IMPACT OF SPLENIC ISCHEMIC PRECONDITIONING ON SPLENIC INJURY INDUCED BY HEPATIC ISCHEMIA/REPERFUSION IN ALBINO RATS

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ABSTRACT:

Background: *Hepatic ischemia reperfusion injury (IRI) occurs during liver transplantation setting that might cause graft rejection and could result in multi-organ dysfunction. Aim: detect the possible effects of splenic ischemic preconditioning on splenic injury induced by hepatic IRI in albino rats.*

Material and methods: *Thirty adult albino rats were divided into three groups, ten rats each. Group I (control): ten rats were divided into negative control was left untreated and sham control was subjected to open abdominal wall without any intervention. Group II (IRI group): ten rats were subjected to hepatic ischemia for 45 minutes followed by 60 minutes of reperfusion. Group III (SIPC+IRI group): ten rats were subjected to intermittent splenic artery clamping in two sessions of 5 min ischemia followed by 5 min reperfusion before liver ischemia. Spleen specimens from all groups were obtained, and processed for histological, immunohistochemical and transmission electron microscopic examination.*

Results: *After hepatic IRI, the spleen showed marked congestion in red pulp with shrunken depleted lymphatic follicles in white pulp. Electron microscopic examination showed plasma cells with degenerative changes in the form of shrinkage of the nucleus, nuclear membrane separation, dilatation of rER, condensation in Golgi apparatus, and dilatation in its vesicles. Macrophages with numerous electron-dense material and secondary lysosomes were observed. There was a significant decrease in number of both CD3 and CD20 immunoreactive positive cells compared to control group. After SIPC, a relative improvement in spleen structure was observed.*

Conclusion: *Splenic ischemic preconditioning improves the splenic injury induced by hepatic ischemia/reperfusion.*

Keywords*: Splenic ischemic preconditioning, immune system, spleen*

INTRODUCTION:

Ischemic pre-conditioning is a medical therapeutic approach in which short repeated attacks of ischemia protect against injury induced by complete artery occlusion[1]. Moreover, the remote ischemic conditioning refers to practicing short episodes of non-lethal ischemia on other tissues or organs to protect against acute ischemia/reperfusion injury[2]. Such therapeutic interventions were documented to be beneficial in patients undergoing surgical procedure[3].

It is well known that the spleen plays multiple supporting roles in immune response[4]. During infectious status, the spleen creates a suitable medium for the

activation of cytotoxic T-cells and natural killer cells through antigen - presenting cells[5]. Moreover, recent researches reported that spleen has another functions as production of growth factors, regulation of portal venous pressure and non-specific immune response[6]. Evidences suggest that spleen plays a vital role in the pathophysiology of hepatic ischemia/ reperfusion injury (IRI) which occurs during setting of liver transplantation. For example, congestion of the spleen during hepatic IRI stimulates excretion of splenic interleukins which results in leukocyte infiltration with subsequent hepatic parenchymal damage[7]. Consequently, splenectomy or ligation of the splenic artery before hepatic IRI was found to inhibit this collateral reaction, thus decreasing both histopathological and biochemical injuries[8&9]. Interestingly, a recent study showed that splenic ischemic preconditioning (SIPC), as intermittent splenic pedicle clamping, can reduce renal IRI[10]. Furthermore, **Chen *et al.*[11]** described immunomodulatory effect of the spleen during remote ischemic conditioning (RIC), affecting mainly the lymphocytes derived from the spleen that afforded neuroprotection against cerebral-ischemia. There is a preclinical evidence for effective and protective role of SRIC. However, the exact underlying mechanisms and cellular pathways are still not fully determined[12].

AIM OF THE WORK:

Thus, this work was designed to detect the possible effect of splenic ischemic preconditioning on splenic injury induced by hepatic ischemia/reperfusion injury in albino rats.

MATERIAL AND METHODS:

Animals:

Thirty adult male albino rats (six-months old), weighing 180-250 gm, housed at anatomy department, Faculty of Medicine, October 6 University were used in this study. Rats were housed with regular dark/light cycles, every 12 hours and were fed the standard rat diet and supplied water *ad libitum*. All rats were kept in the same circumstances throughout the experiment.

Experimental design:

The rats were divided into three groups, ten rats each:

Group I (control group): ten rats were further divided into two subgroups (5 rats each):

Group I-a (negative control): five rats were left without any intervention.

Group I-b (sham control): five rats were subjected to open the anterior abdominal wall without hepatic ischemia or clamping of splenic artery.

Group II (IRI group): rats were subjected to hepatic ischemia for 45 minutes followed by 60 minutes of reperfusion[13].

Group III (SIPC+IRI group): ten rats were subjected to intermittent splenic artery clamping in two sessions of 5 min ischemia followed by 5 min reperfusion (20 min total) before liver ischemia[13].

Surgical technique:

The animals were anesthetized with intraperitoneal injections of urethane at a dose of 1.2 g/kg dissolved in water. Then, a midline laparotomy incision was performed. Liver ischemia was induced by clamping the hepatic pedicle using a microvascular clamp. Hepatic IRI was performed for 45 minutes ischemia followed by 60 minutes reperfusion by removing the clamp. During the procedure, the intestine was protected with a gauze heated saline to avoid fluid loss[14].

In SIPC group, splenic artery was dissected posterior to stomach. SIPC was performed by intermittent clamping of the splenic artery with a microvascular clamp in two sessions of 5 min ischemia and 5 min

reperfusion (20 min total) before liver ischemia [10 & 13].

Specimen collection:

At the end of experiment, rats were anesthetized by ether inhalation then sacrificed and the spleens were dissected out, collected, processed and prepared for examination using the following techniques:

I- For light microscopic study

Some specimens of spleen were fixed in 10% neutral formalin and processed for paraffin blocks. Paraffin sections (5μm thick) were prepared and stained with haematoxylin and eosin (Hx. & E) [15]. The sections were examined with an Olympus light microscope in Anatomy department and were photographed.

