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Article

Hepatic Histopathological Alterations induced by L-Arginine and/or Dexamethasone in Adult Male Albino Rats

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Abstract: The liver is critical organ for metabolic homeostasis and toxic substance clearance and plays an important role in the systemic response to critical illness. Acute panreatitis (AP) progresses with a local production of inflammatory mediators, eventually leading to systemic inflammatory response syndrome. Knowing that almost all pancreatic mediators released from the pancreas to the blood stream may pass through the liver before their dilution in the systemic circulation, it would be reasonable to assume a determinant role of this organ in development of the inflammatory response associated with acute pancreatitis. **Objectives:** The study aimed to investigate the time courses of the effects of the exogenous glucocorticoids agonist dexamethasone on microscopical changes occurring in the liver of rats used as a model of AP induced by L-Arginine. **Materials and Methods:** 60 adult male albino rats weighing 150-200 gm were used. They were divided into 3 groups: Control group: Which is also divided into 2 subgroups (a & b) each of animals of the first were IM injected with 0.5ml/100gm B.W saline and those of second were injected by 0.5mg/100gm B.W dexamethasone. L-Arginine group: which received L-Arginine to induce AP. The animals of this group were divided into 3 subgroups a, b and c the animals of which were sacrificed 3 days, 2 weeks and 1 month after L-Arginine injection respectively. Dexamethasone and L-Arginine group: in which the animals were injected with both L-Arginine and dexamethasone. They were also divided into 3 subgroups a, b and c, the animals of which were sacrificed 3 days. 2 weeks, one month after the injection of the drugs. The liver of the scarified animals were dissected out and prepared for microscopical examination. **Results:** The histopathological changes that occurred in the livers of acute pancreatitis (AP) model animals started in the periphery of the classic hepatic lobules and progressively extended in a centripetal manner to involve all the cells of the lobules in the late period of the experiment. These changes were in the form of ballooning of the hepatocytes, progressive vacuolation of their cytoplasm most propably with fat globules and depletion of the PAS+ve glycogen granules. Injection of dexamethasone in AP model animals did not improve the case, but on the contrary it made the changes more intense, severe, and rapid. One month after injection of L-Arginine and dexamethasone, the hepatocytes all over the hepatic lobules were severely affected. They were markedly ballooned with severely vacuolated cytoplasm which was completely depleted from its PAS +ve glycogen granules, indicating severe fatty degeneration of the liver. **Conclusion:** From the previous data, it can be concluded that treatment of AP with dexamethasone is caused a late bad effect on the liver, where it causes its late fatty liver changes.

Keywords: Acute Pancreatitis, Hepatic Histopathological Changes, L-Arginine, Dexamethasone, Adult Male Albino Rats

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1. Introduction

Acute pancreatitis (AP) is sudden acute inflammatory processes of pancreas [1]. The majority of patients of AP present with mild disease, however, approximately 20% run a severe course and require appropriate management in an intensive care unit [2].

The current ideal drug for the treatment of severe acute pancreatitis is still in need and standardized treatment of consensus is only confined to fluid therapy, nutritional support, treatment of necrosis and infection, and endoscopic procedure of biliary stones [3].

AP is a multi-system disease with alterations not only in the pancreas, but also in liver, lungs and kidneys, which may lead to distant organ dysfunction and death. The liver is critical organ for metabolic homeostasis and toxic substance clearance and plays an important role in the systemic response to critical illness [4].

Although its exact nature is still unknown, acute panreatitis progresses with a local production of inflammatory mediators, eventually leading to systemic inflammatory response syndrome. Knowing that almost all pancreatic mediators released from the pancreas to the blood stream may pass through the liver before their dilution in the systemic circulation, it would be reasonable to assume a determinant role of this organ in development of the inflammatory response associated with acute pancreatitis. Thus, recent studies have shown the involvement of the liver in the complex network of events triggering the multi-organ dysfunction associated with the disease [5].

2. Objectives

The study aimed to investigate the time courses of the effects of the exogenous glucocorticoids agonist dexamethasone on microscopical changes occurring in the liver of rats used as a model of AP induced by L-Arginine.

3. Materials and Methods

3.1. Animals

Sixty adult male albino rats weighing 150-200 gm were used in this study. The animals were maintained in medical research center in Ain Shams University.

They were housed in plastic cages with mesh wire covers and allowed water and standard rat chow adlibitum for the period of experiment. The practical work was performed in accordance to guide for care and use of laboratory animals and approved by the animal ethical committee of Ain Shams University.

3.2. Induction of pancreatitis

Pancreatitis was induced by L-arginine hydrochloride powder of highest purity. It was supplied by med pharma. 20% L-Arginine hydrochloride solution was prepared by dissolving 2gm L-arginine hydrochloride in 8ml saline. PH was adjusted to 7 and then the volume was increased to 10 ml solution.

Animals were weighted accurately and injected by L-arginine using a dose of 500 mg / 100 gm body weight (2.5ml/100gm). The calculated dose for each animal was divided into two halves (1.25 ml/100gm), each one of them was injected followed by the second dose after an hour interval. The same procedure was repeated in following day [6].

3.3. Animals groups

The animals were divided into three groups**:**

Group I (control group): It included 10 male albino rats. This group was subdivided into 2 subgroups, 5 rats each. Sub group IA: It included 5 male albino rats received IM injection of normal saline 0.5 ml/100gm body weight. Sub group IB: 5 male albino rats received IM injection of dexamethasone 0.5mg/100gm of body weight in single dose [7].

