The present study was designed to evaluate the possible hepatoprotective effect of certain antioxidants; for example, thymoquinone (TQ) and quercetin (QR) compared to N-acetylcysteine (NAC) against hepatic ischemia-reperfusion (IR) injury in rats and to assess inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), eNOS trafficking inducer (NOSTRIN) and inositol -3-phosphate synthase (IPS) protein expressions, as a possible mechanism of their hepatoprotective effect.

 Rats were randomly divided into five groups each consisted of 10 rats. The first group served as sham-operated control (received normal saline and 1% tween80 orally), and underwent operation similar to the other groups without actual clamping of the vessels. Group 2 was subjected to ischemia-reperfusion injury (30 minutes ischemia followed by 30 minutes reperfusion). Groups 3, 4 and 5 were pretreated with N-acetylcysteine (300 mg/kg freshly dissolved in normal saline), thymoquinone (20 mg/kg suspended in normal saline and 1% tween80) and quercetin (20 mg/kg suspended in normal saline and 1% tween80), respectively. Rats were daily administered their respective doses per oral (p.o) for 10 days before subjection to IR.

 Hepatic ischemia was surgically performed by occlusion of hepatic pedicle (hepatic artery, portal vein, bile duct) which supplied the left and medial lobes (approximately 70% of the total liver mass), for 30 minutes. with mini-clamp followed by releasing the clamp and the liver was reperfused for 30 minutes.

 The effects of NAC, TQ or QR-pretreatment were evaluated by measurement of liver function tests (serum ALT and AST), oxidative stress biomarkers (hepatic MDA and reduced GSH contents). Furthermore, localization of iNOS, eNOS, NOSTRIN, IPS in liver tissues was assessed by immunofluorescence analysis. Also, hepatic MPO activity and NO production were determined in addition to histopathological examination.

**The Main Findings of the Present Study can be summarized as Follow:**

**1- Effect of IR on the Liver of Normal Rats:**

Hepatic ischemia for 30 minutes followed by reperfusion for 30 minutes resulted in the following findings:

a- Significant increase in serum activity of AST and ALT.

b- Significant increase in hepatic myeloperoxidase activity.

c- Significant increase in hepatic malondialdehyde as a biomarker of oxidative stress in liver.

d- Significant decrease in hepatic glutathione content.

e- A notable histopathological changes with massively dilated and congested central vein as well as dilated congested blood sinusoids lined with activated kupffer cells. Also, many hepatocytes show pyknotic nuclei, cytoplasmic degeneration and infiltration of inflammatory cells.

f- Significant increase in iNOS protein expression with significant increase in hepatic nitric oxide production.

g- Significant decrease in eNOS protein expression along with significant increase in NOSTRIN protein expression

h- Significant increase in IPS protein expression.

2- **Effect of Pretreatment of N-acetylcysteine on IR Induced Liver Injury:**

 Pretreatment of N-acetylcysteine (300mg/kg/day) for 10 days before subjection to IR resulted in the following findings:

a- Significant decrease in serum activity of AST and ALT.

b- Significant decrease in hepatic myeloperoxidase activity.

c- Significant decrease in hepatic malondialdehyde as a biomarker of oxidative stress in liver.

d- Significant increase in hepatic glutathione content which decrease oxidative stress.

e- Marked improvement of histopathological picture. Meanwhile, central vein still dilated and congested.

f- Significant decrease in iNOS protein expression with significant decrease in hepatic nitric oxide production.

g- Significant increase in eNOS protein expression along with significant decrease in NOSTRIN protein expression.

h- No significant effect on IPS protein expression.

3- **Effect of Pretreatment of Thymoquinone on IR Induced Liver Injury:**

 Pretreatment of thymoquinone (20mg/kg/day) for 10 days before subjection to IR resulted in the following findings:

 a- Significant decrease in serum activity of AST and ALT.

b- Significant decrease in hepatic myeloperoxidase activity.

c- Significant decrease in hepatic malondialdehyde as a biomarker of oxidative stress in liver.

d- Significant increase in hepatic glutathione content which decrease oxidative stress.

e- The majority of hepatic lobules retained the normal architecture with dilated and congested central vein.

f- Significant decrease in iNOS protein expression with significant decrease in hepatic nitric oxide production.

g- Significant increase in eNOS protein expression along with significant decrease in NOSTRIN protein expression.

h- Significant decrease in IPS protein expression.

4- **Effect of Pretreatment of Quercetin on IR Induced Liver Injury:**

 Pretreatment of quercetin (20mg/kg/day) for 10 days before subjection to IR resulted in the following findings:

 a- Significant decrease in serum activity of AST and ALT.

b- Significant decrease in hepatic myeloperoxidase activity.

c- Significant decrease in hepatic malondialdehyde as a biomarker of oxidative stress in liver.

d- Significant increase in hepatic glutathione content which decrease oxidative stress.

e- Marked improvement of histopathological picture. Meanwhile, central vein still dilated and congested.

f- Significant decrease in iNOS protein expression with significant decrease in hepatic nitric oxide production.

g- Significant increase in eNOS protein expression along with significant decrease in NOSTRIN protein expression.

h- Significant decrease in IPS protein expression.

**Based on the previous results, we can conclude the following:**

1- Hepatic ischemia-reperfusion resulted in a significant liver injury due to oxidative stress, nitrosative stress and inflammatory responses.

2- Thymoquinone and quercetin exhibited a more pronounced hepatoprotection as they successfully restored liver function and architecture. Also, they inhibited oxidative and nitrosative stress induced by IR compared to N-acetylcysteine.

3- In addition, the hepatoprotective mechanism of thymoquinone and quercetin, at least in part, by modulating iNOS, eNOS, NOSTRIN and IPS protein expressions.

4- Still, further investigations should be done to investigate their potential uses in clinical trial.