II- For immunohistochemistry technique

Five μm thick sections were deparaffinized and washed with phosphate buffered saline. The sections were incubated overnight in a humidified chamber with the primary antibody; rabbit polyclonal CD3 antibodies (1:200; pharmaceuticals, USA) for detection of T lymphocytes and CD20 antibodies (1:100 dilution; Sigma-Aldrich, USA) for detection of B lymphocytes[16]. Then, the spleen sections were rinsed with buffered saline and treated with the biotinylated antibody for one hour. The sections were then incubated with streptavidin combined to horseradish peroxidase (Sigma, USA) and finally the reaction was established using DAB (3,3-diaminobenzidine tetrahydrochloride, Fluca). The sections were stained with Mayer's haematoxylin, the reaction appeared as brown cytoplasmic dots. Stained spleen sections were examined using Olympus binocular microscope and photographed using a Canon camera connected to an IBM computer system.

III- For Transmission electron microscopic study

Other specimens of spleen were cut into $1mm^3$ pieces and dipped in 2.5% glutaraldehyde immediately and kept in the refrigerator at 4°C for 2 hrs. After wash with phosphate buffer, they were post-fixed in 1% buffered osmium tetra-oxide. The specimens were dehydrated in ascending grades of ethyl alcohol. Specimens were cleared in propylene oxide followed by propylene oxide and epoxy resin for at least one hour. Finally, the specimens were embedded in gelatin capsules filled with fresh epon. Semithin sections were cut (1μm in thickness) using an ultra-microtome and stained with 1 % toluidine blue. Ultrathin sections were obtained and stained with uranyl acetate followed by lead citrate[17]. The ultrathin sections were examined with JEOL JEM 1010 transmission electron microscope (Jeol Ltd, Tokyo, Japan) in the Regional Center of Mycology and Biotechnology, Al Azhar University, Egypt.

IV- For morphometric study and statistics analysis:

Counting the number of the immunoreactive CD3 and CD20 positive cells in all groups was done in five microscopic fields per slide (400 X magnification), five slides per rat. In addition, H&E stained sections at 400 X magnification, were examined to measure the area percentage (%) of the white pulp. Measurements in all groups were recorded using an image analyzer and the collected data were statistically analyzed using *SPSS statistical package version 13*. One-way ANOVA test followed by Tukey's multiple comparison tests was used to compare the means of the various groups. P value <0.05 was considered significant and P value < 0.001 was considered highly significant[18].

RESULTS:

I - Light microscopic results:

Group I (control group): Light microscopic examination of spleen sections from sham group revealed a similar histological picture as the negative control group. The parenchyma of the spleen

consisted of lymphoid tissue that was differentiated into white and red pulps. The white pulp consisted of lymphoid tissue which was seen in three discrete zones namely, the periarterial lymphatic sheath (PALS), the lymphatic follicles (splenic Malpighian corpuscles) and marginal zone. The PALS consisted of closely packed small lymphocytes surrounding the central arterioles (Fig.1a). The lymphoid follicles were oval or rounded aggregates mainly of small lymphocytes; most of them possessed condensed darkly stained nuclei (Figs. 1a, 1b and 1c). Peripheral to the lymphatic follicles, marginal zone appeared separating the white pulp from the red pulp. The marginal zone was a thin rim of less populated small lymphocytes with few large lymphocytes (Figs. 1c and 1d). Plasma cells were also seen showing the characteristic cart wheel appearance of their nuclei (Fig.1d). The red pulp was composed of branching and anastomosing splenic cords (Billroth cords) and blood sinusoids in between (Figs. 1a and 1d).

Group II (IRI group): Light microscopic examination of sections of the spleen from hepatic ischemia / reperfusion group showed marked congestion in the red pulp with shrunken depleted lymphatic follicles and around the central arterioles in the white pulp (Fig.2a). Areas of decreased cellularity of the follicles around the central arterioles were observed with ill-defined marginal zone (Fig.2b). Some darkly stained pyknotic nuclei surrounded by empty space were observed (Fig.2c). Marked sinusoidal dilatation and congestion were detected in the red pulp (Figs. 2d & 2e).

Group III (SIPC+IRI group): Examination of spleen sections from splenic ischemic preconditioning group showed evident preservation of lymphocytes of PALS and follicles with dark basophilic lymphocytes (Fig.3a). The white pulp was populated by a large number of lymphocytes with darkly stained nuclei. The marginal zones were well defined and contained large proliferating lymphocytes with euchromatic nuclei (Fig.3b). Moreover, numerous plasma cells were encountered. They had rounded nuclei with cart wheel appearance and abundant cytoplasm (Figs. 3c and 3d).

II- Immunohistochemical results:

Immunoreactive CD3 positive cells were noted in the control group as brown spots mainly distributed in PALS and in lymphoid follicles (T cell region) (Fig.4a). In IRI group, areas of weak reaction to CD3 around central arteriole were observed (Fig.4b), whereas the CD3 positive cells were remarkably increased in splenic ischemic preconditioning group compared to IRI group (Fig.4c).

Brownish coloration of B lymphocytes CD20 positive cells was detected in the peripheral part of the lymphoid follicles of the control group (Fig.5a). In IRI group, weak CD20 expression of B lymphocytes was detected in marginal zone compared to the control group (Fig.5b). Splenic ischemic preconditioning group showed numerous positive CD20 immunoreaction in contrast to IRI group (Fig.5c).

III- Electron microscopic results

Group I (control group): Electron microscopy of the spleen sections of the control group revealed lymphocytes with circular or elliptical outline. Their nuclei were large euchromatic with plenty of fine chromatin and clumps of condensed heterochromatin lining the nuclear membrane. The cytoplasm showed ill-defined organelles. The plasma cell appeared with multiple dense areas of heterochromatin (the characteristic cartwheel arrangement). The most characteristic feature of these cells was the well-developed rough endoplasmic reticulum channels (Fig.6a). Moreover, the cytoplasm of the macrophages displayed some cellular organelles, multiple lysosomes and many mitochondria (Fig.6b).