Group II (L-argnine group): Twenty five rats received 2 intraperitoneal injections of L-Arginine (250 mg/100gm BW) as one hour apart. The same dose was repeated in following day. They were subdivided into three subgroups: Subgroup IIA: Five animals were scarified after three days from last injection of L-Arginine. Subgroup IIB: Five animals were scarified after two weeks after last injection of L-Arginine. _Subgroup IIC: Six animals were scarified one month after last injection of L-Arginine.

Group III (dexamethasone and L-Arginine group): It's included 25 male albino rats that received IM injection of dexamethasone (0.5mg/100gm BW) one hour after receiving 2 intra-peritoneal injection of L-Arginine (250mg/100gm BW) one hour apart. The same dose was repeated in following day [7]. They were subdivided into 3 subgroups: Sub group IIIA: 5 animals were scarified after 3 days from last injection of dexamethasone. Sub group IIIB: 5 animals were scarified after 2 weeks from last injection of dexamethasone. Sub group IIIC: 14 animals were scarified after 1 month after last injection of dexamethasone.

3.4. Methods

At the end of the experimental of each group, the animals were sacrificed by decapitation. Livers of animals were dissected out and were put immediately in fixation. The liver was fixed in buffered formol for 7 days. The paraffin sections of 5µm thick were prepared and stained by the following stains (H&E, Periodic acid schiff reaction (PAS) [8]. Maisson's trichrome stain [9], Immuno-histochemical study, Proliferating cell nuclear antigen (PCNA) [10], and Capsae-3 Technique [11].

3.5. The survival rate:

In group I, no animals died throughout the study, with a survival rate 100%. In group II, nine animals died and sixteen animals survived out of 25 animals with a survival rate 64%. In group III one animal died and 24 animals survived out of 25 with a survival rate 96%.

4. Results

4.1. The survival rate:

In group I, no animals died throughout the study, with a survival rate 100%. In group II, nine animals died and sixteen animals survived out of 25 animals with a survival rate 64%. In group III one animal died and 24 animals survived out of 25 with a survival rate 96%.

4.2. The liver

4.2.1. Control group I (A+B) (A; animals injected with normal saline, B; animals injected with dexamethazone):

Sections of the livers of animals of subgroup (A) were showing the general characters of normal liver.

The liver appeared mainly formed of liver cells (hepatocytes) arranged in special manner forming classic hepatic lobules. Each lobule is a hexagonal or pentagonal mass of liver tissue traversed centrally by a central vein. The lobules are not demarcated from each other due to the absence of interlobular septa. At the corners of the lobules, portal areas may be identified, each is formed of stroma of C.T and contain blood and lymph vessels as well as bile ducts.

In each lobule, the hepatocytes are arranged in the form of branching and anastomosing cords radiating from the central vein to the periphery of the lobule. The cords are separated by blood sinusoids, lined by flat endothelial cells.

Each hepatocyte appeared as polygonal cell with central rounded vesicular nucleus. The cytoplasm was acidophilic and showed a moderate degree of vacuolation (Figure 1 A+B).

The hepatocytes by PAS stain appeared moderately populated by PAS +ve granules and moderate content of well circumscribed vacuoles (Figure 1 C).

Masson trichome stain showed the minimal content of collagenous stroma which appeared only around the central veins and in the portal tracts (Figure 1 d).

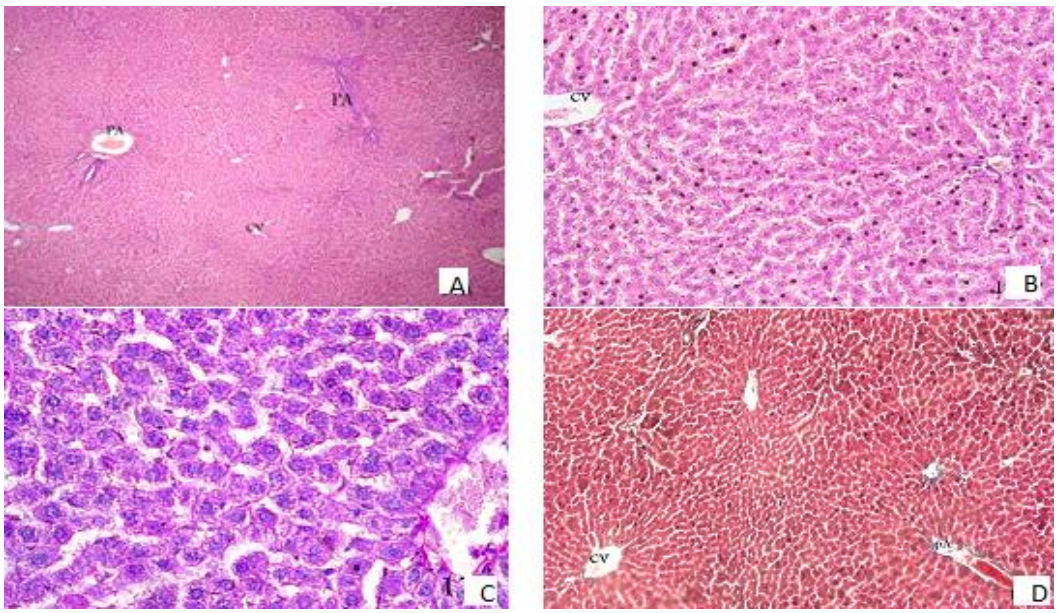

Figure 1. Photomicrographs of sections of the livers of animals of subgroup IA showing the normal control structure of the liver. CV = central vein, P.A. Portal area. Notice the minimal collagenous fibres content in the stroma, especially in portal areas. (P.A) [figure D]. Also moderate content of PAS +ve glycogen granules is appeared in the hepatocytic cytoplasm [figure B]. A - H&E × 40, B - H&E × 200, C - PAS × 400, D - Masson tricome × 100.

