Effect of HBOT on Acute liver injury & Survival rate in a sepsis animal model

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Declaration of conflict interest

- I have **no conflict of interest** to disclose with regard to the subject matter of this presentation.

- Ajou University Medical Center, Institutional Animal Care and Use Committee approved the experimental procedures

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BACK GROUND

1 Sepsis is up-regulated systemic inflammation and HBOT provides several adaptive mechanisms in sepsis

2 Previous studies have suggested HBO had beneficial effects on animal sepsis model

3 Relatively less severe sepsis model (LPS, E-coli inoculum, CLP) & short experimental period

4 Not suggested mode of action for survival enhancement

5 ROS regulation & Apoptotic cell death pathway

PREVIOUS STUDY

• **Hepatic injury of septic rat model** **Effects of hyperbaric oxygen treatment on liver functions, oxidative status and histology in septic rats**. (2005).	Rat, Cecal ligation & puncture (CLP) model Survival rate **53.3% :73.3%** TBARS, Antioxidant enzyme (**SOD**, CAT)
• **Fecal slurry model** **Dose-dependent mortality and organ injury in a cecal slurry peritonitis model**. (2016).	Rat, cecal slurry model **Fecal slurry** 5-15 mL/kg
• **Anti-oxidative, Apoptotic signaling**	
Proapoptotic stimuli induce nuclear accumulation of glycogen synthase kinase-3 beta. (2001).	In vitro(human neuroblastoma cell) **GSK-3β** activity contributes to apoptotic signaling
Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte.(2000).	Mouse, CLP model, **caspase -3,** stem cell recombination caspase3-/- mice
Hyperbaric oxygen promotes osteogenic differentiation of bone marrow stromal cells by regulating Wnt3a/β-catenin signaling—Anin vitro and in vivo study.(2014)	In vitro(BMSC), Rabbit **HBOT promotes Phosphorylation of Akt(Ser473) & GSK-3β(Ser9**) => Decrease the activity of GSK-3 **Akt-GSK-3β survival pathway**