Group II (IRI group): Electron microscopic examination of the spleen sections from hepatic ischemia / reperfusion group showed numerous plasma cells with degenerative changes in the form of shrunken chromatin clumped nucleus (Figs.7a & 8a), nuclear membrane separation (Figs.7a & 8a), vacuolated cytoplasm (Fig.8a) and dilatation of rough endoplasmic reticulum (rER) (Figs.7a & 7b). Other plasma cells appeared with a condensation in the Golgi apparatus near the nucleus and dilatation in its vesicles (Fig.7b). Active macrophages containing secondary lysosomes with different electron densities were observed (Figs. 7a and 7c). Degenerated cells appeared with nuclear condensation and vacuolated cytoplasm (Fig.8a). Moreover, the dilated congested blood sinusoids were lined with irregular outlined endothelial cells (Fig.8b).

Group III (SIPC+IRI group): Electron microscopic examination of the spleen sections from splenic ischemic preconditioning before hepatic ischemia-reperfusion injury group revealed that most of the cells had preserved their normal appearance. Plasma cells were frequently seen with characteristic cart wheel nuclei and regularly arranged rough endoplasmic reticulum (Fig.9a). The lymphocytes appeared with well-defined cell membrane and intact nuclei (Figs.9a). Moreover, the cytoplasm of the macrophages contained some cellular organelles and many mitochondria (Fig.9b).

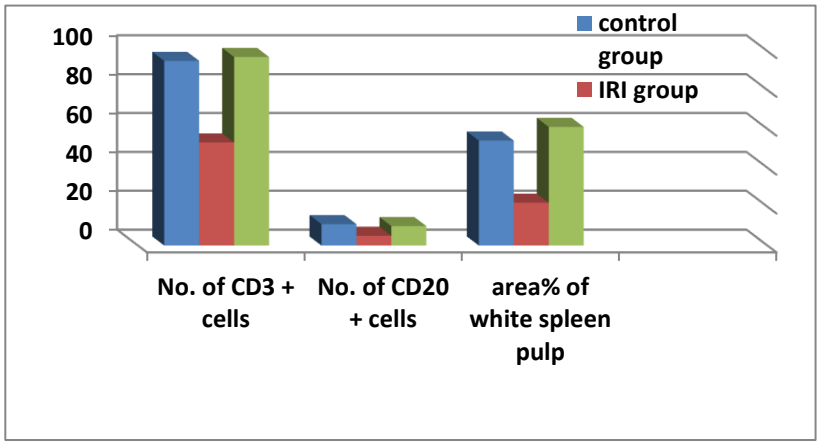
IV-Statistical results:

Upon computer image analysis, the mean number of CD3 and CD20 immuno-positive cells and the mean area percentage (%) of spleen white pulp in hepatic IRI group were significantly decreased compared to the control group. In contrast, SIPC group showed significant increase when compared to hepatic IRI group (Table 1, Histogram 1).

Table. 1: Comparison between all groups as regards the number of CD3 and CD20 immuno-reactivity per high microscopic field and the mean area % of spleen white pulp

Groups	No. of CD3 + cells	No. of CD20 + cells	area% of white spleen pulp
Control group	95.2±9.4	10.2±0.31	54. 33 ± 9.8
Hepatic IRI group	53.5±7.8*	5.7±2.3*	22.43 ± 7.2*
SIPC group	97.6±8.2 #	9.3±0.4#	61.4 ± 11.5 #

Values are mean±SD * P < 0.05 compared to control group. # P < 0.05 compared to hepatic IRI group.



Histogram. 1: Comparison between all groups as regards the mean number of CD3 and CD20 immunoreactivity per high microscopic field and the mean area% of spleen white pulp