Sections of the livers of the animals of subgroup (b) showed almost the structure similar to that seen in subgroup (A) with some minor differences. In subgroup (b) the hepatocytes appeared more ballooned with severely vacuolated cytoplasm (Figure 2 A). The cytoplasm was also intensly packed with PAS +ve granules (Figure 2 B).

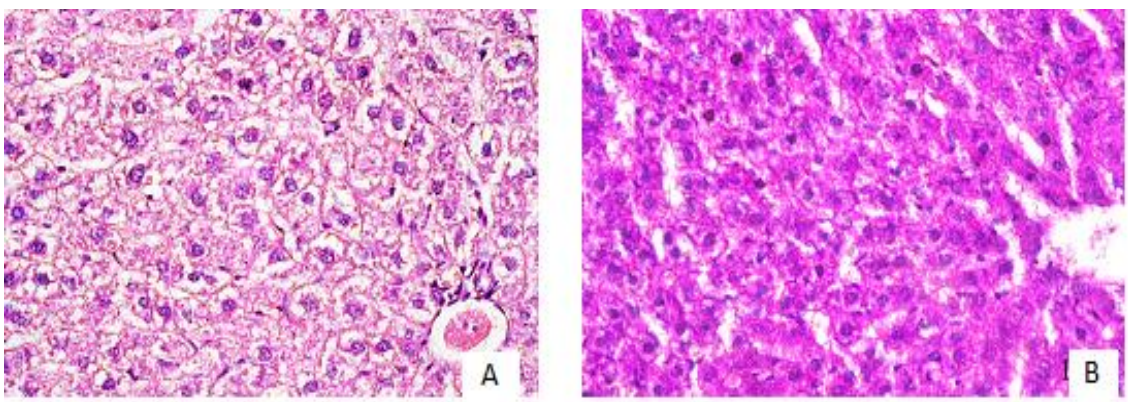

Figure 2. Photomicrographs of sections of the livers of animals of subgroup IB showing the ballooned hepatocytes with their severely vacuolated cytoplasm (A) which is intensely packed with PAS +ve glycogen granules (B). a - H&E × 400, b - PAS × 400

4.2.2. Group II (Acute pancreatitis) by L-Arginine

4.2.2.1. Subgroup II (A) 3 days after injection of L-arginine

Sections of the livers of the animals of this subgroup showed that the hepatocytes of the periphery of the lobules (zone I & II) were more ballooned and more vacuolated then those of the central areas of the lobule (zone III) (Figure 3).

PAS stain showed that the hepatocytes of the periphery of the lobules (zones I & II) are relatively depleted from their content of PAS +ve granules but had a cytoplasm more vacuolated with well circumscribed vacuoles compared to the central area (zone III) (Figure 4).

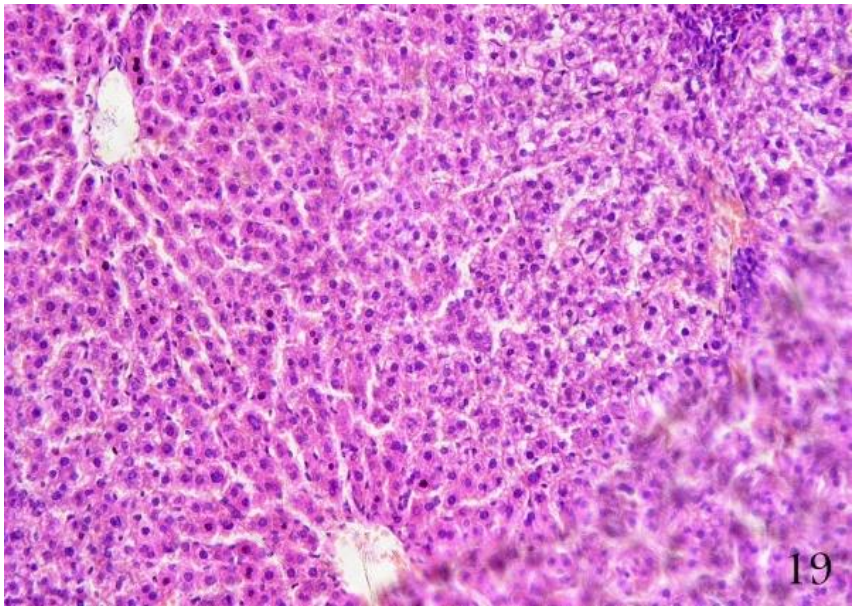

Figure 3. A photomicrograph of section of the liver of an animal of subgroup IIA showing the increased ballooning and vacuolation of hepatocytes at the periphery of the hepatic lobules (zone I&II). H&E × 200

