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EFFECT OF N-ACETYLCYSTEIN (NAC) IN EXPERIMENTAL BLUNT LIVER INJURY

General Surgery

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ABSTRACT

Introduction and Aims: Nowadays, blunt liver injury is increasingly preferred to be managed non-operatively. Therefore, wound heal progressing agents are widely investigated. N-Acetylcystein (NAC), which has antioxidant effect, is an amino aside derivate and a glutathione precursor as well. Aim of this study is to investigate the effect of NAC at liver injured bluntly.

Materials and Methods: A total of 6 groups to be sham, NAC intraperitoneally (IP), and NAC intramuscular (IM) administrated 6 rats in each group. These groups were separated for day 3 (Sham-3, IM-3 and İP-3) and day 7 (Sham-7, IM-7 and IP-7) terminations. Blunt abdominal trauma was applied to all groups. The dosage of NAC was 50mg/kg and 200mg/kg for intramuscular and intraperitoneal pathways respectively. Histopathological assessment was done by scoring inflammation severity between 0-3, apoptosis index(AI) by the method of tunnel and Ki-67 proliferation index (PI).

Results: AST and ALT levels were less in the seventh day groups than that of the third day groups. This decrease was significant in Sham-7 and IM-7 groups. Among the same day groups, AST, ALT and LDH levels were significantly low in IM and IP groups according to the Sham groups. However, there was no evident difference between IM and IP groups. Inflammation severity did not differ between the day 3 and 7 groups. But, it was significantly low in IM and IP groups compared to the Sham-3. Day 7 groups also showed difference. Ki-67 PI's of the IM-3 and IP-3 groups were prominently higher than that of the IM-7 and IP-7 groups. AI, the seventh day of the third day, groups, IM and IP groups did not differerences significantly. AI groups' of the Sham-3, IM-3 groups were the differences between these groups were definite. Seventh-day AI groups, a significant differences between the sham group was higher in IM.

Conclusion: When all our results are examined, NAC is effective in the healing and regeneration of liver tissue after blunt liver trauma. However, further studies are required.

KEYWORDS

Blunt Liver Injury, N-Acetyl Systein, Ki-67

INTRODUCTION

Abdominal trauma is third only to trauma of the head and chest (1). Blunt abdominal traumas cause mostly common liver, spleen and pancreatic injuries, respectively. Out of these 70 - 90% liver traumas are simple and 10 to 30% comprise of complex liver injuries, accompanied by fragmentation. The developed diagnostic methods of today have helped with the non-operative, conservative treatment and follow-up of liver simple injuries, decreasing liver-related mortality rate to 10% (2). The idea of researching the substances that contribute to helping this recovery time has attracted the attention of researchers and many studies have been done on this subject (3).

NAC (N-acetylcysteine) is a mucolytic agent made up of a thiol compound. Active metabolites comprise of cysteine, methionine, disulphides and reduced glutathione. Since glutathione is precursor, it has a protective effect on the cell against toxic effects by reducing H2O2 level (4 – 7). Since understanding the detoxifying of free radical effect of NAC, it has been popularized in the use of cancer, heart disease, metal toxicity and liver; as in the case acetaminophen toxicity (5). NAC extends cell life by regulating the activities of apoptosis and various proteins, reducing endothelial dysfunction, reducing the formation of invasion, fibrosis, inflammation, promoting acetaminophen detoxification and decreasing the need for transplantation (8).

The aim of our study is to investigate the effects of NAC on liver tissue healing after blunt liver trauma as it is known to have antioxidant properties and be pro-tissue regeneration.

MATERIALS AND METHODS

The study was held at Abant Izzet Baysal University following Experimental Animals Research Laboratory approval number: 2012/23 of the local Ethics board of Animal research at Abant Izzet Baysal University. 36 Wistar Albino type rats with weights ranging from 250 to 300g were kept in cages in groups of six. Standard pellet feed and municipal drinking water were used. The rats were maintained under constant temperature and humidity. Blunt abdominal trauma was applied to all groups. The subjects were starved for six hours before the trauma and before surgery.