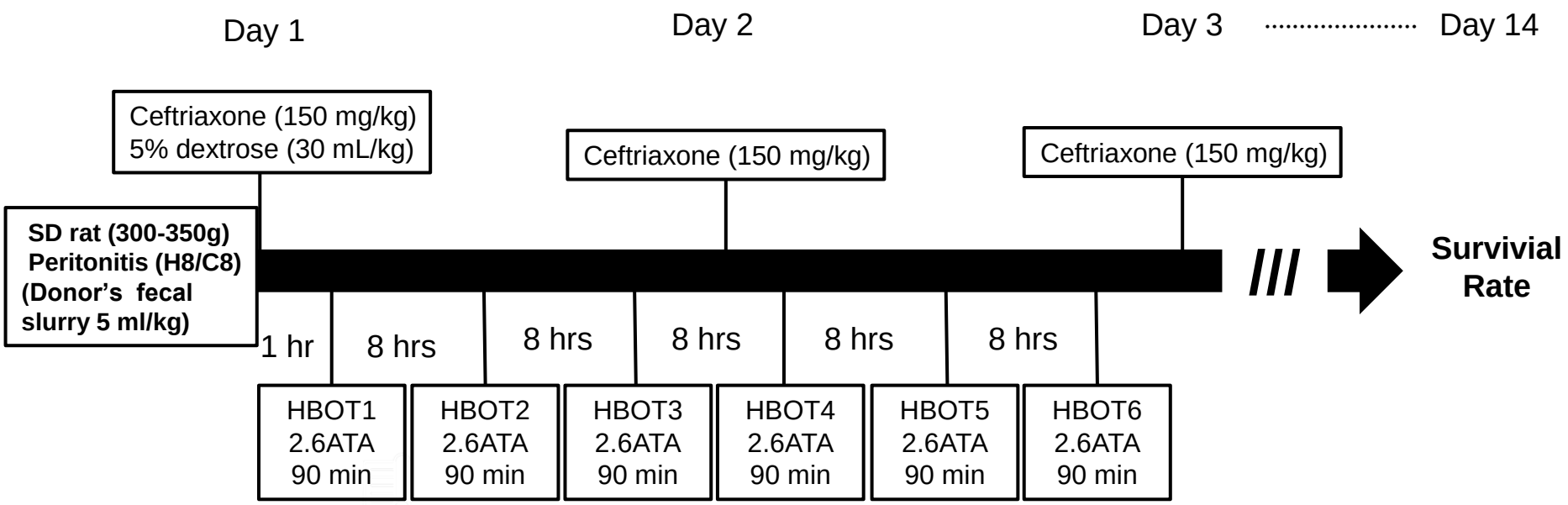
OBJECTS

To evaluate the Effect of HBOT on

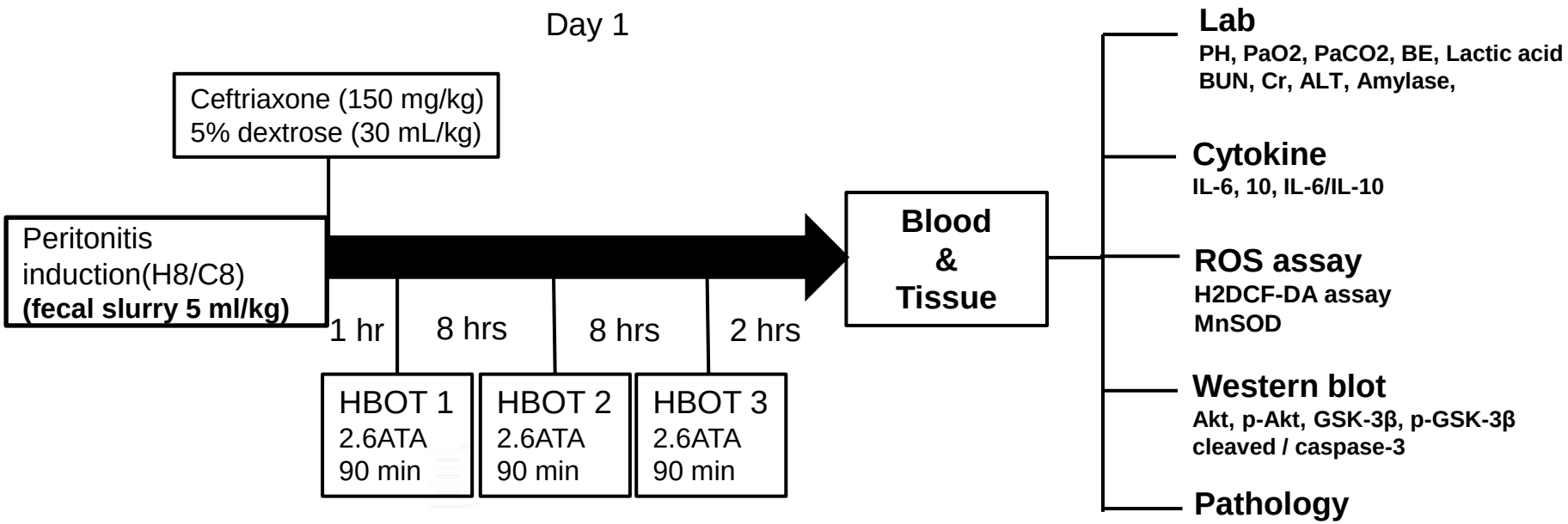
- Acute Liver Injury
- Survival rate

in a Cecal Slurry Peritonitis Rat Model

Experimental protocol (1)



Experimental protocol (2)