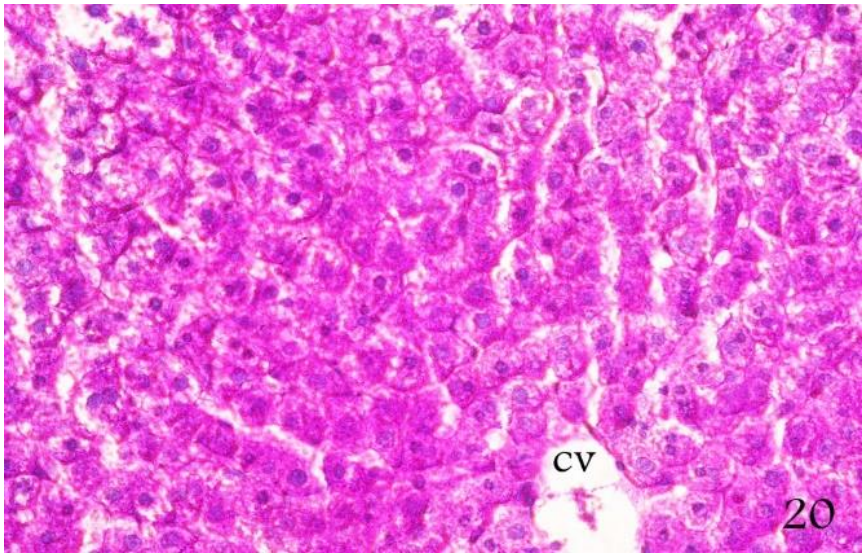

Figure 4. A photomicrograph of section of the liver of an animal of subgroup IIA showing the hepatocytes of the periphery of the hepatic lobules more depleted and vacuolated with well circumscribed vacuoles compared to the central zone. PAS × 200

No change of the collagen fibers content was observed in this subgroup compared to the control (Figure 5).

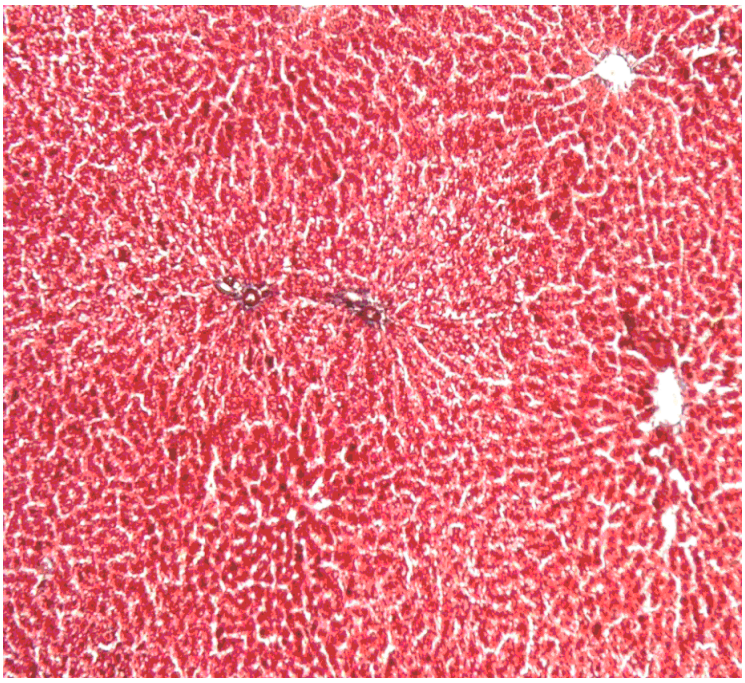

Figure 5. A photomicrograph of section of the liver of an animal of subgroup IIA showing the minimal content of collagenous fibers stroma. Masson trichome × 100

4.2.2.2. Subgroup II(b) 2 weeks after injection of L-arginine:-

In sections of the livers of the animals of this subgroup, the blood sinusoids appeared dilated and the hepatocytes were generally of vacuolated cytoplasm (Figure 6). On the other hand, the hepatocytes were relatively depleted from their content of PAS +ve granules inspite of the vaculation of the cytoplasm with well circumscribed vacuoles as seen by PAS stain (Figure 7).

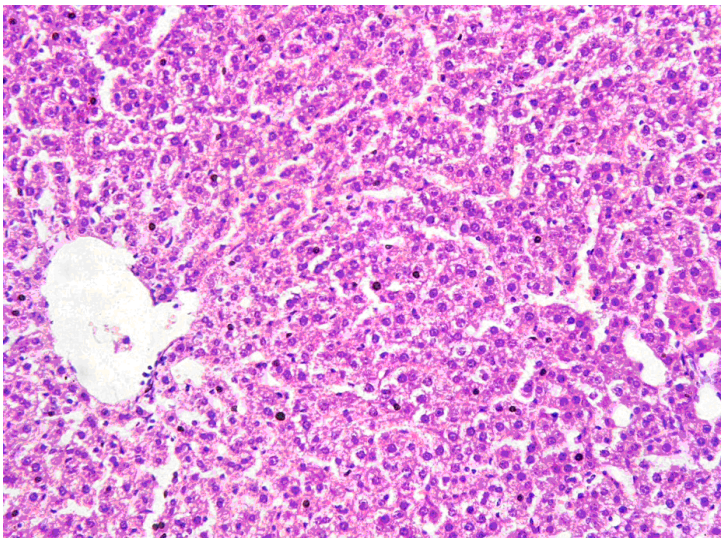

Figure 6. A photomicrograph of section of the liver of an animal of subgroup IIB showing the dilated blood sinusoids and the vacuolated hepatocytes all over the lobules. H&E × 200.

