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Study of peritoneal macrophages, liver macrophages (KC) and spleen cells (splenic macrophages) in streptozotocin (STZ) induced mice

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Abstract

Diabetes mellitus (DM) is a major health problem worldwide. Streptozotocin (STZ) has been extensively used to induce diabetes for various studies. The present study was designed to demonstrate the morpho-functional changes of peritoneal macrophages, macrophages of liver and spleen cells in STZ induced mice. Mice were divided into experimental and their respective control groups. In our experiment STZ was administered by i.p. injection in doses of 45 (group A), 50 (group B), and 60 mg/kg body weight (group C) dissolved in normal saline (0.9% NaCl) to overnight-fasted mice. Normal saline (0·9% NaCl) was injected into mice peritoneum and the aspirate was taken for macrophage study. Spleen and liver were removed using the forceps and mashed through the cell strainer into the petridish containing 0·1 M phosphate buffer saline (PBS, pH 7·2) in presence of trypsin- EDTA. Spleen and liver cell suspensions were used to study. A significant percentage of peritoneal macrophages, liver macrophages and splenic cells became pyknotic and necrotic in group B and C mice. Increased peritoneal macrophage aggregation and increased tendency of macrophage fusion was noticed in both group B and C mice which may relate inflammatory reaction. Some studies indicated hyperglycemia aggravated hepatic injury by inhibiting liver-resident macrophage M2 polarization. Considering that macrophage is an important component of immune system, suppression of macrophage viability may explain, the increased susceptibility of diabetic patients to infection.

Key words : Streptozotocin (STZ), Diabetes, Macrophage, Spleen Cells, Liver Cells, Kupffer cells (KCs).

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Diabetes has emerged as an important public health issue nowadays. The balance of cellular anti-inflammatory and pro-inflammatory responses is broken during diabetes[13]. Type 1 diabetes patients present high mortality and morbidity rates in part due to an increase in the susceptibility to infections[5]. Diabetes causes dysregulation in signal transduction pathways in immune cells leading to an impaired immunity to pathogens. It has been shown that diabetic macrophages expressed impaired phosphoinositide 3-kinase (PI3K) pathways[21].

Streptozotocin (STZ) has been extensively used to induce diabetes. The common mechanism of action of streptozotocin includes degradation of pancreatic islet beta-cells[20].

The aim and objective of the present investigation was to study of peritoneal macrophages, liver macrophages (KC) and spleen cells (splenic macrophages) in streptozotocin (STZ) induced mice.

It has been shown that in diabetic subjects, *in vitro* and *in vivo* immune responses are significantly reduced[1,8] and the degree of impairment is directly related to the blood glucose level[16]. The increased incidence of bacterial and fungal infections in poorly controlled diabetic animals is due to impaired phagocytic activities of granulocytes and macrophages[15].

Monocytes from diabetic subjects exhibit enhanced adherence to the endothelium suggesting an increased tissue infiltration under diabetic conditions[4]. Previous studies stated macrophages possess specific insulin binding sites on their surfaces and the saturation of these receptors by insulin is critical for functions of macrophages[3]. Alloxan induced high-degree diabetes significantly reduces macrophage activity. Insulin treatment of diabetic animals restores macrophage activity to the normal level[16].

Mice were divided into experimental and their respective control groups. Each group consisted of 7 mice. In our experiment STZ was administered by i.p. injection in doses of 45 (group A), 50 (group B), and 60 mg/kg body weight (group C) dissolved in normal saline (0.9% NaCl) to overnight-fasted mice[2,7].

Normal saline (0·9% NaCl) was injected into mice peritoneum and the aspirate was taken for macrophage study. After incubation the nonadherent cells were removed by washing with PBS. The adherent macrophages were fixed by methanol and stained by Giemsa, methylene blue (MB) and observed under light microscope. Cell counting was performed by hemocytometer. Activated charcoal particles was injected into mice peritoneum and the aspirate was taken for phagocytosis study.

