5. RESULTS

5.1. RESULTS OF PHASE – I STUDY, TO ESTABLISH THE MODEL OF NASH:

5.1.1. Liver biopsy remains a gold standard against the other methods used to diagnose and confirm the presence or absence of NASH. Histological analysis of liver tissue specimens was carried out and the photomicrographs of the histological analysis of the liver tissues were shown in Fig. 2(A-C). The rats that were fed with normal rat feed for 4 weeks (group A1) (Fig. 2A) and rats that were fed with standard diet for 4 weeks (group A2) (Fig. 2B), showed normal architecture of liver tissue on histopathological evaluation. Whereas the rats that fed with high fat diet for 4 weeks (group A3) (Fig. 2C), showed slightly enlarged hepatocytes with feathery degeneration of cytoplasm (early change).

5.1.2. Liver histological analysis of the rats that fed with normal rat feed, standard diet and high fat diet for 8 weeks, were shown in Fig. 3(D-F). The rats that were fed with normal rat feed for 8 weeks (group B1) (Fig. 3D) and rats that were fed with standard diet for 8 weeks (group B2) (Fig. 3E), showed normal architecture of liver tissue on histopathological evaluation. Whereas the rats that were fed with high fat diet for 8 weeks (group B3) (Fig. 3F) showed diffused fatty infiltration of hepatocytes (steatosis) with mono nuclear inflammatory infiltrate (inflammation), confirming the development of NASH.
5.1.3. Liver histological analysis of the rats that fed with normal rat feed, standard diet and high fat diet for 12 weeks, were shown in Fig. 4(G-I). The rats that were fed with normal rat feed for 12 weeks (group C1) (Fig. 4G) and the rats that were fed with standard diet for 12 weeks (group C2) (Fig. 4H), showed normal hepatocytes. Whereas the rats that were fed with high fat diet for 12 weeks (group C3) (Fig. 4I) showed hepatic macrovesicular steatosis, obvious fibrosis, larger lipid accumulation (fatty cysts & larger fatty vacuoles).
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FIG. 2 (A-C): PHASE – I: HISTOPATHOLOGICAL STUDIES IN GROUP A (WEEK 4) (20x MAGNIFICATION)

Fig. 2A: Group A1 (Control) - Showed normal hepatocytes

Fig. 2B: Group A2 (Standard diet) - Showed Normal Hepatocytes, no pathological changes observed

Fig. 2C: Group A3 (High fat diet) - Showed slightly enlarged hepatocytes with feathery degeneration of cytoplasm (early change).
**FIG. 3 (D-F): PHASE – I: HISTOPATHOLOGICAL STUDIES IN GROUP B (WEEK 8) (20x MAGNIFICATION)**

**Fig. 3D:** Group B1 (Control) - Showed normal hepatocytes

**Fig. 3E:** Group B2 (Standard diet) - Showed Normal Hepatocytes, No pathological changes observed

**Fig. 3F:** Group B3 (High fat diet) - Showed diffused fatty infiltration of hepatocytes with mono nuclear inflammatory infiltrate.
Results

FIG. 4 (G-I): PHASE – I: HISTOPATHOLOGICAL STUDIES IN GROUP C
(WEEK 12) (100x MAGNIFICATION)

Fig. 4G: Group C1 (Control) - Showed normal hepatocytes

Fig. 4H: Group C2 (Standard diet) - Showed normal hepatocytes, no pathological changes observed

Fig. 4I: Group C3 - (High fat diet) showed hepatic macrovesicular steatosis, obvious fibrosis, larger lipid accumulation (fatty cysts & larger fatty vacuoles).
5.2. RESULTS OF PHASE II STUDIES: COMPARATIVE STUDY OF PROTECTIVE ROLE OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID (HCA):

5.2.1. Histopathological evaluation, which remains the sole method of distinguishing disease progression and regression of NASH was measured after the treatment of pioglitazone, quercetin, and hydroxy citric acid. The photomicrographs are shown in Fig. 5 (A-H). The rats in group 1 (control) that were fed with standard diet for 8 weeks (Fig. 5A), represented normal architecture of rat liver tissue on histopathological evaluation.

The rats in group 2 (NASH induced) that were fed with high fat diet for 8 weeks showed diffused fatty infiltration of hepatocytes (steatosis) with mono nuclear inflammatory infiltrate (inflammation) (Fig. 5B), confirming the induction of NASH.

Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control) (Fig. 5C), with quercetin (group 4; quercetin control) (Fig. 5D) and with hydroxy citric acid (group 5; HCA control) (Fig. 5E) showed normal hepatocytes and no pathological changes were observed in rats that were present in these three drug control groups. The rats that were present in experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone) (Fig. 5F) showed no fatty degeneration.