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AQ-9912SD

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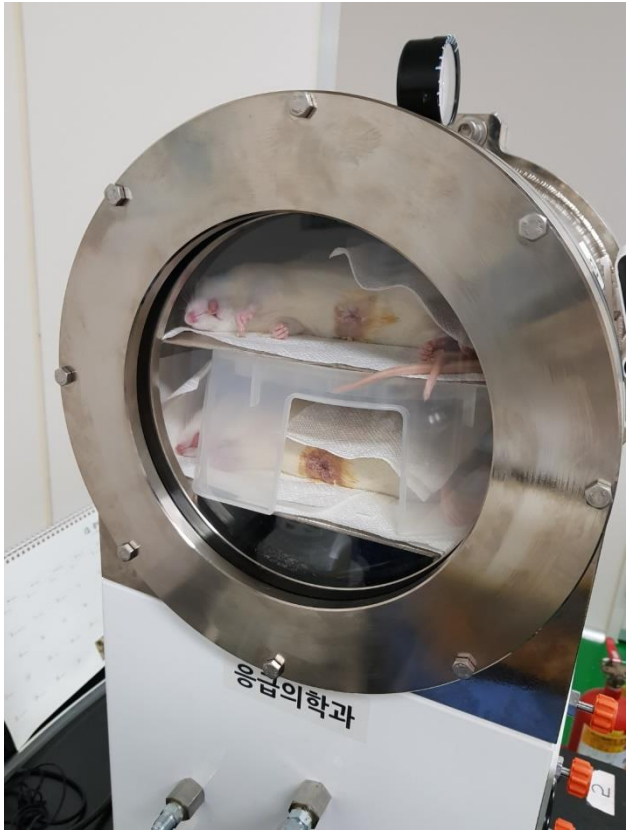
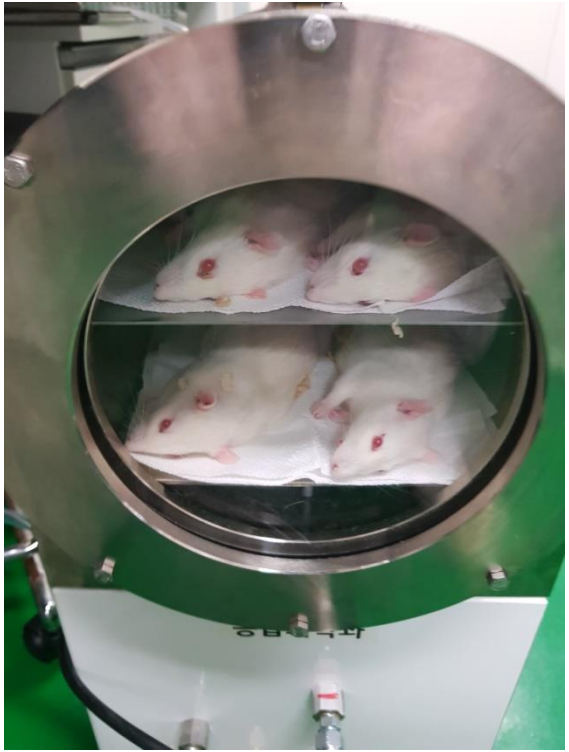