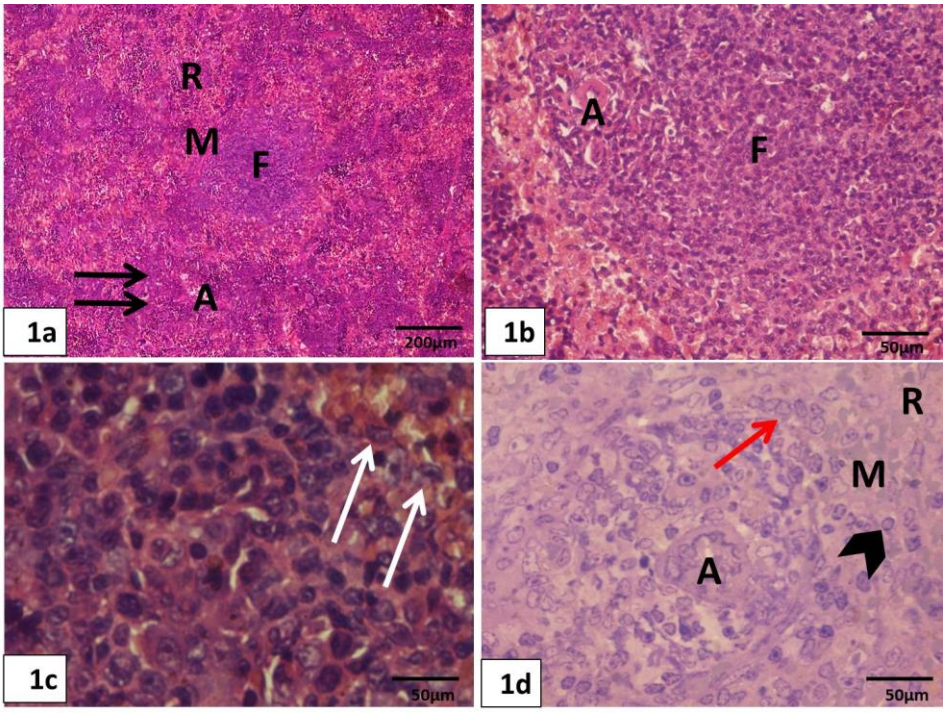


Fig.1: Photomicrographs of spleen sections of the control group showing (1a) the white pulp containing the periarterial lymphatic sheath (black arrow) consists of closely packed small lymphocytes surrounding the central arteriole (A). Notice the lymphoid follicles (F), marginal zone (M) and the red pulp (R). (1b) lymphoid follicles (F) containing condensed darkly stained nuclei of small lymphocytes. Notice periarterial lymphatic sheath around the central arteriole (A) (1c) marginal zone with less populated lymphocytes (white arrow). (1d) the marginal zone (M) with less populated lymphocytes (arrow head) separating the white pulp from the red pulp (R). Plasma cells are also seen showing the characteristic cart wheel appearance of their nuclei (red arrow). Notice the central arteriole (A).

Scale bar: (1a) Hx&E.; X100. (1b) Hx&E.; X400. (1c) Hx&E.; X1000. (1d)Toluidine blue; X1000

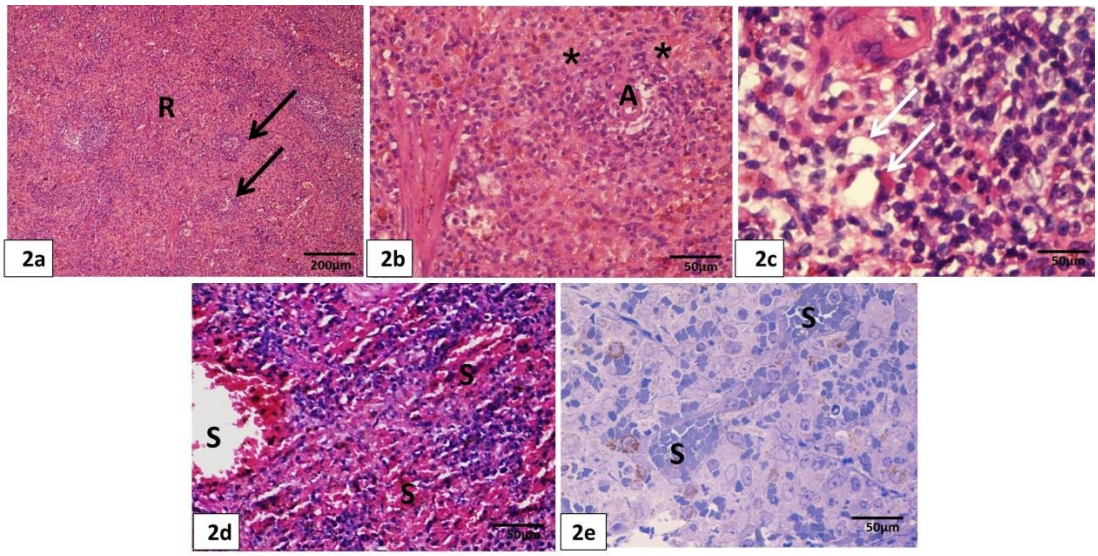


Fig. 2: Photomicrographs of spleen sections of IRI group showing (2a) marked congestion in red pulp (R) with shrunken depleted lymphatic follicles (black arrow). (2b) areas of decreased cellularity of the follicles (asterisks) around the central arteriole (A). (2c) lymphocytes with darkly stained pyknotic nuclei surrounded by empty space (white arrow). (2d) marked blood sinusoidal dilatation and congestion (S) are detected in the red pulp. (2e) sinusoidal dilatation and congestion (S) are observed. Scale bar: (2a) Hx&E.; X100. (2b) Hx&E.; X400. (2c) Hx&E.; X1000. (2d) Hx&E.; X400. (2e &2f) Toluidine blue; X1000.

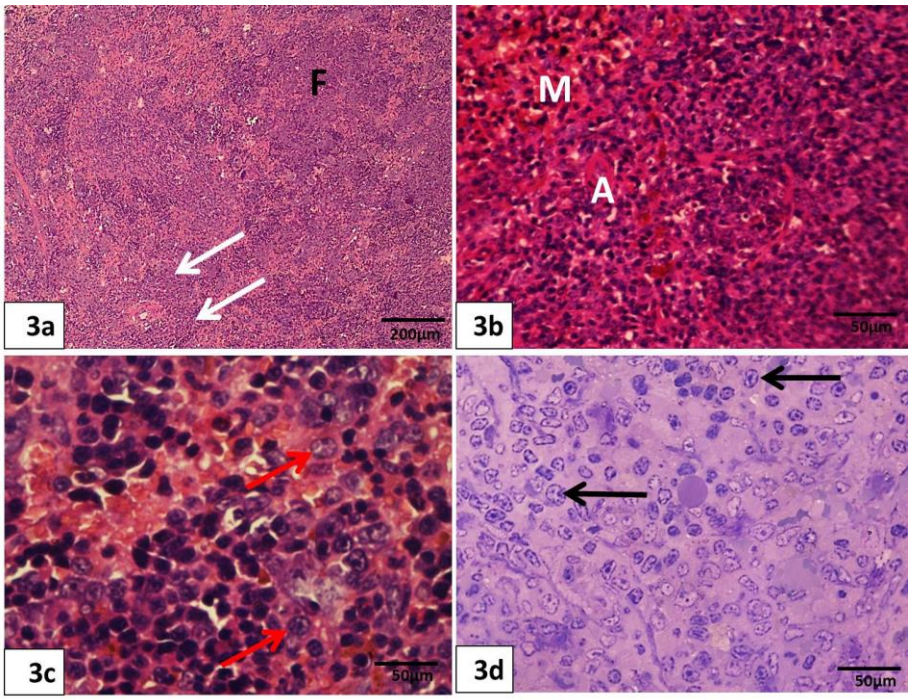


Fig. 3: Photomicrographs of spleen sections of SIPC+IRI group showing (3a) preservation of lymphocytes of PALS (white arrow) and lymphatic follicles (F) (3b) the white pulp with large number of lymphocytes around central arteriole (A). Notice the well-defined marginal zone (M). (3c) numerous plasma cells with cartwheel rounded nuclei (red arrow) (3d) numerous plasma cells with cartwheel rounded nuclei (black arrow). Scale bar: (3a) Hx&E.; X100. (3b) Hx&E.; X400. (1c) Hx&E.; X1000. (1d)Toluidine blue; X1000