In group 7 rats, which were treated with quercetin (NASH+quercetin) (Fig. 5G) hepatocytes appear normal and no
obvious fatty infiltration and inflammation were seen. There observed local hepatocyte necrosis with inflammatory collections in the rats present in experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA) (Fig. 5H).

5.2.2. Scanning Electron Microscopy (SEM) photomicrographs are shown in Fig. 6 (A-H). The rats in group 1 (control) that were fed with standard diet for 8 weeks (Fig. 6A), represented the epithelium and layers of the liver with normal architecture. The rats in group 2 (NASH induced) that were fed with high fat diet for 8 weeks showed accumulation of fat on liver tissues (steatosis), with cellular destruction showing pathological changes with mono nuclear inflammatory infiltrate (inflammation) (Fig. 6B), confirming the induction of NASH. Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control) (Fig. 6C), with quercetin (group 4; quercetin control) (Fig. 6D) and with hydroxy citric acid (group 5; HCA control) (Fig. 6E) showed the epithelium and layers of the liver with normal architecture and no pathological changes were observed in rats that were present in these three drug control groups. The rats that were present in experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone) (Fig. 6F) showed marked reduction in the size of fat accumulation. In group 7 rats that were treated with quercetin (NASH+quercetin) (Fig. 6G) hepatocytes appear normal and no
obvious fatty infiltration and inflammation were seen. The rats that were present in experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA) (Fig. 6H) there observed local hepatocyte necrosis with inflammatory collections but showed reduction in the size of fat accumulation.

5.2.3. Table - 2 and Fig. 7(A-F) showed the effect of pioglitazone, quercetin and hydroxy citric acid on the levels of serum liver marker enzymes in experimental NASH. The experimental rats in NASH group (group 2) produced severe liver injury by significantly increasing the serum levels of ALT, AST, GGT and LDH compared with that of the control. However, the experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone), with quercetin (group 7; NASH+quercetin) and with hydroxy citric acid (group 8; NASH+HCA) showed an obvious decrease in ALT, AST, GGT and LDH levels when compared with that of NASH induced group (group 2). Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control), with quercetin (group 4; quercetin control) and with hydroxy citric acid (group 5; HCA control) does not show any significant effect on the liver marker enzymes compared to control group (group 1).

5.2.4. Table - 3 and Fig. 8(A-E) depicts the effect of pioglitazone, quercetin and hydroxy citric acid on the levels of other biochemical parameters such as serum albumin, total bilirubin,
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creatinine, urea, uric acid and glucose in experimental NASH. A significant increase in the levels of albumin, creatinine, urea, uric acid, glucose & total bilirubin was noticed in experimentally induced NASH group (group 2) when compared to rats in control group (group 1). The experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone), with quercetin (group 7; NASH+quercetin) and with hydroxy citric acid (group 8; NASH+HCA) showed significant reduction in serum albumin, total bilirubin, creatinine, urea, uric acid and glucose levels when compared with that of NASH induced group (group 2). Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control), with quercetin (group 4; quercetin control) and with hydroxy citric acid (group 5; HCA control) does not show any significant effect on serum albumin, total bilirubin, creatinine, urea, uric acid and glucose compared to control group (group 1).

5.2.5. Table - 4 and Fig. 9(A-F) shows the effect of pioglitazone, quercetin and hydroxy citric acid on the lipid profile levels in experimental NASH induced rats. The levels of serum lipid profile parameters such as total cholesterol, free cholesterol, esterified cholesterol, phospholipids, triglycerides and free fatty acids levels were raised significantly in experimental NASH induced rats (group 2) when compared to control group (group 1). The experimental NASH rats treated with pioglitazone
(group 6; NASH+pioglitazone), with quercetin (group 7; NASH+quercetin) and with hydroxy citric acid (group 8; NASH+HCA) showed marked reduction in levels of lipid profile parameters when compared with that of NASH induced group (group 2). Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control) does not show any significant effect on levels of total cholesterol, esterified cholesterol, phospholipids, free fatty acids but showed significant reduction in free cholesterol and triglyceride levels when compared to control group (group 1). On the other hand, rats fed with standard diet simultaneously with quercetin (group 4; quercetin control) does not show any significant effect on all the lipid profile parameters compared to control group (group 1). Whereas, rats fed with standard diet simultaneously with hydroxy citric acid (group 5; HCA control) does not show any significant effect on levels of total cholesterol, esterified cholesterol, triglycerides, free fatty acids but showed significant reduction in the levels of free cholesterol and phospholipids when compared to control group (group 1).