응급의학과

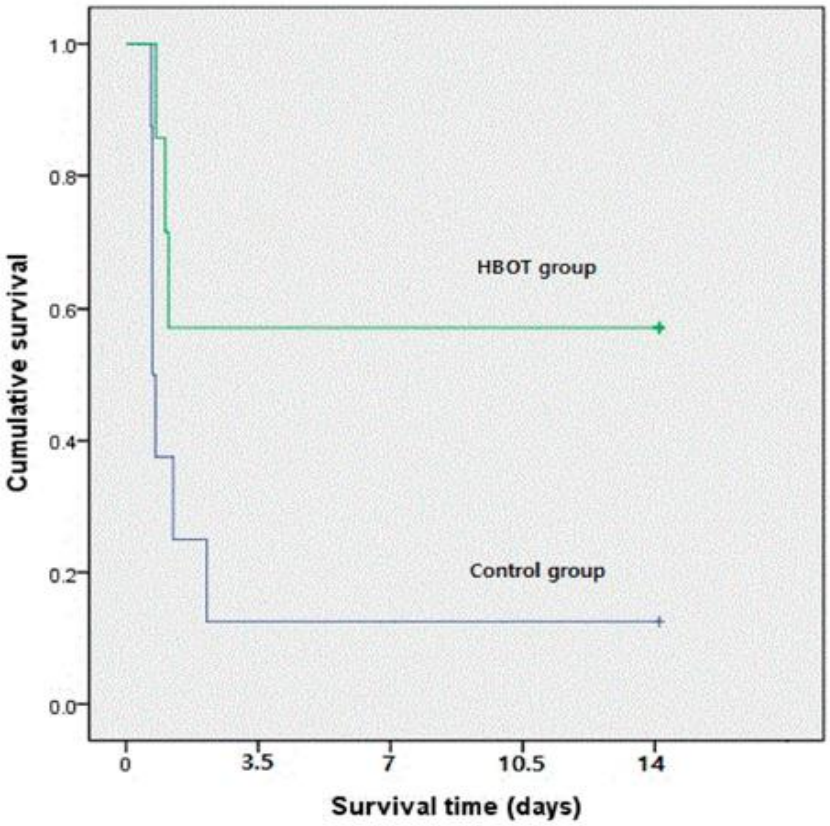


응급의학과

Results (1)

- Survival rate at 14 days
  - HBOT group – 57.1%
  - Control group – 12.5%

(*P=0.029*, *Kaplan-Meier analysis*)



Results (2)

	HBO group	non-HBO group	P-value
Albumin(g/dl)	3.8 (3.6-4.0)	3.8 (3.6-3.9)	0.906
ALT(IU/L)	34.0 (31.0-38.0)	46.5 (37.3-163.8)	0.02
Amylase(IU/L)	730.0 (634.0-1194.0)	976.0 (742.5-1137.0)	0.268
BUN(mg/dl)	55.0 (51.0-65.0)	66.0 (54.5-71.3)	0.352
SCr(mg/dl)	0.5 (0.4-0.7)	1.0 (0.7-1.5)	0.022
Glucose(mg/dl)	121.0 (115.0-137.0)	101.5 (65.3-111.8)	0.037

Data are presented as median (IQR)

Results (3)

	HBO group	non-HBO group	P-value
PH	7.4(7.31-7.42)	7.3(7.2-7.4)	0.248
PCO_2	35.2(25.0-37.6)	26.9(19.4-45.8)	0.736
PO_2	103.3(92.2-114.63)	94.3(58.8-112.3)	0.441
HCO_3	18.2(15.6-22.0)	15.7(11.4-18.0)	0.102
Lactate	2.8(2.3-3.9)	5.9(3.5-6.6)	0.015
Base excess	-6.3(-9.5--3.2)	-10.6(-14.7--7.6)	0.034