- ***Group I*** (n=6) (Sham-3): Followed-up for three days post-trauma. Laparotomy done and rat resutured.
- ***Group II*** (n=6) (NAC(IP)-3): Intraperitoneal application of 200mg/kg of NAC was administered for three days post-trauma.
- ***Group III*** (n=6) (NAC (IM)-3): Intramuscular application of 50mg/kg NAC given for three days post-trauma.
- ***Group IV*** (n=6) (Sham-7): Followed-up for seven days post-trauma.
- ***Group V*** (n=6) (NAC (IP)-7): Intraperitoneal application of 200mg/kg of NAC was administered for seven days post-trauma.
- ***Group VI*** (n=6) (NAC (IM)-7): Intramuscular application of 50mg/kg NAC given for seven days post-trauma.

Anesthesia and Infliction of Trauma

General anesthesia was intramuscularly given in the form of ketamine HCl (Ketalar 50mg/ml, 40 mg/kg dose Eczacıbaşı, Istanbul) and 5 mg/kg intramuscular doses of Xylazine (Rompun 20mg/ml, Bayer, Istanbul). 0.784 joule of kinetic energy was applied with a fixed weight of 200gr from a height of 40 cm which was administered on the specially manufactured platform, being located to the right lateral abdominal wall of the rats as identified on the table (Fig. 1) (9).

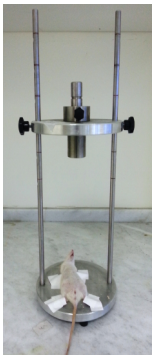


FIGURE 1: Custom Made Platform

General anesthesia and approximation to the table position was done following an abdominal shave and sterilization followed by approximately 2.5cm medium line incision with laparotomy; three days post-traumatically for Groups I, II, III, and seven days post-traumatically for Groups IV, V, VI. 2 cc of intra-cardiac blood was removed to check for AST, ALT, LDH. This was dispatched to the s biochemistry lab. Then all the subjects were sacrificed, and hepatic tissue was resected in total and delivered to the histology and embryology laboratory in 10% formaldehyde.

Biochemical Evaluation

Serum samples were obtained by using the Olympus device in the Biochemistry laboratory, and the levels of ALT, AST, LDH were studied in all groups.

Histological Evaluation

5 micrometer thickness of paraffin blocks prepared sections based on the method Wintzer et al. being evaluated to determine the Ki-67(10). 150 to 500 cells were counted on the slides corresponding to an area magnified to 400. Apoptotic bodies were evaluated in light microscope in sections stained by terminal Deoxy transferase-mediated DUTP-biotin nick-end labeling (TUNEL) method. In sections of the area where most of the staining was seen at x400 magnification (OlymposCX41) 10 consecutive apoptotic cell areas were counted. Ki-67, the number of cells showing apoptotic staining, was calculated as a percentage of the total cell count. The inflammation of the specimens was evaluated with H&E staining in 5 micrometer thickness sections of the tissues (Table 7).

TABLE 7: Classification Of Inflammation

Inflammation	Extent
No inflammation	0
Minimal inflammation	1
Moderate inflammation	2
Severe inflammation	3

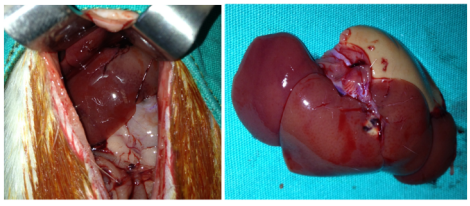
Statistical Evaluation

The data was calculated in the form of an average ± standard deviation (SD). Analysis of data was done using SPSS version 16.0 program for Windows. Differences between AST, ALT, LDH, levels of each group obtained by Mann-Whitney U test and the differences between the levels of apoptosis and inflammation were evaluated by the Ki-67 test; $P < 0.05$ values were considered meaningful.