Liver and spleen were removed using the forceps and mashed through the cell strainer into the petridish containing 0·1 M phosphate buffer saline (PBS, pH 7·2) in presence of trypsin- EDTA. Cell suspension was subjected for centrifugation at 800xg for 3 minutes. Supernatant was discarded and pellet was resuspended in PBS. Cell suspension was taken for study. Liver cells and spleen cell suspension were placed and smeared directly on sterilized glass slides. The nonadherent cells were removed by washing

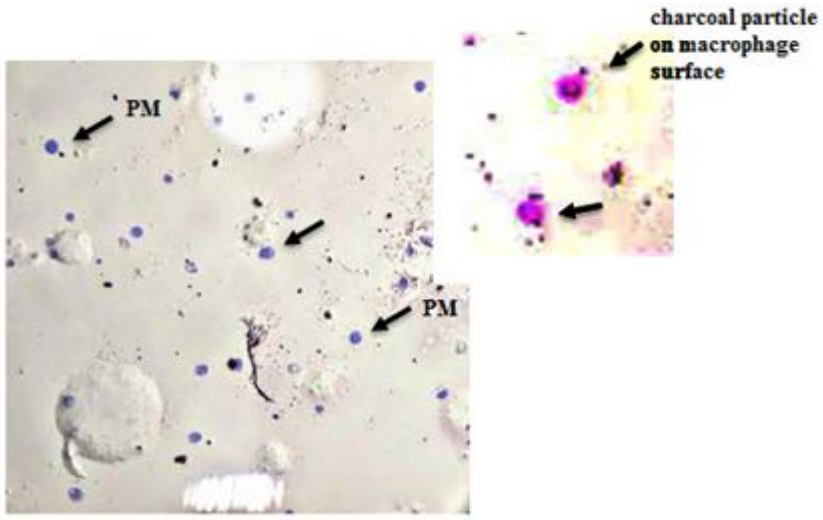


Fig. 1 (A)

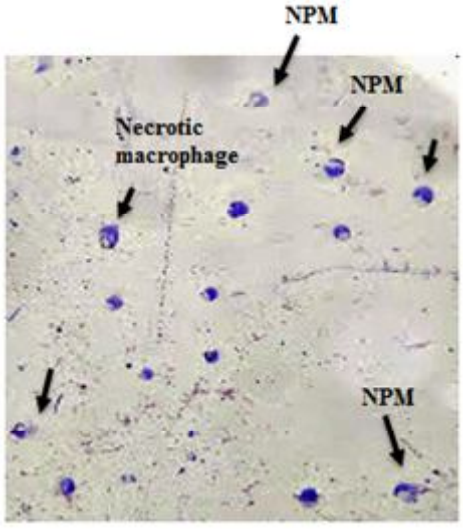


Fig. 1 (B)

Fig. 1. (A, B). (A) Giemsa stained peritoneal macrophages of control mice (x 100). (B) STZ treated Giemsa stained pyknotic peritoneal macrophages (group C mice) (x 100)

with PBS and adherent macrophages on glass slides were stained by Giemsa.

The phagocytic efficiency was examined by calculating phagocytic index. Peritoneal macrophage death was also confirmed by trypan blue staining.

Normal morphology was predominately found in the control group cells.

Significant number of STZ treated macrophages became pyknotic (Fig. 1). Increased cell aggregation and increased tendency of macrophage fusion was noticed in both group B and C mice (Fig. 5). Mean phagocytic index was significantly reduced and mean mortality index was significantly increased in diabetic group C mice (Fig. 3 and 4). Increased STZ treated pyknotic liver macrophages (KC) was found in group C mice (Fig. 7). Increased tendency of splenic cell fusion was found more in group C mice than group B mice (Fig. 8C). In present study, mean number of liver pyknotic cells was significantly increased in STZ treated mice (group A, B and C mice) in comparison with control mice (where P value was 0.0216) (Fig. 10).