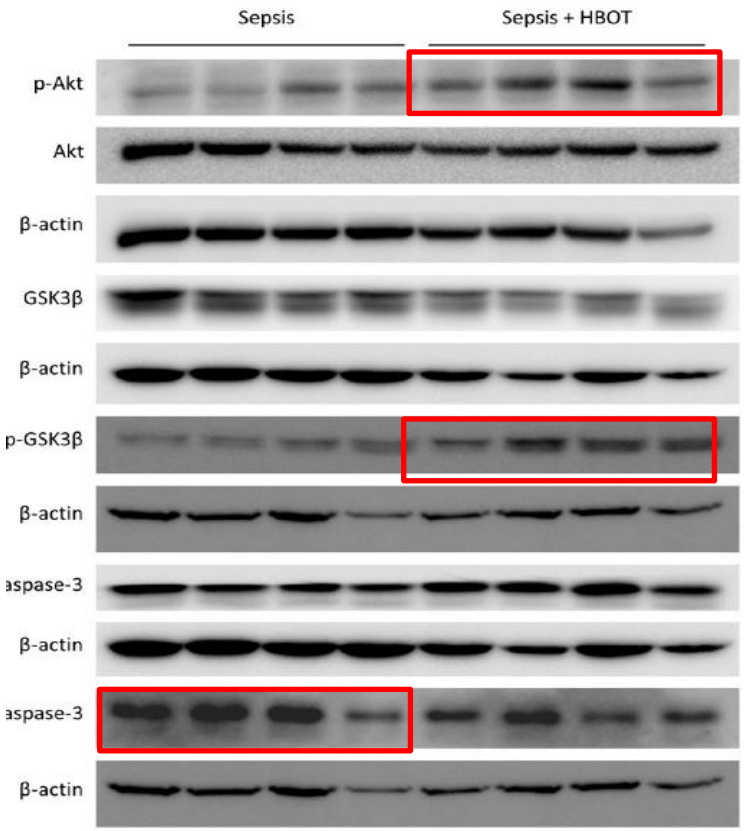
Data are presented as median (IQR)

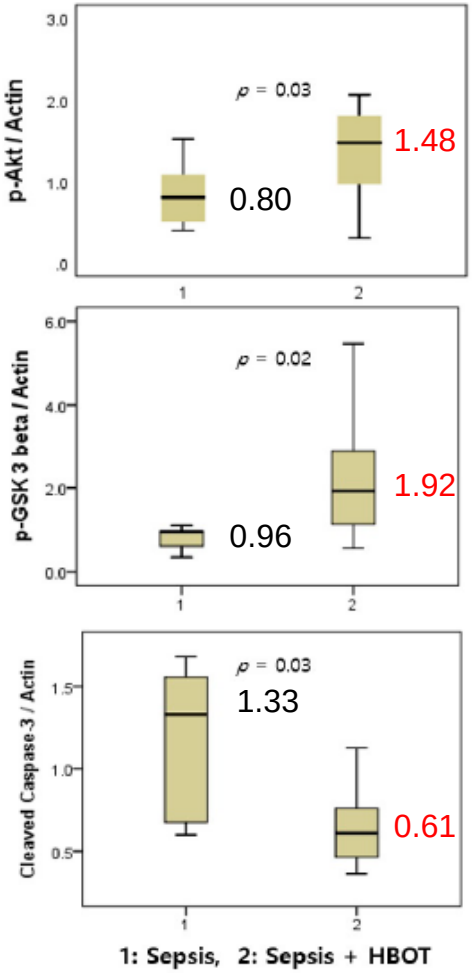
Results (4)

	HBO group	non-HBO group	P-value
IL-6	$4.5*10^3$ ($3.7*10^3$- $8.2*10^3$)	$23*10^3$ ($88*10^3$- $298*10^3$)	0.011
IL-10	$3.3*10^3$ ($3.0*10^3$- $4.2*10^3$)	$4.3*10^3$ ($3.6*10^3$- $6.6*10^3$)	0.037
IL-6/IL-10 ratio	1.5 (1.1-1.9)	3.9 (1.8-5.6)	0.037

