0,0001
Hour 2 (entry-exit)	5,83±1,17	1,67±1,03*,≠	2,50±2,35*	6,83±1,33+,&	1,67±1,03*,?,≠	0,001

*: p<0,05 (When compared with Group C), +: p<0,05 (When compared with Group P), &: p<0,05 (When compared with Group OMP),?:p<0,05 (When compared with Group OM), ≠: p<0,05 (When compared with 1st week measurements)

Table 3. Data about NSE and S 100 β of the groups [Mean \pm SD].

	Group C (n=6)	Group P (n=6)	Group OMP (n=6)	Group OM (n=6)	Group IPMP (n=6)	P
NSE ($\mu\text{g/L}$)	743,20 \pm 53,90	1085,80 \pm 374,28*	884,00 \pm 198,85	698,50 \pm 99,57+	631,50 \pm 346,36	0,030
S 100 β ($\mu\text{g/L}$)	5,36 \pm 3,35	10,60 \pm 9,65	5,47 \pm 1,01	6,00 \pm 1,70	6,50 \pm 5,58	0,414

*: p<0,05 (When compared with Group C), +: p<0,05 (When compared with Group P)

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Correccion made in article

1. In abstract-matherial method section group abbreviations have been renamed.
2. In abstract -result section, p values added
3. In matherial method section, groups are seperated
4. In matherial method section groups, 'To avoid tissue damage, the time to remain in the' hot plate was limited to 25 sec)' is added
5. The 7th literature is renamed.
6. The 14 th literature is changed