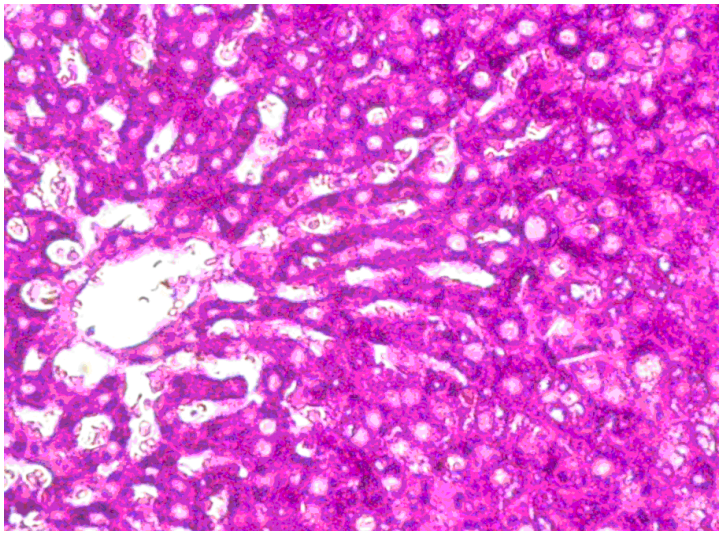

Figure 7. A photomicrograph of a section of the liver of an animal of subgroup IIB showing that the hepatocytes are relatively depleted from the PAS +ve granules content and vacuolated with well circumscribed vacuoles. PAS × 400

Some few focal areas of cellular necrosis were detected in livers of animals of this subgroup. No change of the collagenous fibers content was observed in this subgroup compared to the control (Figure 8).

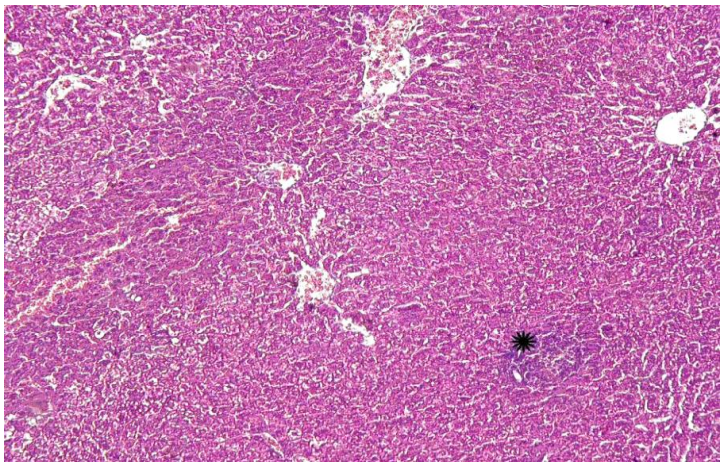

Figure 8. A photomicrograph of section of the liver of an animal of subgroup IIB showing the minimal content of collagenous fibres stroma. Notice a focal area of necrosis (*). Masson trichome × 100

4.2.2.3. Subgroup II(c) 1 month after injection of L-arginine:

Section of the livers of the animals of this subgroup showed marked ballooning of the hepatocytes and vaculation of their cytoplasm as well as depletion of its PAS +ve granules specially in the cells of the periphery of the lobules and extending to all of its zones (I, II, III). The vacuoles were well circumscribed (Figure 9). Mild increase in collagen fibers content was also observed especially in the portal areas compared to the control, II(A), and II(B) subgroups (Figure 10).

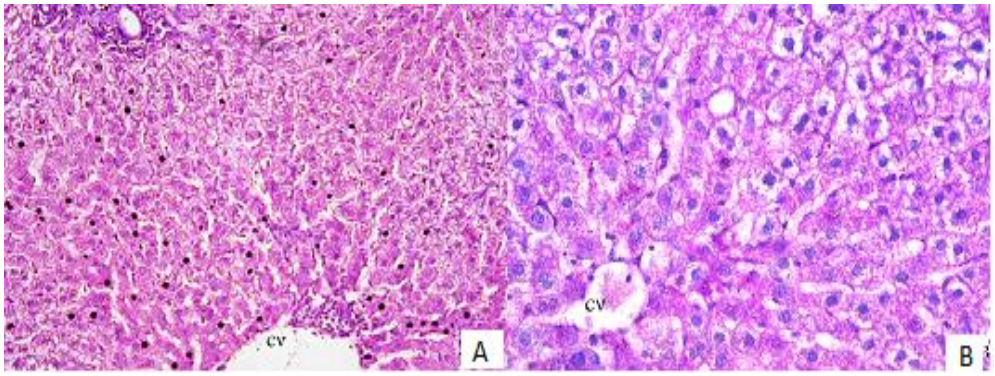

Figure 9. Two photomicrographs of sections of the livers of animals of subgroup IIC showing marked ballooning of the hepatocytes and vaculation of their cytoplasm with well circumscribed vacuoles as well depletion of the cytoplasmic PAS +ve glycogen granules specially in the periphery of the hepatic lobules. A – H&E × 200, B – PAS × 400.

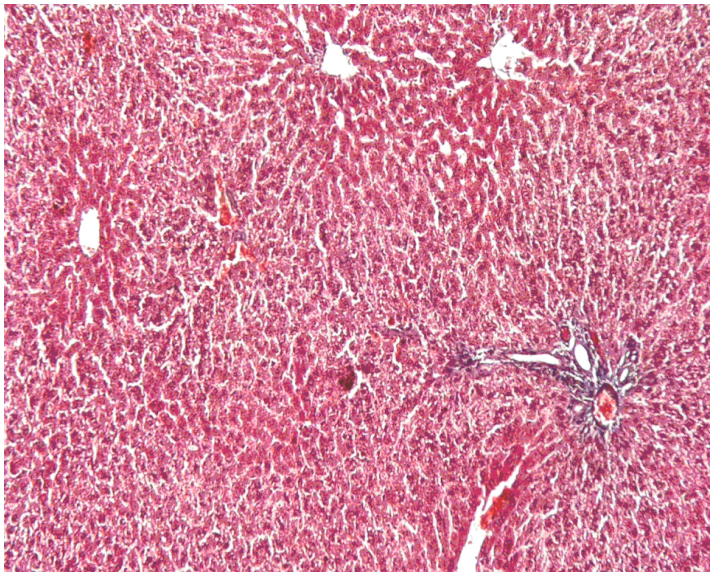

Figure 10. A photomicrograph of a section of the liver of an animal of subgroup IIC showing mild increase in the collagen fibers content in the stroma compared to the control specially in the portal area. Masson trichome × 100