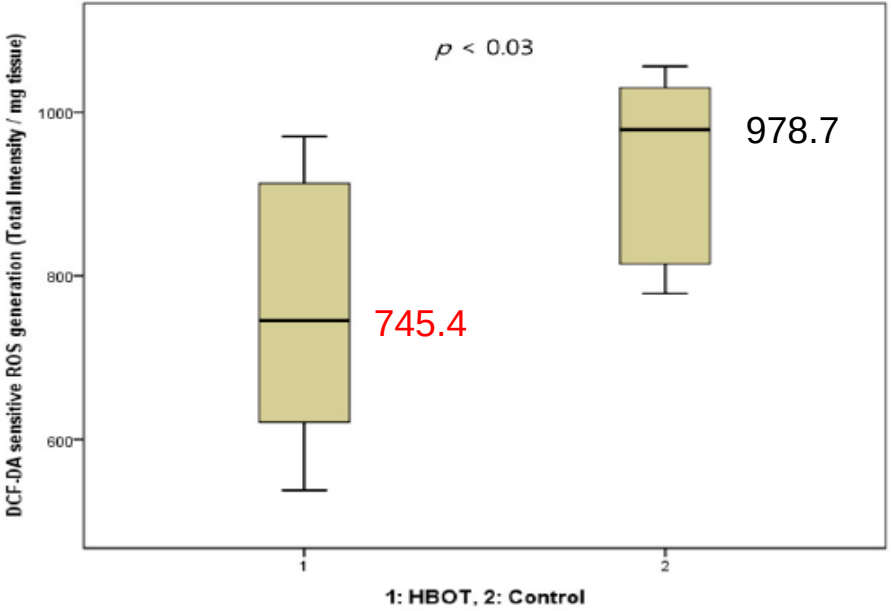
Data are presented as median (IQR)

Results (5)

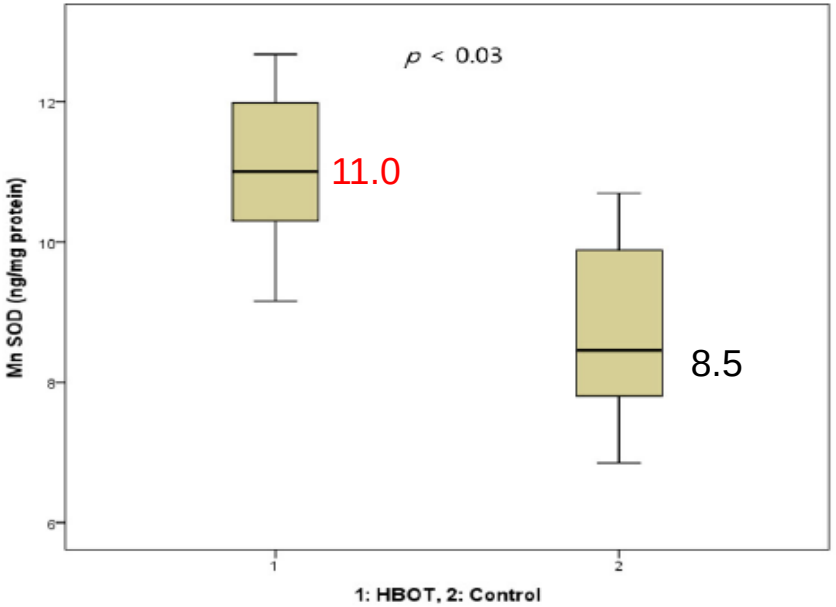

Sepsis
Sepsis + HBOT
p-Akt
Akt
β-actin
GSK3β
β-actin
p-GSK3β
β-actin
aspase-3
β-actin
aspase-3
β-actin


p-Akt / Actin
3.0
2.0
1.0
.0
p = 0.03
0.80
1.48
1
2
p-GSK 3 beta / Actin
6.0
4.0
2.0
0.0
p = 0.02
0.96
1.92
1
2
Cleaved Caspase-3 / Actin
1.5
1.0
0.5
p = 0.03
1.33
0.61
1
2
1: Sepsis, 2: Sepsis + HBOT

Results (6)