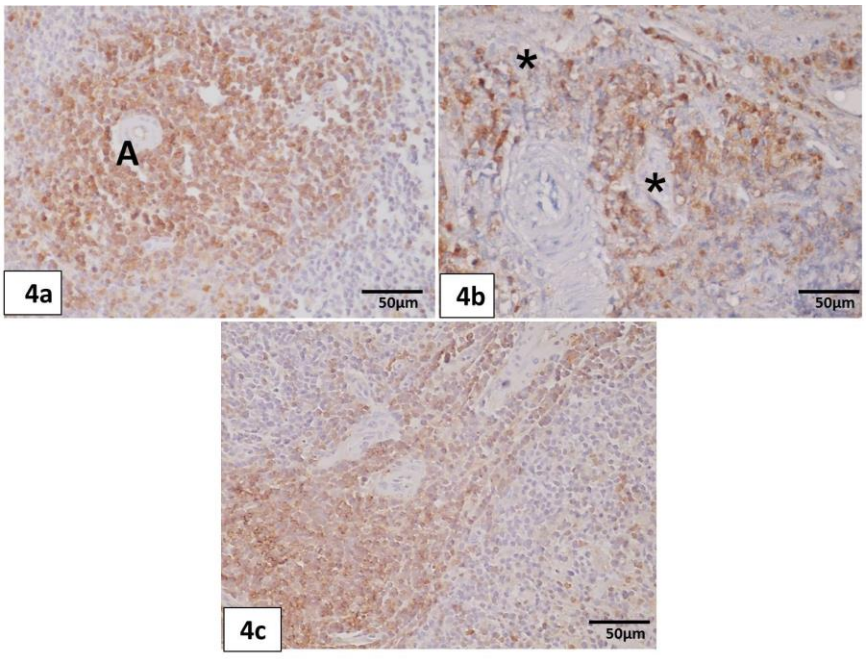


Fig. 4: Photomicrographs of CD3 immunostaining of spleen sections of all groups showing (4a) control group with brown spots of CD3 immunopositivity reaction around central arteriole (A). (4b) IRI group with areas of weak reaction to CD3 (asterisks). (4c) splenic ischemic preconditioning group with remarkably increase in CD3 immunoreactivity. (CD3 IHC × 400)

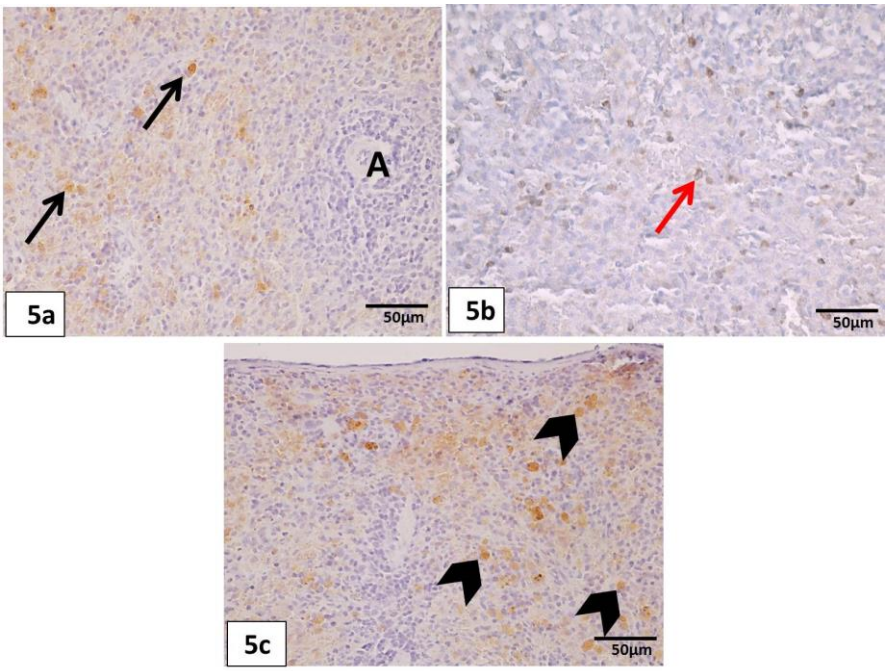


Fig. 5: Photomicrographs of CD20 immunostaining of spleen sections of all groups showing (5a) control group with brownish coloration of B lymphocytes CD20 positive cells in the peripheral part of the lymphoid follicles (black arrow). Note the central arteriole (A). (5b) IRI group with weak CD20 expression (red arrow) in the marginal zone. (5c) splenic ischemic preconditioning group with numerous positive CD20 expression (arrow head) in the marginal zone. (CD20 IHC × 400)