RESULTS

Group I was observed to have a Grade II injury in the liver of rats on laparotomy to determine the severity of trauma (Fig. 2). Only one rat was exitus in group V with all rats in other groups being viable.

FIGURE 2: Grade II liver laceration (a) in trauma-induced rats (a), healed liver in group V, who underwent post-trauma laparotomy on the seventh day (b)



a b

Group I (sham3) and Group IV (sham7) which had undergone laparotomy in days three and seven days post traumatically had the highest average levels of AST, ALT and LDH. It was discovered that in Groups II (İP-3) and III(İM-3) AST, ALT, LDH levels were seen to be statistically significantly lower compared in relationship to those in Group I(Sham-3) (P=0.004, P=0.010, P=0.004, P=0.004, P=0.004, P=0.004; respectively) (Table 8). Group V (İP-7) and Group VI (IM-7) showed a statistically significantly lower level of AST, ALT, LDH compared to Group IV (Sham-7) (P=0.006, P=0.006, P=0.006, P=0.004, P=0.006, P=0.004; respectively) (Table 8). Intraperitoneal and intramuscular groups given NAC showed statistical significance for AST, ALT and LDH between Group III(IM-3) and Group VI(IM-7), regarding levels of AST and ALT, but not for LDH (P=0.020, P=0.16, P=0.78; respectively) (Table 8).

TABLE 8. Post-laparotomy levels mean levels and standard deviation for AST,ALT,LDH in all groups.

	AST(Mean ±SD)	ALT(Mean ±SD)	LDH(Mean ±SD)
Grup I (Sham-3)	878,6±495,8	466,5±189,9	3449±3534,6
Grup II (İP-3)[a]	246,8±97,4	123,1±32,7	774,8±424,1
Grup III (İM-3)[bc]	275,5±131,8	142,6±46,8	588±234,5
Grup IV (Sham-7)	316,3±80,6	153,6±30,0	2159,6±294,1
Grup V (İP-7)[d]	145±33,5	93,8±10,3	691,8±286,5
Grup VI (İM-7)[ef]	141±23,0	85±10,1	855,5±273,3

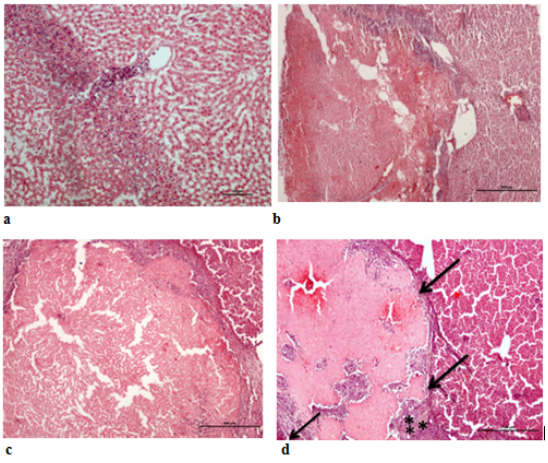
[a b d e] Comparing Group I with Groups II and III ; and Group IV for AST,ALT, LDH levels were significant $P<0.05$

[c f] Comparing Groups II and Grup III; Groups V and VI did not reveal a significant level difference in AST,ALT, LDH $P>0.05$

Histopathological Evaluation

When compared for extent of liver inflammation, rats sacrificed three days post-trauma in Group I, when compared to Groups II and III, and when Group V was compared to Groups VI and VII was seen to be significant (P=0.046, P=0.014, P=0.001, P=0.001; respectively). There was no significance in the two therapeutic groups II and III and V-VI when inter-compared (P=0.558, P=1.00; respectively) (Table 9, Figure 3).