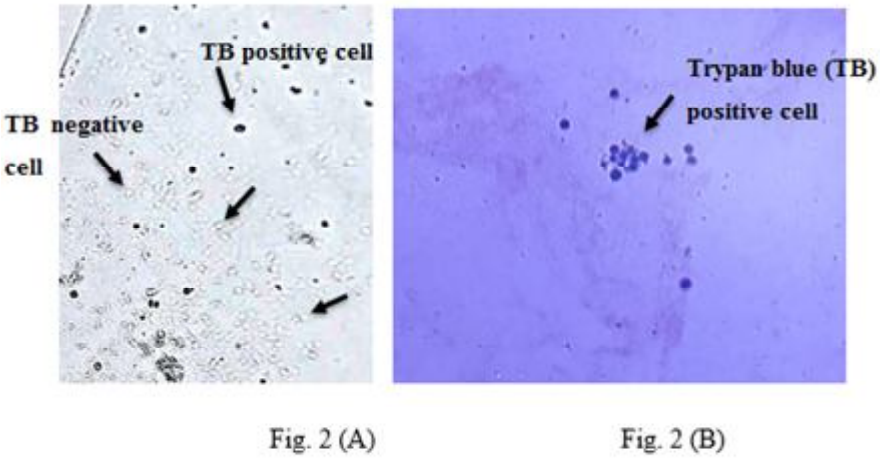


Fig. 2. (A, B). (A) Control mice viable peritoneal macrophages. (B) STZ treated diabetic mice dead peritoneal macrophages stained by trypan blue (group C mice)

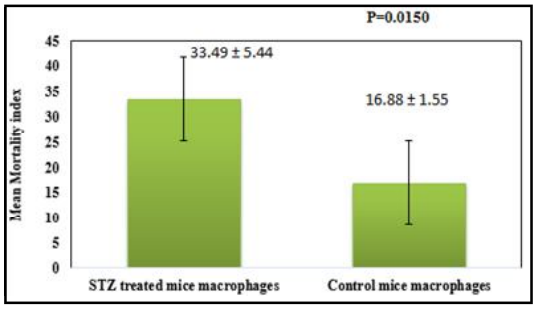


Fig. 3. Mean mortality index in control and diabetic group (group C mice). Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

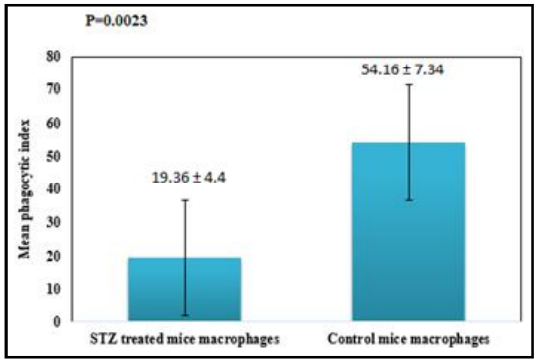


Fig. 4. Mean phagocytic index in normal and diabetic group (group C mice). Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

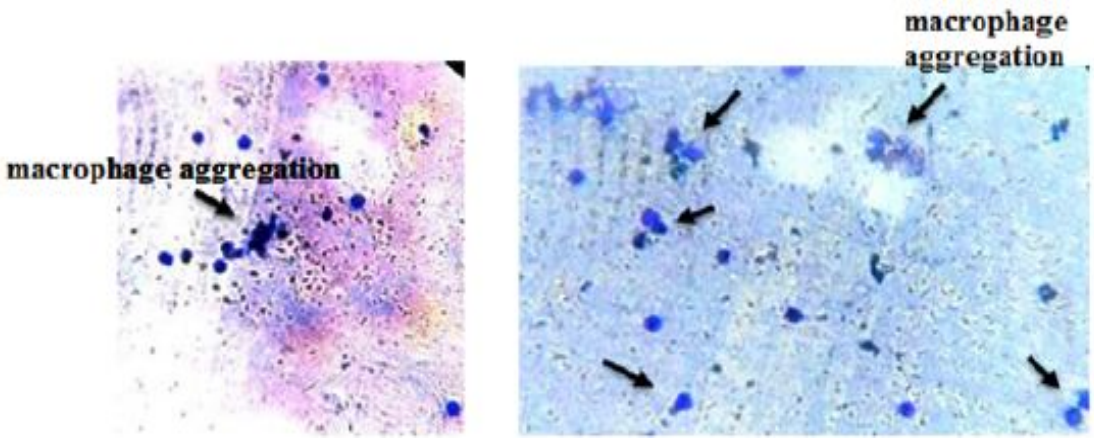


Fig. 5 (A) Fig. 5 (B)

Fig. 5. (A, B). (A) MB stained control peritoneal macrophages aggregation. (B) MB stained diabetic peritoneal macrophage aggregation (group B mice). Arrow indicated cell fusion/aggregation.