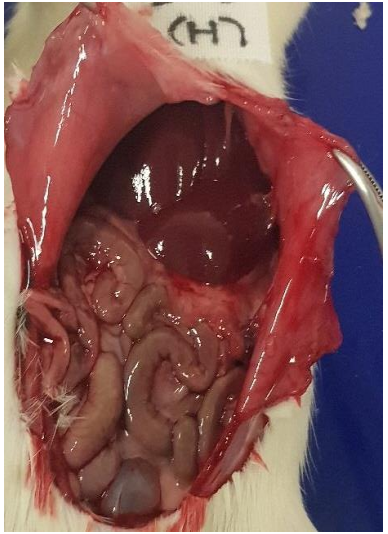
(a) ROS generation



(b) Antioxidant capacity

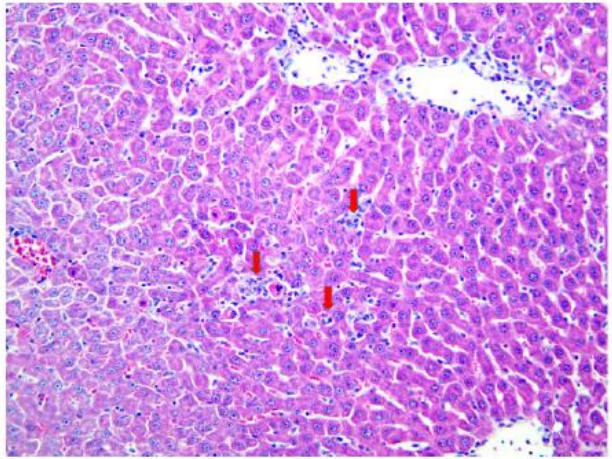
Results (7)

Non-HBOT

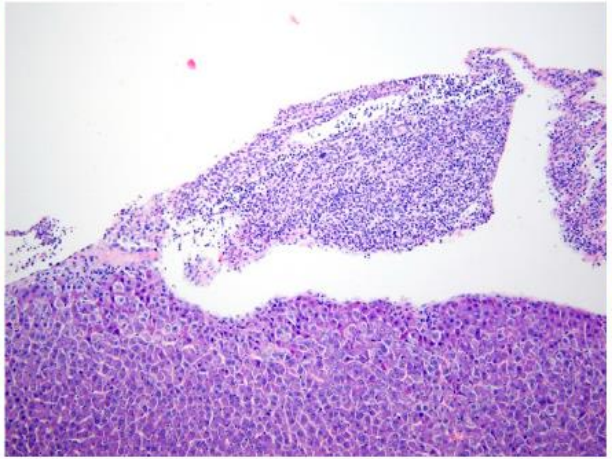


HBOT

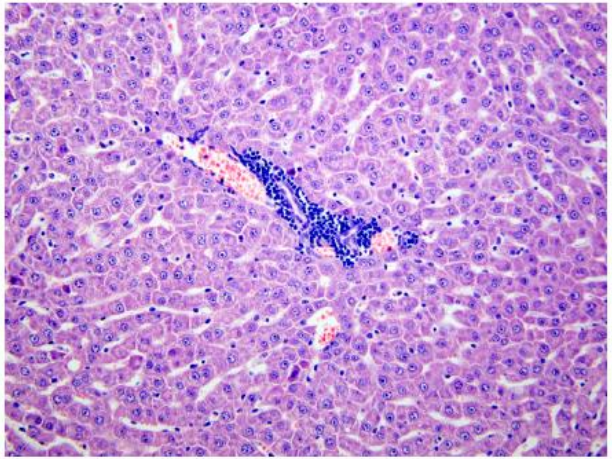
Results (8)



(a) Spotty necrosis (arrows). H&E 30x.



(b) Capsular inflammation. H&E 20x.



(c) Portal inflammation. H&E 30x.

Hepatic injury score HBOT 5[4.25-6.75] Vs Control 7[4.0-7.0]

Conclusions

1 HBOT showed beneficial effect on Acute liver injury and survival rate in a cecal slurry peritonitis model.

2 HBOT decrease intracellular ROS and oxidative status

3 HBOT enhancing the Akt signaling and inhibit GSK-3β pathway

4 HBOT attenuate apoptosis pathway (cleaved caspase-3)

5 Future goal - GSK3β-mediated pathways
HBOT for the treatment of human sepsis