4.2.3. Group III (acute pancreatitis induced by L-Arginine and treated by dexamethazone):

4.2.3.1. Subgroup III(A) 3 days after injection of L-arginine

Compared to the sections of the livers of the animals of subgroup II(A), sections of this subgroup (IIIA) showed the affection of the hepatocytes extended to occupy wider zone in the periphery of the lobules. In this wide area, the hepatocytes were ballooned and had deeply stained eccentric nuclei. The cytoplasm was occupied with well circumscribed vacuoles and depleted from its PAS +ve granules (Figure 11).

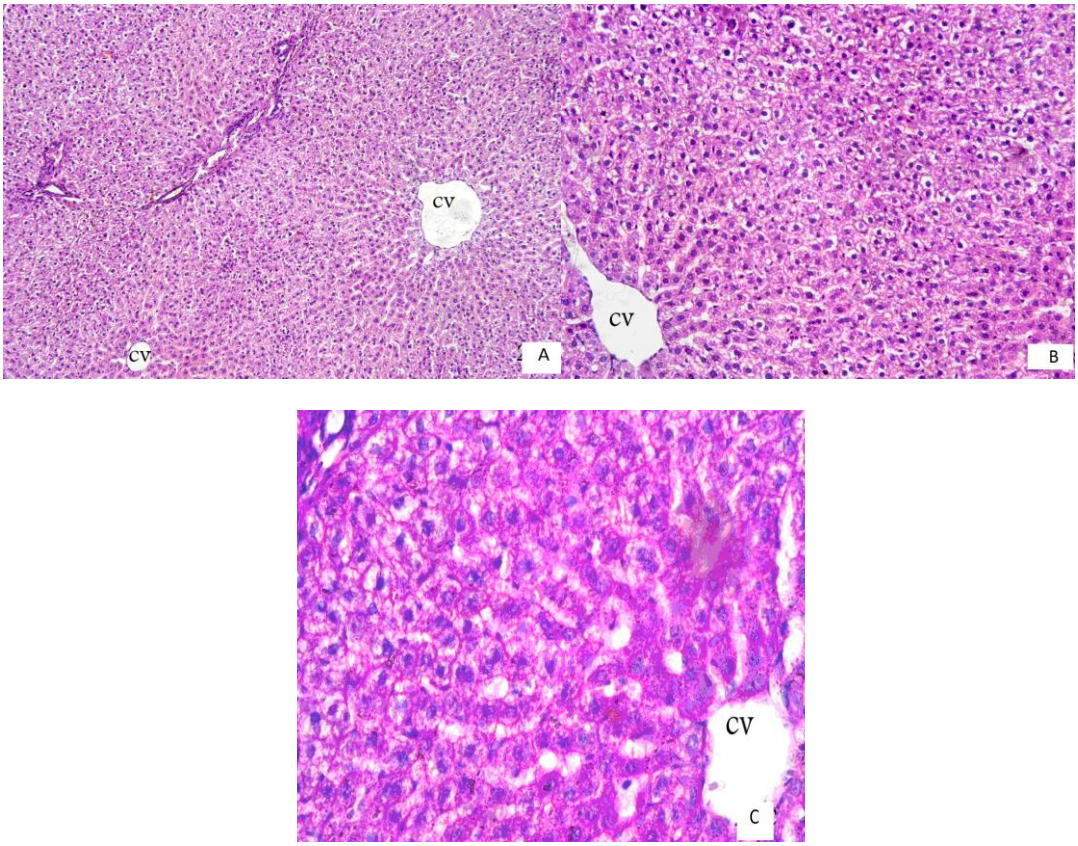

Figure 11. Photomicrographs of sections of the livers of animals of subgroup IIIA showing that the hepatocytes in a wide area of the periphery of the hepatic lobules are ballooned and has deeply stained eccentric nuclei. The cytoplasm is occupied by well circumscribed vacuoles and depleted from its PAS +ve glycogen granules. A: H&E × 100, B: H&E × 200, C: PAS × 400

No increased fibrosis was detected in the sections of this subgroup (Figure 12).

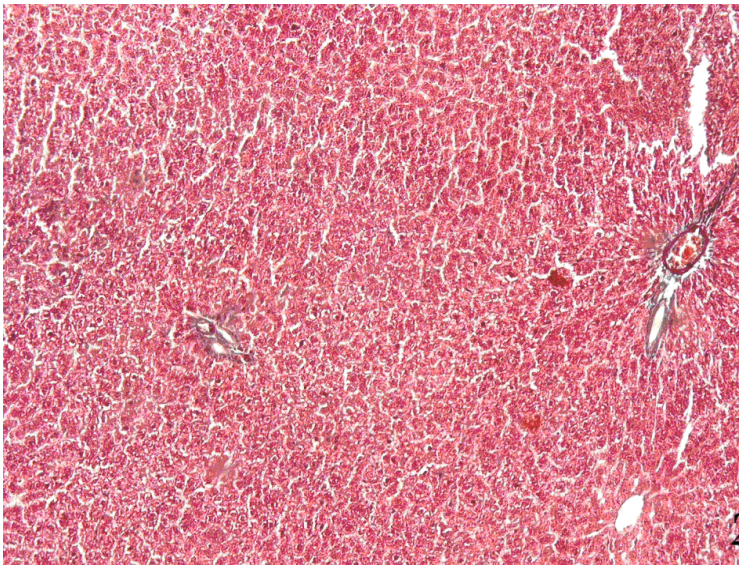

Figure 12. A photomicrograph of a section in the liver of an animal of subgroup IIIA showing the minimal collagen fibers content in the stroma comparable with the control group. Masson tricome × 100

4.2.3.2. Subgroup III(B) 2 weeks after injection of L-Arginine

Examination of the sections of the livers of the animals of this subgroup showed that ballooning, vacuolation and depletion of PAS +ve granules extended to involve hepatocytes all over the lobules in a marked degree (Figure 13).