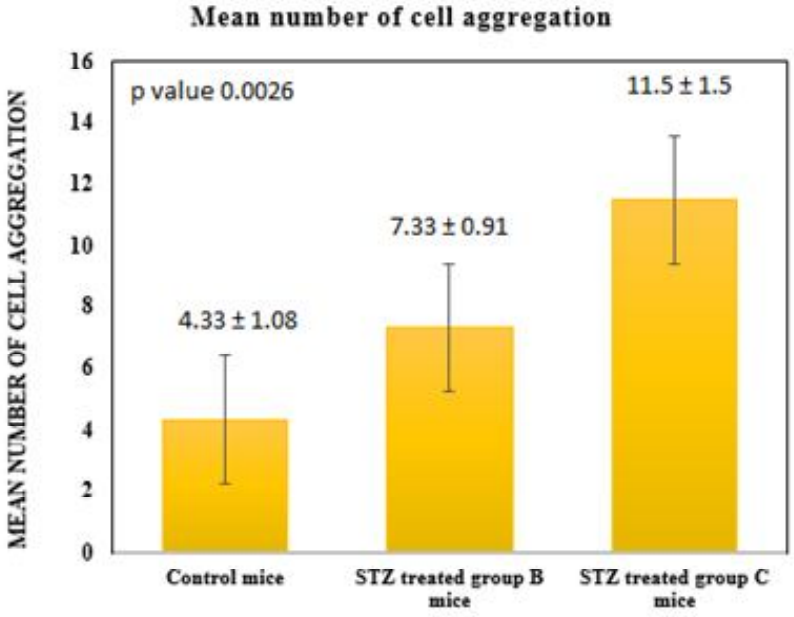


Fig. 6. Mean number of macrophage aggregation in normal and diabetic group (group B and C mice). Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

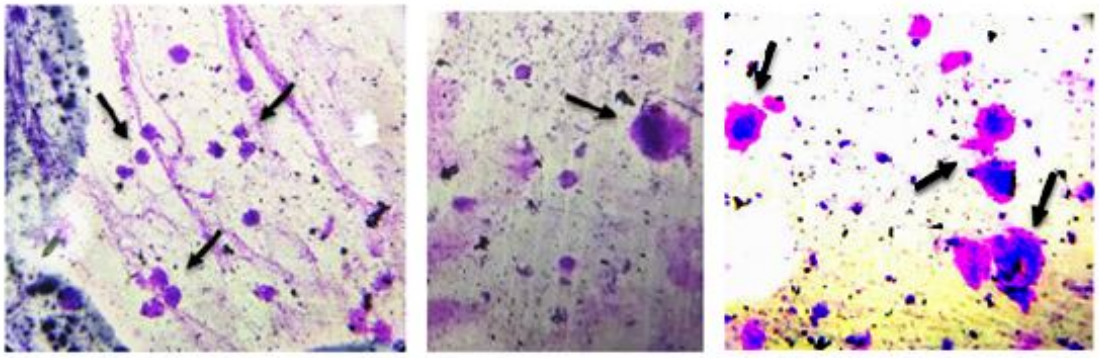
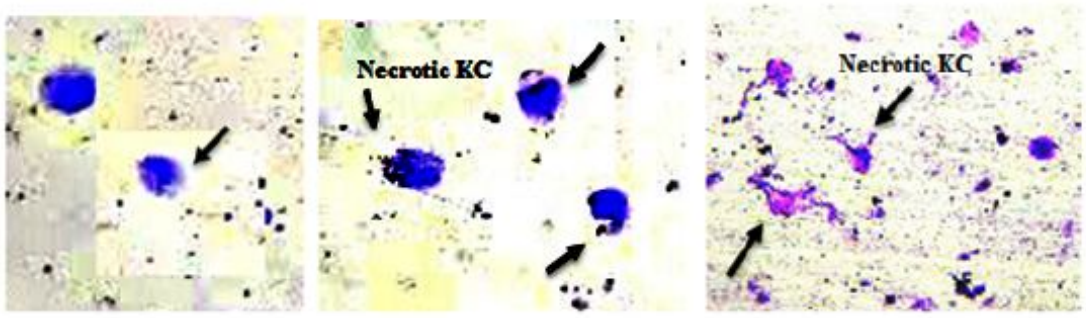


Fig. 7(A)



(group A) (group B) (group C)

Fig. 7(B)

Fig. 7. (A, B). (A) Control mice Giemsa stained liver macrophages (x 400). (B) STZ treated Giemsa stained necrotic cells or pyknotic liver macrophages (KC) (group A, B and C mice)

Generally different dosages of STZ are used in the experiment (45-70 mg/kg) and route of administration (i.p., i.v.), to induce diabetes mellitus in rats[19]. The highest STZ dose (70 mg/kg) is lethal to the animals, the doses of 50 and 60 mg/kg induce persistent hyperglycaemia[7].