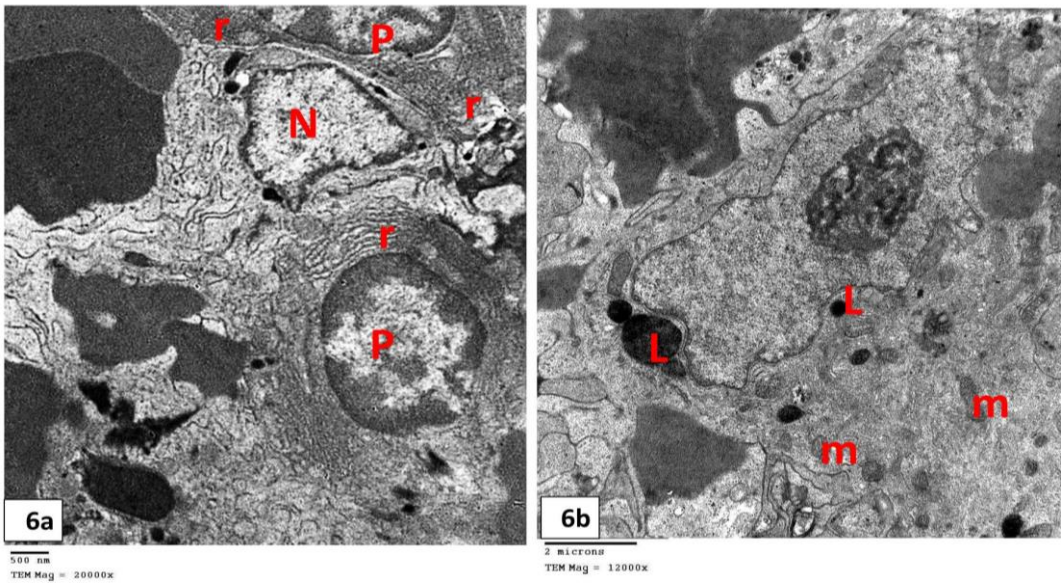


Fig. 6: Electron photomicrographs of spleen ultrathin sections of control group showing (6a) lymphocyte nucleus (N) with plenty of fine chromatin and clumps of condensed heterochromatin lining the nuclear membrane. The plasma cell (P) appears with multiple dense areas of heterochromatin and well-developed rough endoplasmic reticulum (r). (6b) macrophage with multiple lysosomes (L) and many mitochondria (m).

(Uranyl acetate& lead citrate; (6a): X20,000 & (6b):X12,000)

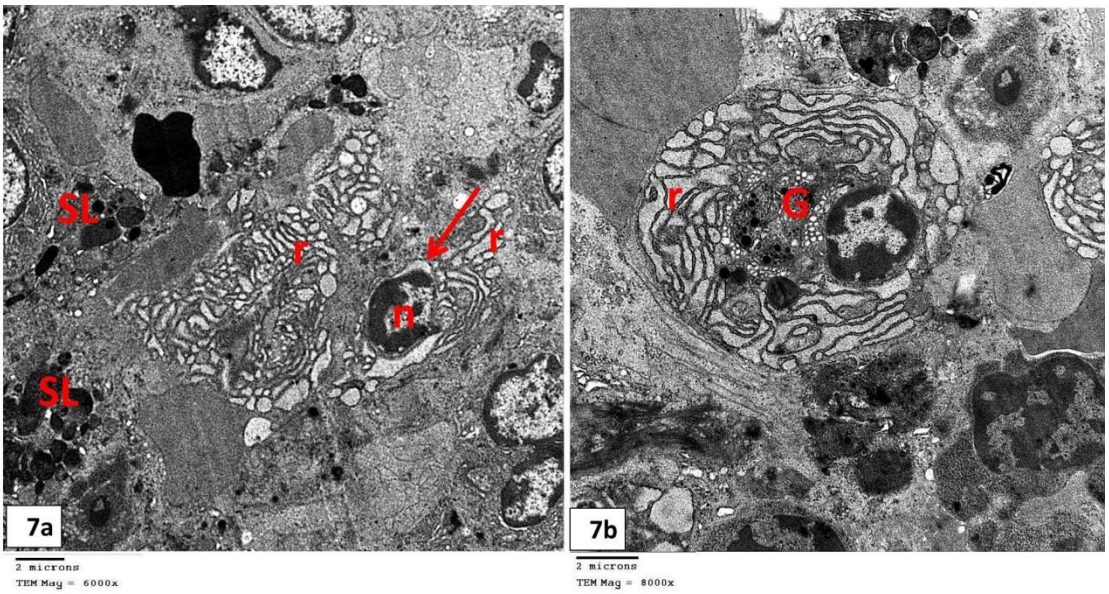


Fig. 7: Electron photomicrographs of spleen ultrathin sections of IRI group showing (7a) plasma cells with shrunken chromatin clumped nucleus (n), nuclear membrane separation (arrow), dilated rough endoplasmic reticulum (r). Notice the macrophages secondary lysosomes with different electron densities (SL). (7b) plasma cell with dilated rough endoplasmic reticulum (r) and dilated Golgi apparatus (G).

(Uranyl acetate& lead citrate; (7a): X6,000 & (7b): X8,000)