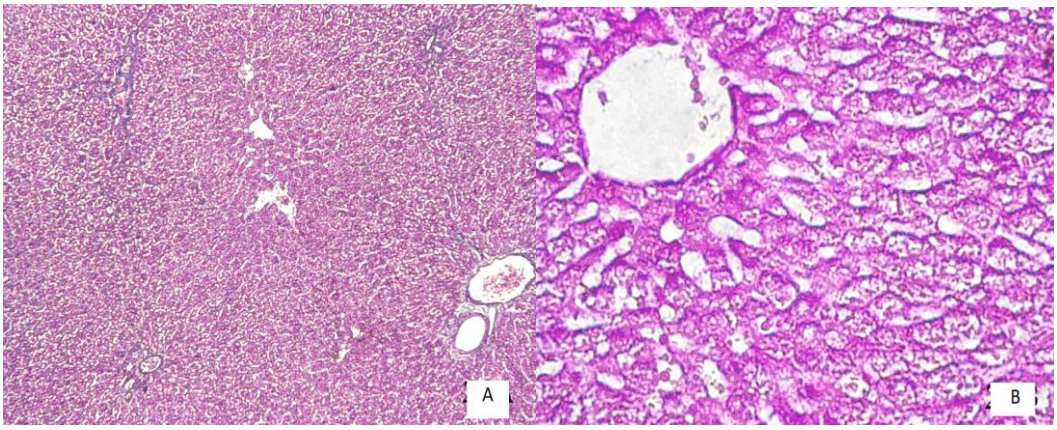

Figure 13. Two photomicrographs of sections of the livers of animals of subgroup IIIB showing the extension of the hepatocytic ballooning, vacuolation and depletion of PAS +ve granules all over the lobules. a - Masson trichome × 100, b - PAS × 400

4.2.3.3. Subgroup III(C) 1 month after injection of L-arginine:

Examination of the sections of the livers of the animals of this subgroup showed the severe affection of the hepatocytes all over the hepatic lobules. The cells were severely ballooned, had vacuolated cytoplasm and completely depleted from their content of PAS +ve granules. Relative increase in the collagenous fibrosis specially in the portal areas and extended in-between the cells of the lobules (Figure 14).

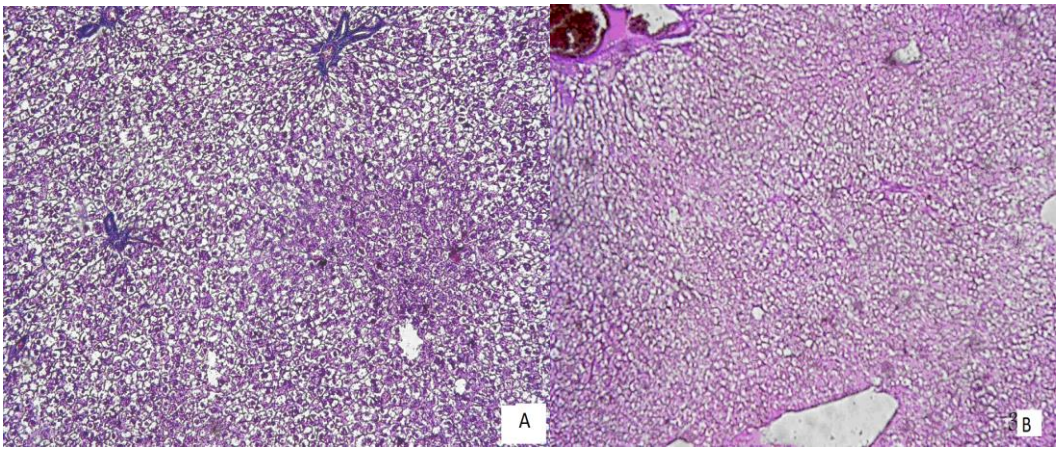

Figure 14. Two photomicrographs of sections of the livers of animals of subgroup IIIC showing the severe affection of the hepatocytes all over the hepatic lobules. The cells appear severely ballooned, vacuolated and completely depleted from their PAS +ve granules. A relative increase in collagenous fibrosis of the stroma is appearent. a- Masson trichome × 100, b- PAS × 100

5. Discussion

Folch-Puy, [5] mentioned that AP progresses with a local production of inflammatory mediators, eventually leading to systemic inflammatory response syndrome. Almost all

pancreatic mediators released from the pancreases to the blood stream may pass through the liver before their dilution in the systemic circulation, so it would be reasonable to assume a determinant role for this organ in development of the inflammatory response associated with acute pancreatitis.