Guria *et al.*,[10] exhibited that STZ treated rat showed atrophy of pancreatic islets and damages of liver[10]. Previous study clearly indicated that alloxan treated diabetes adversely affects peritoneal macrophages in rat [12]. Previous study showed alloxan treated liver was associated with low hepatic glycogen levels which may be related with decrease of glucose tolerance[11].

Guria[9] clearly indicated that STZ adversely affects peritoneal macrophages,

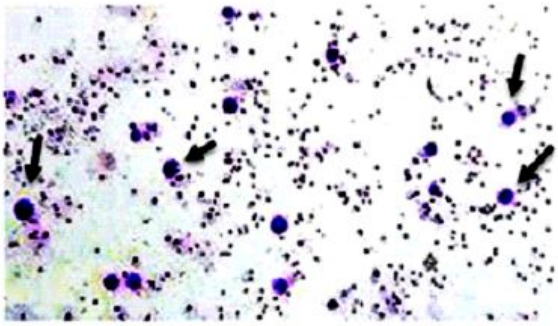
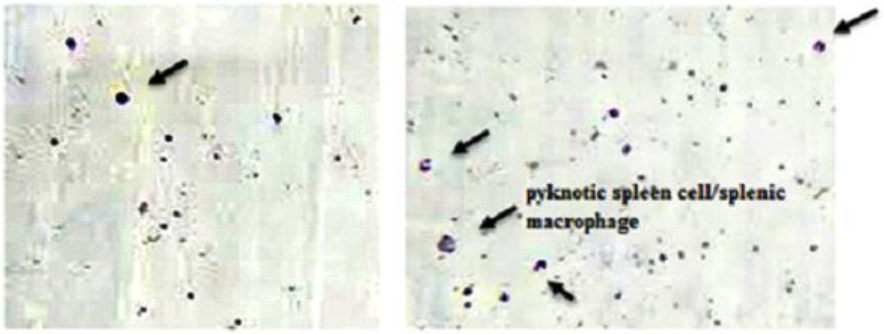
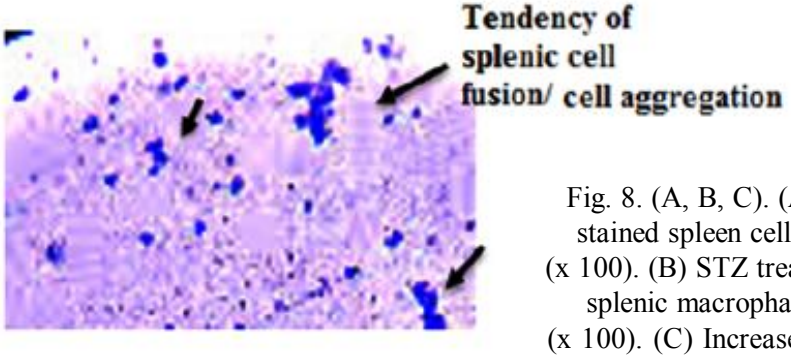


Fig. 8(A)



(group A) (group B)

Fig. 8(B)



(group C)

Fig. 8(C)

Fig. 8. (A, B, C). (A) Control mice Giemsa stained spleen cells /splenic macrophages (x 100). (B) STZ treated pyknotic spleen cells/ splenic macrophages (group A, B mice) (x 100). (C) Increased STZ treated methylene blue (MB) stained spleen cells aggregation (group C mice) (x 100). Arrow indicated cell aggregation.