Figure 3. In the Trauma group II, many inflammatory cells in the liver tissue are seen to be dispersed from the veins into the parechymal tissue (a), and in group III, the cellular area that lead to necrosis is seen to be smaller than the control (b). In group V, like group III, there is an increase in fibrotic tissue around the area of damage (c). In group VI, the fibrotic tissue that restricts the necrotic area is evident (arrows), bile duct proliferation (*) is observed (d). HE staining



a b c d

TABLE 9: Evaluation of the degree of liver tissue inflammation in all groups.

inflammation degree	0	1	2	3
Grup I (Sham-3)	0	0	0	6 (%100)
Grup II (İP- 3)	0	0	3 (%50)	3 (%50)
Grup III (İM- 3)	0	0	4 (%66,6)	2 (%33,3)
Grup IV (Sham- 7)	0	0	4 (%66,6)	2 (%33,3)
Grup V (İP- 7)	0	0	5(%100)	0

Sacrificed rats following three days post-trauma showed no significant differences in Groups II, III and I when Ki-67 immunostaining was carried out in the liver tissue (P=0.105, P=0.017; respectively). There was a significant difference in comparison between the Groups II and III (P = 0.029). Sacrificed rats following seven days post-trauma showed no significant differences in Groups VI, V and VI when Ki-67 immunostaining was carried out in the liver tissue (P=0.621, P=1, P=0.621; respectively) (Table 10) (Figure 4, 5).

Figure 4. Group I, II, III immunization of Ki-67 in experimental groups. Hepatocyte (Arrow) (a), Group III Ki-67 (+) stained Hepatocites (arrows) (b), which are in the group Ki-67 (+), in group III, in the portal area, the bile duct Ki-67 (+) stained epithelial cells (c), Ki-67 (4) stained hepatocytes (arrows) observed in Group II (d)

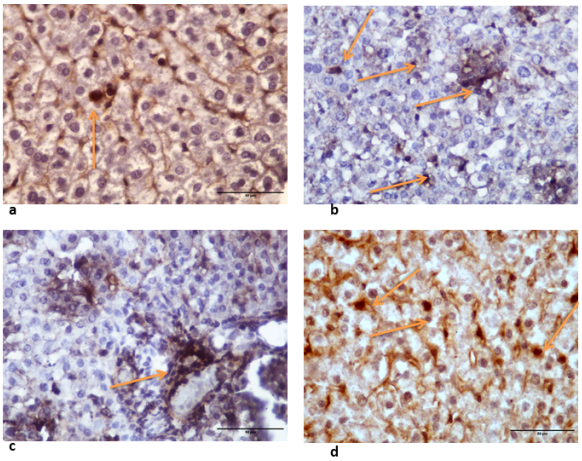


Figure 5. Group II, V the immunostaining with Ki-67 in intraperitoneal NAC groups. In group II, in hepatocytes around the portal area, Ki-67 (+) staining (arrows) (a), group II hepatocytes KiI-67 (+) staining (arrows) (b), in Group V, the Ki-67 (+) painted hepatocytes (arrows) (c), in Group V, in the bile duct epithelial cells in the portal area-67 (+) Staining (arrow) (d) is observed

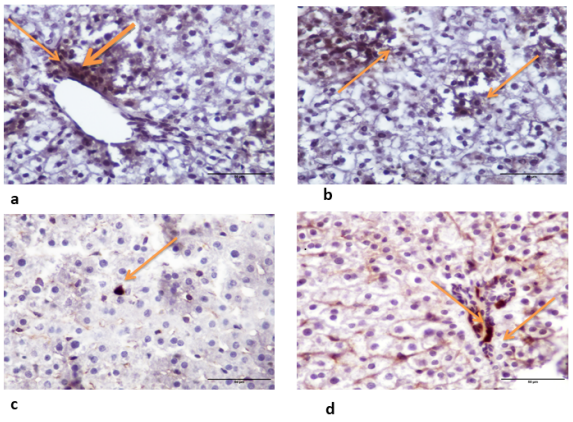


TABLE 10 : Ki-67 Value In All Groups.