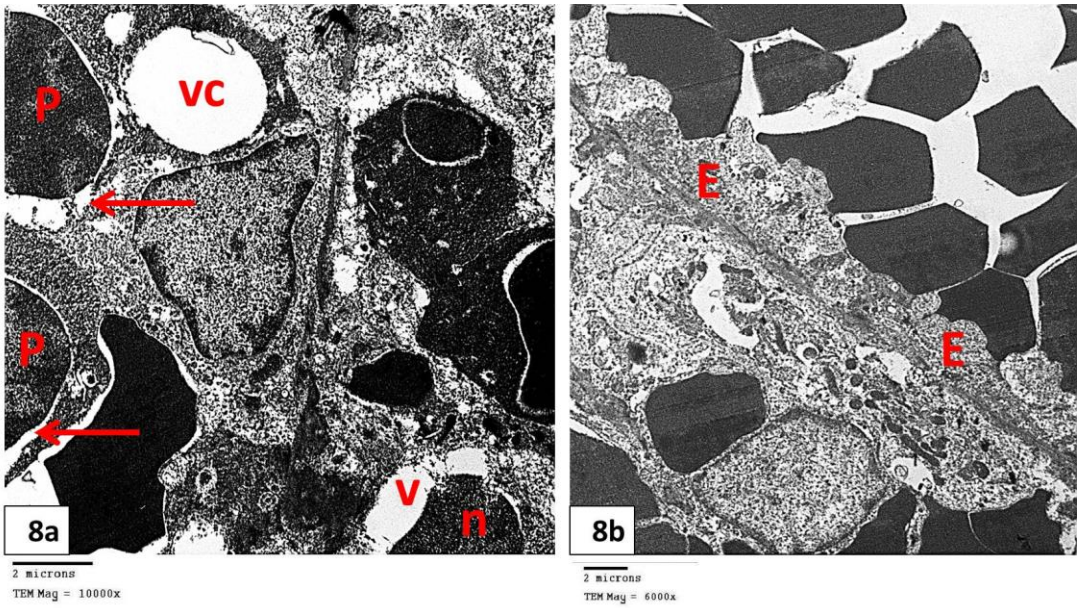


Fig. 8: Electron photomicrographs of spleen ultrathin sections of IRI group showing (8a) plasma cells with shrunken chromatin clumped nucleus (P), nuclear membrane separation (arrow), vacuolated cytoplasm (vc). Notice the degenerated cells with nuclear condensation (n) and vacuolated cytoplasm (v) (8b) dilated congested sinusoid with irregular outlined endothelial cells (E).

(Uranyl acetate& lead citrate; (8a): X10,000 & (8b): X 6,000)

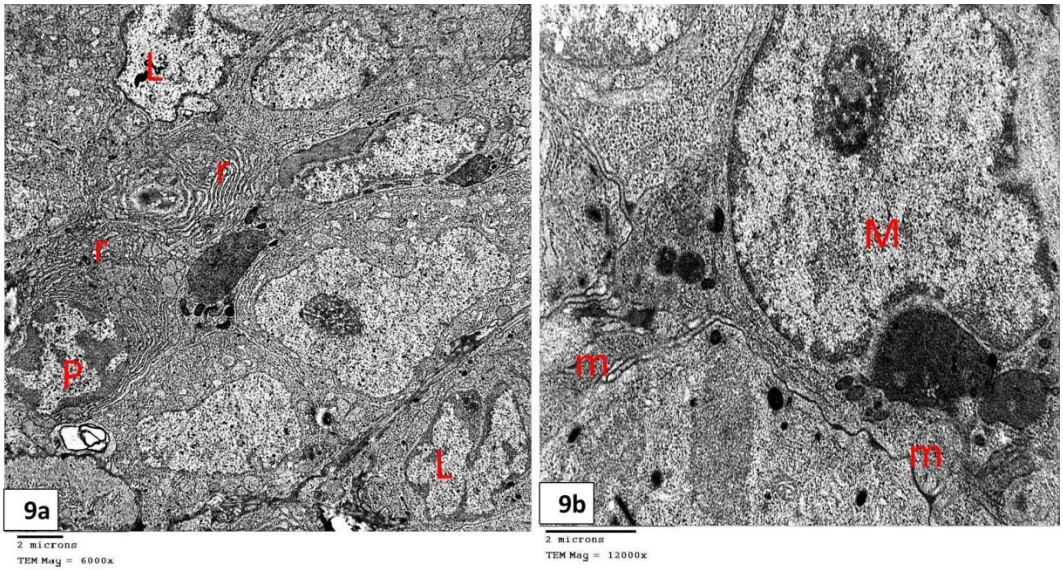


Fig. 9: Electron photomicrographs of spleen ultrathin sections of SIP+IRI group showing (9a) plasma cell (P) with regularly arranged rER (r). lymphocytes (L) with well-defined cell membrane and intact nuclei. (9b) macrophage (M) with some cellular organelles and many mitochondria (m). (Uranyl acetate& lead citrate; (9a): X6,000 & (9b): X 12,000)

DISCUSSION:

There is a link between the liver and the spleen. Both organs are vital to immunological homeostasis and are significant components of the portal circulation[19]. A liver-spleen axis has been considered a point of interface between immunity, pathogen elimination, and metabolic activity in a variety of disorders, including chronic liver disease[20]. Splenectomy was once used to treat the devastating complications of portal hypertension caused by liver cirrhosis. Many individuals, like those who suffer from thrombocytopenia, seek a substitute to splenectomy. Despite the unavailability of such possibilities, ischemia preconditioning with many transient ischemic bouts may create new paths for therapeutic strategies. Thus, the current investigation was conducted to evaluate the splenic ischemic preconditioning as therapeutic intervention that modulating spleen injury due to hepatic ischemic-reperfusion injury.

Multiple immune cell subgroups are dispersed differently within the white and red pulp and marginal zone sections of the spleen. T lymphocytes dominate in white-pulp zones, whilst B cells are mostly seen in the periphery of splenic follicle. Macrophages are typically seen in the marginal zone and red pulp zones. Immune cell migrating among these regions is required for pathogenic eradication[21].

In the current investigation, the spleen showed significant sinusoidal congestion associated with parenchymal degeneration following hepatic IRI. This was in accordance with **Wen et al.,**[22] who observed congestive spleen in pre-hepatic portal hypertensive rats. Spleen plays an important role in accelerating hepatic IRI, because ischemia/reperfusion promote splenic IL-2 excretion and macrophage infiltration within the liver, which in turn aggravate hepatic injury[7].

