6. Summary and conclusions

6.1. Summary

Fulminant hepatic failure is a dramatic clinical syndrome resulting from massive hepatocyte death, whereas concomitant administration of D-galactosamine with lipopolysaccharide can lead to an experimental model of this disease. Oxidative stress and its resulting intracellular actions of apoptosis may be the main root of this liver damage. FoxO3a is an evolutionarily conserved transcription factor involved in apoptosis and oxidative stress resistance that has been implicated in the pathogenesis of oxidative stress-related diseases such as cardiovascular diseases, hepatic disorders, and alcohol-induced liver injury. It also changed in hepatitis C infection, fatty liver and some hepatocellular carcinomas.

FoxO3a is regulated by various external stimuli, positively by oxidative stress/JNK, and negatively by PI3K/Akt and MAPK/Erk pathways. Akt/ PKB is considered the main limiting system for FoxO3a as this can be achieved through phosphorylation and then inactivation of FoxO3a, which results in holding of FoxO3a in the cytoplasm and hang-up target gene transcription. The reverse is dephosphorylation of FoxO3a which causes nuclear translocation and activation of its transcriptional activity.

Antioxidant and apoptotic markers together with liver function and pAkt, Erk and pFoxO3a were estimated under the influence of both D-GalN and LPS and drugs (silibinin and vitamin E) treatments in order to estimate their curative and prophylactic effectiveness in modifying the oxidative stress and apoptosis which are thought to have the principle role in fulminant hepatic failure.

Our interest has been focused on the changes observed in activities and levels of these parameters, with special significant attention to pFoxO3a as a new diagnostic biomarker to liver injury.

Sixty male Wister albino rats were used in this study. Rats were divided into six groups; each group included ten animals.

Group 1: Untreated control healthy group as their rats were received normal diet and injected with saline (1 ml/kg).

Group 2: D-GalN and LPS group as their rats were administered a single dose of both D-GalN (500 mg/kg) and LPS (50 μg/kg) by intraperitoneal injection.

Group 3: The curative silibinin group in which the rats were administered a single dose of both D-GalN (500 mg/kg IP) and LPS (50 μg/kg IP) and they were then received silibinin (100 mg/kg IP) daily for 2 weeks.

Group 4: The curative vitamin E group in which the rats were administered a single dose of both D-GalN (500 mg/kg IP) and LPS (50 μg/kg IP) and they were then received vitamin E (400 mg/kg orally) daily for 2 weeks.

Group 5: The prophylactic silibinin group in which the rats were administered silibinin (100gm / Kg daily) intrapretoneally for 2 weeks and they were then administered a single dose of both D-GalN (500 mg/kg IP) and LPS (50 μg/kg IP).

Group 6: The prophylactic vitamin E group in which the rats were administered Vitamin E (400 mg /Kg daily orally) for 2 weeks and they were then administered a single dose of both D-GalN (500 mg/kg IP) and LPS (50 μg/kg IP).

At the end of the experimental period, the blood samples were collected from medial canthus blood capillaries of the eye from each animal in all groups into sterilized tubes

containing EDTA for plasma separation for measurement of ALT and AST. The rats were then anesthetized and scarified. Then, their liver tissues were separated and were cut into small slices which were used for determination of the expression levels of pFoxO3a, pAkt, Erk, TLR4, TrxII, mGSH and MnSOD. Likewise liver tissue was used for determination of apoptosis by measuring caspase-8 and caspase-3 gene expression. They were used for measurement of GSH content, MDA level and activities of the SOD and CAT. Sections of liver tissues were stained with hematoxyline and eosin for histopathological examination.

The results revealed that:

In D-GalN and LPS administered group, antioxidant power in which mitochondrial (TrxII, mGSH and MnSOD) and non-mitochondrial (SOD, CAT and GSH) were decreased with subsequent increase in MDA content compared to the normal control group. In addition, D-GalN and LPS administered group showed a significant decrease in pFoxO3a expression compared to that of the normal healthy control group. On the other hand, D-GalN and LPS treated group showed a significant increase in tissue expression of pAkt, Erk and TLR4 in addition to apoptotic markers (caspase-3 and caspase-8). The changes induced by D-GalN and LPS were supported by the histopathological examination of liver of the rats intoxicated with D-GalN and LPS that showed slightly congested central veins and vacuolated hepatocyte with pyknotic nuclei. In that group some hepatocytes lost their architecture with apparent cellular damage and cellular death as well as most of hepatic sinusoids appeared degenerated.

In contrast, administration of either silibinin or vitamin E to D-GalN and LPS treated rats showed a significant restoration in mitochondrial (TrxII, mGSH and MnSOD) and non-mitochondrial (SOD, CAT and GSH) antioxidants with subsequent decrease in MDA content compared to D-GalN and LPS treated animals. Additionally the plasma ALT and AST activities

were also reduced in curative and prophylactic groups of silibinin and vitamin E compared to D-GalN and LPS treated animals. On the other hand, the expression of pFoxO3a was increased compared to D-GalN and LPS treated animals, while the expression of pAkt, Erk and TLR4 in addition to apoptotic markers (caspase-3 and caspase-8) were decreased compared to D-GalN and LPS treated animals. Histopathological images of liver sections of rats intoxicated with D-GalN and LPS and treated with either silibinin or vitamin E appeared more or less resembling normal with an improvement in the liver architecture compared to D-GalN and LPS treated group.

6.2. Conclusion

In conclusion, this study demonstrated that Phosphorylated Forkhead box O3 (pFoxO3a) was found to be an excellent diagnostic marker for detection of fulminant hepatic failure induced by D-GalN and LPS. Additionally, supplementation of either silibinin or vitamin E to D-GalN and LPS induced fulminant hepatic failure in rats, has been found to have a valuable protective effect against liver failure through their antioxidant, anti-apoptotic, and hepatoprotective effects, but the best results were obtained from the curative groups rather than the prophylactic groups of both silibinin and vitamin E, and data obtained from silibinin were better than that obtained from vitamin E.