Ki-67	1	2	3	4	5
Grup I (Sham-3)	3 (%50)	3 (%50)	0	0	0
Grup II (İP- 3)	0	5 (%83,4)	1 (%16,6)	0	0
Grup III (İM- 3)	0	0	3 (%50)	1 (%16,6)	2(%33,3)
Grup IV (Sham- 7)	3 (%50)	3 (%50)	0	0	0
Grup V (İP- 7)	4 (%80)	1 (%20)	0	0	0
Grup VI (İM- 7)	4 (%66,6)	2 (%33,3)	0	0	0

TABLE 11 : Apoptosis Index(ai) In All Groups.

Apoptosis index	1	2	3
Grup I (Sham-3)	6 (%100)	0	0
Grup II (İP- 3)[a]	0	2 (%33,3)	4 (%66,6)
Grup III (İM- 3)[bc]	0	0	6 (%100)
Grup IV (Sham- 7)	5 (%83,4)	1 (%16,6)	
Grup V (İP- 7)[d]	0	3 (%60)	2 (%40)
Grup VI (İM- 7)[ef]	0	1 (%16,6)	5 (%83,4)

[a]P >0,05 when group I and II compared; bP <0,05 when group I ve III compared
[c]P >0,05 when group II and III compared; dP >0,05 when group I veII compared
[e]P <0,05 when group I and III compared; fP >0,05 when group II and III compared

DISCUSSION

The tissue healing and regeneration ability of the liver has been the curious subject of research for many years. The feasibility of finding a compound to contribute to the recovery time of the liver has attracted the attention of researchers and a lot of work has been done on this subject (3, 11, 12).

Blunt Abdominal Trauma Model (BATM) on experimental animals is important in the investigation of substances to be used to treat blunt abdominal traumas. All conditions kept constant, BATM may differ even when the same energy is held constant, due to each animal's unique properties, such as body tissue quantities and integrity. To offset this short-coming of the BATM, the experiment can be created by grouping the subjects according to the severity of abdominal trauma (13). Karamercan et al. found that 1.0J and above in the BATM created a major injury while application of 0.6j to 0, 8j, to the solid organ caused minor injury (2). In our study, that Grade II injury was seen on laparotomy which was performed three days after trauma in the group I which was applied 0.8J of impact energy in BATM. This was found to be consistent with the work of Kamercan et al.

The difference between the Ki-67 and any other method is that it corresponds to all the growth phases of the cycle, rather than just the S-phase. This classification a good indication of the cellular proliferation activity (14,15). Uzun et al. concluded that the treatment of NAC increased liver regeneration following partial hepatectomy in non-alcoholic fatty liver (16). Silva et al. showed that ischemia reperfusion after hepatectomy improved liver regeneration when treated with NAC (17).

Yang et al. in their hepatotoxication model in rats with acetaminophen, determined that after long-term NAC, liver regeneration regressed (18). In our study, in comparison with Group II and III, the Ki-67 regeneration rates (RR) are statistically meaningful (p = 0.017, p = 0.029; respectively). Uzun et al., accordance with the work done by Silva et al., showed were in accordance with the findings of Yang et al. The absence of RR difference in comparison with control groups of IP groups supports the operation of Yang et al. However, it is our conclusion that the findings of Yang et al. are not compatible with the application model of NAC (18). Comparing Group V and VI there is not a statistical difference between RR compared to the Group IV. Therefore, this is incompatible with the operation of Yang et al. and is not compatible with the work of the Uzun et al. (18). The difference is that the long-term use of NAC, as reported by Yang et al., the formation of delayed liver regeneration.

Yang et al., however, after long-term NAC treatment in hepatotoxic mice performed with acetaminophen, showed a significant decline in ALT and AST following therapy, higher ratio of ALT/AST was recorded at the end of NAC treatment. In our study, the ALT/AST ratio of Group II and Group III was on average 0.5, while Group V and group VI had decreased lower levels values compared to group II and Group III, later this ALT/AST ratio of Group V and Group VI increased to 0.6. Compared to this rate group I and group IV the ALT/AST ratio was observed to decline from 0.5 to 0.4. These results are consistent with the work of Yang et al. and we postulate that NAC has a long-term effect of increasing ALT/AST ratio.