In this study, sections of the livers of the animals of subgroup I(A) were showing the general characters of the normal liver. The hepatocytes were moderately populated with PAS +ve glycogen granules and moderate content of well circumscribed vacuoles most probably due to fat droplets. Sections of livers of the animals of subgroup I(B), where the animals were injected with dexamethazone, showed almost the same structure similar to that seen in subgroup I(A) with some minor difference where the hepatocytes in subgroup I(B) appeared more ballooned with severely vacuolated cytoplasm and intensely packed with PAS +ve glycogen granules. This is explained by Brunton *et al.,* [12] who mentioned that the main metabolic effects of glucocorticoids are on carbohydrate and protein metabolism. The hormones cause both a decrease in the uptake and utilization of glucose and an increase in gluconeogenesis, resulting in a tendency to hyperglycaemia. There is a concomitant increase in glycogen storage which may be due to insulin secretion in response to the increase in blood sugar.

Examination of the livers of the animals injected with L-Arginine only (group II) after 3 days, 2 weeks and one month, showed progressive affection of the hepatocytes extending from the cells of the periphery of hepatic lobules (Zone I) to the central part of the lobules (Zone III). This affection was in the form of progressive ballooning of the hepatocytes, deplation of PAS +ve glycogen granules and increased vaculation with well circumscribed most probably fat vacuoles. Some occasional focal areas of hepato-cellular necrosis were detected in some animals 2 weeks after L-Arginine injection. The blood sinusoids appeared also dilated. Mild increase in the collagenous fibers content was also observed specially in the portal areas mostly after one month of L-Arginine injection.

Maruyama *et al.,* [13] mentioned that pancreatitis may induce a spectrum of venous and arterial vascular complications. However, hepatic infarction complicated with acute pancreatitis seldom occurs because of the unique vascular configuration of the liver.

Esrefoglu *et al.,* [14] found that AP caused hepatocytic necrosis, intracellular vacuolization, vascular congestion and sinusoidal dilation, a picture similar to that observed in this work

Hori *et al.,* [15] and Ueda *et al.,* [16] observed hepatocyte apoptosis in rat acute necrotizing pancreatitis.

In acute pancreatitis, over-expression of cytokines is widely accepted as being indispensable in the occurrence of multiple organ dysfunction syndrome (MODS) [17-19]. Increasing numbers of reports have demonstrated that these cytokines, which contribute to MODS in acute pancreatitis, largely originated from macrophages, especially liver macrophages [20]. The liver macrophages can be activated after they engulf necrotic tissue and endotoxin which often exist in the blood of patient with acute pancreatitis. These macrophages produce proinflammatory cytokines and amplify the level of systemic inflammation [20].

Similar findings were reported by Folch-Puy, [5], who mentioned that; once pancreatic mediators reach the liver, they strongly activate Kupffer cells, the resident macrophages, greatly amplifying the release of cytokines into blood stream and thus contributing to the systemic manifestations of acute pancreatitis. He also added that the pancreas is not the only source of mediators that trigger the deleterious effects of acute pancreatitis, but the liver may orchestrate the final outcome of the disease.

On the other hand, Esrefoglu *et al.,* [14] and Yang *et al,* [21] who reported that a number of studies have suggested that pancreatitis associated ascetic fluid (PAAF) plays critical role in inducing hepatocyte injury by inducing hepatocyte apoptosis. PAAF induces liver injury by direct hepatocyte injury and death independent from locally

produced Kupffer-cell derived cytokines. Ueda *et al.,* [22] reported that the dramatic elevation of hepatocyte Ca^{++} due to PAAF may be closely related to the hepatocellular injury in severe acute pancreatitis and that platelet – activating factor may play a pivotal role in increasing hepatocyte Ca^{++}.

Zhao *et al.,* [23] mentioned that NF-Kappa β plays an important role in pathogenesis of liver injury in rats with severe acute pancreatitis.

It's also worthy to note the finding of Ni *et al.,* [24] that severe acute panreatitis with liver injury is associated with hepatic NF-Kappa B activation leading to production of NF-Kappa B dependent cytokines and chemokines such as TNF-α. Melatonin reduces the apoptosis and necrosis in liver by inhibiting the activity of NF-Kappa β and decreasing the expression of TNF-α.

The deterioration of hisotpathological picture of the liver in the late period of this study is supported by a case report published by Rana *et al.,* [25]. They mentioned that a 46 year old female patient presented with acute pancratitis. She was managed conservatively and improved. An endoscopic ultrasound evaluation performed 1 month later revealed normal gall bladder and normal bile duct. On follow-up, 9 month later, she was found to be having elevated serum alkaline phosphatase with normal aminotransferases. Ultrasound of abdomen revealed prominent central intrahepatic biliary radicles with thrombosed portal and splenic veins. A diagnosis of portal hypertension biliopathy secondary to segmental portal hypertension was made.

Examination of the sections of livers of the rat injected with L-Arginine and treated with dexametasone, revealed progressive deterioration of histopathological picture of the organ.

This means, that treatment of AP with dexametashone apparently ameliorated the histopathological picture of the pancreas, but on the contrary, it speeded the deterioration of hepatic picture. This problem must be further studied.

6. Conclusion

The change that occurred in the livers of AP model animals started in the periphery of the classic hepatic lobules and progressively extended in a centripetal manner to involve all the cells of the lobules in the late period of the experiment. These changes were in the form of ballooning of the hepatocytes, progressive vacuolation of their cytoplasm most propably with fat globules and depletion of the PAS+ve glycogen granules.

Injection of dexamethasone in AP model animals did not improve the case, but on the contrary it made the changes more intense, severe and rapid. One month after injection of L-Arginine and dexamethasone, the hepatocytes all over the hepatic lobules were severely affected. They were markedly ballooned with severely vacuolated cytoplasm which was completely depleted from its PAS +ve glycogen granules, indicating severe fatty degeneration of the liver.

From the previous data, it can be concluded that treatment of AP with dexamethasone is caused a late bad effect on the liver where it causes its late fatty liver changes.

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