In the current work, the hepatic IRI lead to a reduction in CD3 positive cells in T lymphocytes region in the spleen. Moreover, electron microscopic observation demonstrating signs of apoptosis in the form of nuclear condensation and vacuolated

cytoplasm in degenerated cells most probably lymphocytes. This was in accordance with results of **Nugroho,**[23] who stated that patients with liver cirrhosis were considered as immunocompromised and prone to infections. Similarly, **Tanabe et al.**[24] observed that after carbon tetrachloride or thioacetamide-induced hepatic injury in mice, the number of CD4 T cells in the spleen decreased. This decrease is clear evident that splenic T lymphocytes may target the cirrhotic liver in order to promote hepatic fibrosis. Whereas, after splenic ischemic pre-conditioning in the present work, most of the cells had preserved their normal appearance and the lymphocytes appeared with well-defined cell membrane and intact nuclei. In a similar manner, **Chen et al.,**[11] indicated that remote ischemic preconditioning (RIPC), which increased the ratios of cytotoxic T cells (Tc) as soon as an hour later, would increase the proportions of splenic lymphocyte cells. Remarkably, 3 days after remote ischemic preconditioning, RIPC markedly boosted the B cell percentage in the spleen. Additionally, Hall et al.[25] stated that the proliferation of antigen-specific T regulatory cells and T cell-derived cytokines play a critical role in improving liver allograft survival and mediating transplant tolerance. CD25 and CD4 T cells (Treg) in the spleen have been shown to have a role in mice's spontaneously accepting of liver allografts following a liver transplant. The prognosis of a liver transplant appears to be influenced by the proportion of Treg to T effector cells[26].

In the present study, after hepatic IRI, several changes of plasma cells of variable degrees were observed. Many darkly stained nuclei surrounded by empty spaces were observed in H&E-stained sections. Electron microscopic observation confirmed the evident signs of plasma cells degenerative changes; these changes were in the form of shrinkage of the nucleus, nuclear membrane separation and dilatation of rough endoplasmic reticulum (rER). The rER's dilatation implies a malfunction in the secretory substance production process[27]. Moreover, the rER stress appears to be accompanied by protein deposition in the cell[28]. Numerous stress conditions, such as viral infection and hypoxia, contribute in the aggregation of unfolded or misfolded proteins in the endoplasmic reticulum lumen [29]. In response to ERS stimuli, certain proteins in the endoplasmic reticulum membrane are referred to as stress sensors. These proteins activate many signaling pathways to either cause apoptosis of the cells or cell survival under short or long-term stress [30]. A common ultimate pathway for many different disease processes, including cancer, is reactive oxidative mediated cellular damage [31] which may lead to happen in hepatic ischemic-reperfusion injury within the initial few hours of reperfusion [32& 33]. Consequently, oxidative stress caused by hepatic IRI may result in graft rejection in individuals who have had liver transplants, as it induces harmful splenic side effects[13].

The macrophages with multiple secondary lysosomes containing electron-dense material noticed in this study may have played an essential role in the interruption of the upsurge in deteriorated cells following hepatic IRI. Macrophage abilities have been linked to nonspecific responses such as phagocytosis, and secretion of IL-1β, TNF-α and TGF-β1 [34] which may be the leading causes of cirrhotic liver worsening [35]. Furthermore, there is proof that ischemia preconditioning activates G-protein-coupled receptors, which leads to the activation of Protein Kinase B [1]. Protein Kinase B activation inhibits the apoptosis signaling pathway [36].

It may be inferred from the present results that splenic ischemic preconditioning improves the splenic injury induced by hepatic ischemia-reperfusion. Physicians must select the therapeutic intervention that

modulating hepatic IRI as well as minimizing damage.

Ethical approval: The study was approved by the ethics committee of October 6 University, Faculty of Medicine.

Conflict of interest:

The authors declare that they have no conflict of interest.

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تأثير التكيف المسبق لنقص تروية الطحال الدموية على إصابة الطحال الناجمة عن اعادة الامداد الدموي للكبد بعد قطعه في الجرذان البيضاء

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خلفية البحث: إصابة الكبد بسبب الإقفار و إعادة الضخ للدم أثناء عمليات زراعة الكبد قد تؤدي إلى رفض العضو المزروع و قد ينتج عنه الإختلال الوظيفي لكثير من الأعضاء.

الغرض من البحث : تقييم تأثير تكيف الإقفار المسبق على إصابة الطحال بسبب إصابة الكبد نتيجة الإقفار و إعادة الضخ في الجرذان البيضاء.

المواد والطرق المستخدمة: تم تقسيم ثلاثين من الجرذان البيضاء البالغة إلى ثلاث مجموعات تحتوي كل مجموعة على عشرة جرذان. المجموعة الأولى (الضابطة): تم تقسيم عشرة جرذان إلى مجموعة تركت دون علاج ومجموعة أخرى تعرضت لفتح جدار البطن دون أي تدخل. المجموعة الثانية (مجموعة IRI): تعرضت عشرة جرذان لنقص التروية فى الكبد لمدة 45 دقيقة ثم اعادة الضخ لمدة 60 دقيقة. المجموعة الثالثة (مجموعة SIPC + IRI): تعرضت عشرة جرذان لغلق الشريان الطحالي في دورتين من 5 دقائق من نقص التروية و 5 دقائق من اعادة ضخه (إجمالي 20 دقيقة) قبل نقص تروية الكبد. في نهاية التجربة ، تم أخذ عينات من الطحال ومعالجتها من أجل الفحص النسيجي والهيستوكيميائي المناعي و المجهر الإلكتروني النافذ.

النتائج: بعد اعادة الامداد الدموي للكبد بعد قطعه ، أظهر الطحال احتقانًا ملحوظًا في اللب الأحمر مع بصيلات ليمفاوية متقلصة منكمشة في اللب الأبيض. أظهر الفحص المجهري الإلكتروني خلايا البلازما بتغيرات تنكسية على شكل انكماش النواة ، فصل الغشاء النووي , اتساع الشبكة الإندوبلازمية الخشنة ، التكثف في جهاز جولجي واتساع حويصلاته. لوحظ وجود العديد من المواد كثيفة الإلكترون وليسوسومات ثانوية فى الخلايا البالعة. أيضا ، كان هناك انخفاض ملحوظ في عدد كل من الخلايا الموجبة CD3 و CD20 مقارنة بالمجموعة الضابطة. بعد التكيف المسبق لنقص تروية الطحال ، لوحظ تحسن نسبي في بنية الطحال.

الخلاصة: التكيف المسبق لنقص تروية الطحال يحسن من إصابة الطحال التي يسببها اعادة الامداد الدموي للكبد بعد قطعه.