Furuta et al. in a study examining liver regeneration by performing 70% hepatectomy together with 50% pancreatectomy. On day 0, the AST and ALT levels were higher per day but reported to have decreased to 50% on the third day and normalized by the seventh day (19). Leed Ejs et al. performed 30% hepatectomy and used NAC in hepatic ischemia-reperfusion model in mice, although there was no statistically significant difference in the AST levels in the NAC group, there was significantly lower ALT levels (P < 0.05) (20). Fukuzawa et al. reported that NAC had the potential to decrease hepatic ischemia-

reperfusion injury. They infused one group with NAC prior and one after the ischemia-reperfusion injury. It was shown that both NAC groups had lesser ischemia-reperfusion injury compared to the control group which did not receive any NAC. They reported their findings by showing a significant difference in rates of AST and LDH levels (21). The results of our research are compared with the literature; the measured levels of AST and ALT are not supported by Furuta et al., while Fukuzawa et al. showing a decrease in hepatic ischemia-reperfusion injury with NAC is consistent with biochemical values in our study.

We deduce that this difference results from 70% is by hepatectomy extrahepatic and chemical agents, further accelerating regeneration and healing. The study of hepatic ischemia reperfusion with 30% hepatectomy of Leed Ejs et al. and treated with NAC supports our work.

Our experimental study of isolated liver injuries of blunt abdominal trauma is the first study of its type when we examined the literature, as there is difficulty in modelling an isolated liver injury; the absence of any other work on experimentally isolated liver injury is the major limitation of our study.

Toydemir et al. investigated the antiapoptotic and proliferative effects of the anti-toxin, turmeric (*Curcuma longa*) on hepatectomized rats. In the study, the percentage of apoptosis in the hepatectomy group was found to be higher than the hepatectomy group treated with turmeric, and the groups reported a statistically significant difference when compared with the least apoptosis ratio in the sham (no NAC) group (22).

In a study where Zhou et al. studied the protective effects of NAC on gastric mucosal ischemia reperfusion, they found that gastric mucosal cell apoptosis decreased according to the control group of the group treated with NAC (23). While our study does not support the work of Zhou et al., it is similar to the work of Toydemir et al.

CONCLUSION

When all our results are examined, NAC is effective in the healing and regeneration of liver tissue after blunt liver trauma, as well as short-term treatment it is a high performance long-term treatment. There was no significant difference in terms of intramuscular and intraperitoneal administration of the treatment. We hope that the effects of NAC will have a positive effect on humans as well. After blunt liver trauma, short-term treatment in nonoperative patients is an option which can be beneficial in terms of early discharge and treatment costs. However, extensive clinical and experimental studies are still needed.