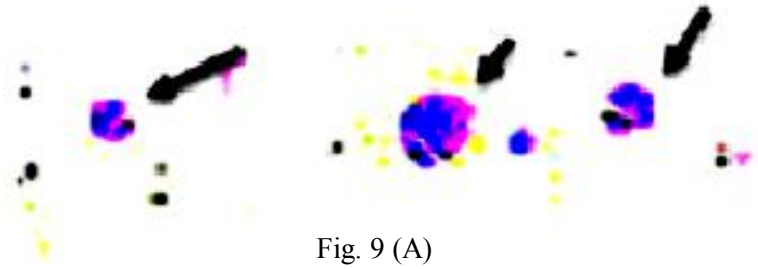


Fig. 9 (A)

Fig. 9 (B)

Fig. 9. (A, B). (A) STZ treated necrotic cells or pyknotic spleen cells (x400) (group B mice). (B) STZ treated necrotic cells or pyknotic spleen cells (x400) (group C mice)

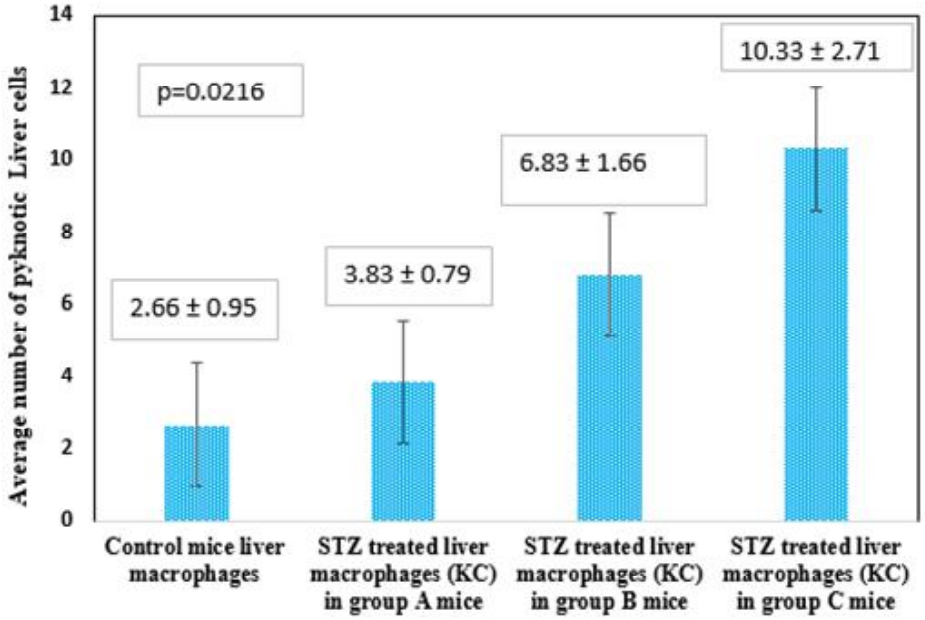


Fig. 10. Mean number of pyknotic liver cells (liver macrophages or KC) in control and STZ treated mice (group A, B and C mice). Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

phagocytosis and cell aggregation which is the central sign of diabetes mellitus[9].

In present study, increased cell aggregation and tendency of peritoneal macrophage fusion was noticed in group B and C mice which may relate inflammatory reaction.

Significant numbers of peritoneal macrophages, liver macrophages (Kupffer cells, KC) and spleen cells (splenic macrophages) were found to be pyknotic in group B and C mice. Mean phagocytic index was significantly reduced and mean mortality index was significantly increased in diabetic group (group C mice). This results corroborated the previous studies.

During obesity there is an imbalance in the ratio of M1/M2 macrophages. Some studies stated M1 "pro-inflammatory" macrophages were enhanced where M2 "anti-inflammatory" macrophages were down-regulated, leading to chronic inflammation in diabetes[14]. 35.3% of DM patients have an increased risk for pyogenic liver abscess, compared with normal people[6]. Rao Z *et al*.,[17] stated, aggravated liver ischemia has been observed in diabetes. Liver-resident macrophages (Kupffer cells, KCs) play vital roles in infection of liver. KCs obtained from hyperglycemic mice secreted higher levels of the pro-inflammatory factors TNF-α and IL-6, while they secreted significantly lower levels of the anti-inflammatory factor IL-10 [17]. Spolarics Z, *et al*.,[18] stated Kupffer cells displayed the most marked response to insulin showing increased in glucose uptake[18]. Significant numbers of liver macrophages (KC) and splenic macrophages were found to be pyknotic in present experimental studies.

Considering that macrophage is an important component of immune system, suppression of macrophage activity may explain, the increased susceptibility of diabetic patients to infection.

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