REFERENCES

1. Shao Y, Zou D, Li Z, Wan L, Qin Z, Liu N, et al. Blunt liver injury with intact ribs under impacts on the abdomen: a biomechanical investigation. Plos One. 2013;8(1):e52366.
2. Karamercan A et al. Künt karın travması: Tanı yöntemlerinin değerlendirilmesi ve cerrahi sonuçlar. Ulus Travma Acil Derg. 2008;14(3):205-210
3. Ramalho FS, Ramalho LN, Castro-E-Silva Júnior O, Zucoloto S, Corrêa FM. Angiotensin-converting enzyme inhibition by lisinopril enhances liver regeneration in rats. Braz J Med Biol Res Rev Bras Pesqui Médicas E Biológicas Soc Bras Biofísica Al. 2001;34(1):125–127.
4. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci Cmls. 2003;60(1):6–20.
5. Jones AL. Mechanism of action and value of N-acetylcysteine in the treatment of early and late acetaminophen poisoning: a critical review. J Toxicol Clin Toxicol. 1998;36(4):277–285.
6. Ziment I. Acetylcysteine: a drug that is much more than a mucokinetic. Biomed. Pharmacother. Biomédecine Pharmacothérapie. 1988;42(8):513–519.
7. Holdiness MR. Clinical pharmacokinetics of N-acetylcysteine. Clin Pharmacokinet. 1991;20(2):123–134.
8. Foresti R, Sarathchandra P, Clark JE, Green CJ, Motterlini R. Peroxynitrite induces haem oxygenase-1 in vascular endothelial cells: a link to apoptosis. Biochem J. 1999;1(1):729–736.
9. Karamercan MA, Sevgili AM, Karamercan A, Atilla P, Balkanci ZD, Karamercan G, et al. Microscopic hematuria as a marker of blunt abdominal trauma in rats: description of an experimental model. J Trauma. 2011;71(3):687–693.
10. Wintzer HO, Zipfel I, Schulte-Mönting J, Hellerich U, von Kleist S. Ki-67 immunostaining in human breast tumors and its relationship to prognosis. Cancer. 1991;67(2):421–428.
11. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. J Hepatol. 2012;57(3):692–694.
12. Ramalho LNZ, Zucoloto S, Ramalho FS, Castro-e-Silva O de Jr, Corrêa FMA. Effect of antihypertensive agents on stellate cells during liver regeneration in rats. Arq Gastroenterol. 2003;40(1):40–44.
13. Reed RL 2nd. Recurrent ventilator-associated pneumonias: the gift that keeps on giving or the guest who won't leave? Surg Infect. 2010;11(3):319–324.
14. Hopf NJ, Bremm J, Bohl J, Perneczky A. Image analysis of proliferating cells in tumors of the human nervous system: an immunohistological study with the monoclonal antibody Ki-67. Neurosurgery. 1994;35(5):917–923.
15. Hall PA, Levison DA. Review: assessment of cell proliferation in histological material. J Clin Pathol. 1990;43(3):184–192.
16. Uzun MA, Koksal N, Kadioglu H, Gunerhan Y, Aktas S, Dursun N, et al. Effects of N-acetylcysteine on regeneration following partial hepatectomy in rats with nonalcoholic fatty liver disease. Surg Today. 2009;39(7):592–597.
17. Silva SM, Carbonel AAF, Taha MO, Simões MJ, Montero EFS. Proliferative activity in ischemia/reperfusion injury in hepatectomized mice: effect of N-acetylcysteine. Transplant Proc. 2012;44(8):2321–2325.
18. Yang R, Miki K, He X, Killeen ME, Fink MP. Prolonged treatment with N-acetylcystine delays liver recovery from acetaminophen hepatotoxicity. Crit Care Lond. Engl. 2009;13(2):55.
19. Furuta, Kakita, Takahashi, Tomiya, Fujiwara. Experimental study on liver regeneration after simultaneous partial hepatectomy and pancreatectomy. Hepatol Res Off J Jpn Soc Hepatol. 2000;17(3):223–236.
20. Lee EJS, Silva SM da, Simões M de J, Montero EF de S. Effect of N-acetylcysteine in liver ischemia-reperfusion injury after 30% hepatectomy in mice. Acta Cirúrgica Bras Soc Bras Para Desenvolv Pesqui Em Cir. 2012 ;27(4):346–349.
21. Fukuzawa K, Emre S, Senyuz O, Acarli K, Schwartz ME, Miller CM. N-acetylcysteine ameliorates reperfusion injury after warm hepatic ischemia. Transplantation. 1995;59(1):6–9.
22. Toydemir T, Kanter M, Erboga M, Oguz S, Erenoglu C. Antioxidative, antiapoptotic, and proliferative effect of curcumin on liver regeneration after partial hepatectomy in rats. Toxicol Ind Health. 2013;52(1): 75-79
23. Zhou X-Y, DU D-S, Ma X-B, Zhang J-F. [The protective mechanism of N-acetylcysteine against ischemia/reperfusion induced gastric injury in rats]. Sheng Li Xue Bao. 2010;62(1):69–72.