5.2.6. The levels of Lipoproteins such as HDL, LDL, VLDL, HDL:LDL, TC:HDL were presented in Table 5 and Fig. 10 (A-E). Raised levels of low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol are pointers to insulin resistance. The disease progression was directly
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proportional to the lipoprotein levels, showed by significant increase of lipoprotein levels in experimentally induced NASH group (group 2) when compared to control rats (group 1). Protective effect of pioglitazone, quercetin and hydroxy citric acid was noticed by decreasing the lipoprotein levels towards normalcy in group 6 (NASH+pioglitazone), group 7 (NASH+quercetin) and group 8 (NASH+HCA), when compared to experimentally induced NASH group (group 2). Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control) and with quercetin (group 4; quercetin control) do not show any significant effect on levels of lipoproteins when compared to control group (group 1). On the other hand, rats fed with standard diet simultaneously with hydroxy citric acid (group 5; HCA control) does not show any significant effect on levels of LDL and VLDL but showed significant increase in the levels of HDL when compared to control group (group 1).

5.2.7. Oxidative stress is one of the key mechanisms responsible for liver damage and disease progression in NASH. Non enzymatic antioxidant such as GSH and antioxidant enzymes such as catalase, SOD, GPx ,GR, and GST were decreased significantly in experimental NASH rats (group 2), compared to that of control group (group 1) as shown in Table 6 and Fig. 11(A-G). The experimental NASH rats treated with pioglitazone
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(group 6; NASH+pioglitazone) showed marked increase in the levels of GSH, catalase and SOD but does not show a significant effect on the levels of GPx, GR and GST levels when compared to the experimentally induced NASH group (group 2). Similarly, experimental NASH rats treated with hydroxy citric acid (group 8; NASH+HCA) showed marked increase in the levels of GSH and catalase but does not show a significant effect on the levels of SOD, GPx, GR and GST levels when compared to the experimentally induced NASH group (group 2).

On contrary to these two drugs viz. pioglitazone and hydroxy citric acid, the experimental NASH rats treated with quercetin (group 7; NASH+quercetin) showed significant increase in the levels of antioxidants viz. GSH, catalase, SOD, GPx, GR and GST when compared with that of NASH induced group (group 2) showing maximum protective effect against NASH. Malondialdehyde (MDA), lipid peroxidation product is an index of lipid peroxidation. The levels of lipid peroxidation products (MDA) have been significantly increased in experimentally induced NASH group (group 2) compared to the control group (group 1).

The experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone), with quercetin (group 7; NASH+quercetin) and with hydroxy citric acid (group 8; NASH+HCA) showed significant reduction in malondialdehyde (MDA) levels when compared with that of NASH induced group (group 2). Rats fed
with standard diet simultaneously with pioglitazone (group 3; pioglitazone control), with quercetin (group 4; quercetin control) does not show any significant effect on the levels of lipid peroxidation products (MDA) and non-enzymatic (GSH) and enzymatic (catalase, SOD, GPx, GR and GST) antioxidants when compared to control group (group 1). On the other hand, rats fed with standard diet simultaneously with hydroxy citric acid (group 5; HCA control) does not show any significant effect on the levels of lipid peroxidation products (MDA) and GSH, GPx, GR and GST but showed significant decrease in the levels of catalase and SOD when compared to control group (group 1).

5.2.8. The imbalanced production of pro- and anti-inflammatory adipokines secreted from fat contributes to the pathogenesis of NASH. Leptin has been proved to be a good predictor of the disease with median levels of NASH. The levels of hyaluronic acid and leptin were increased significantly in experimentally induced NASH group (group 2) compared to control group (group 1); whereas adiponectin, an important adipokine decreased significantly in experimentally induced NASH group compared to control group (group 1). The pattern of hyaluronic acid, leptin and adiponectin were depicted in Table 7 and Fig. 12(A-C). The experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone) showed significant decrease in
the levels of hyaluronic acid and significant increase in the levels of adiponectin levels when compared to experimentally induced NASH group (group 2) but does not show any effect on the levels of leptin, compared to experimentally induced NASH group (group 2). Whereas the experimental NASH rats treated with hydroxy citric acid (group 8; NASH+HCA) does not show any effect on all the three extracellular components (hyaluronic acid, leptin and adiponectin). On contrary to these two drugs viz. pioglitazone and hydroxy citric acid, the experimental NASH rats treated with quercetin (group 7; NASH+quercetin) showed significant decrease in the levels of hyaluronic acid and leptin compared with that of NASH induced group (group 2) and significant decrease in adiponectin levels compared with that of NASH induced group (group 2), conferring maximum protection against NASH, when compared to the other two drugs. Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control), with quercetin (group 4; quercetin control) does not show any significant effect on the levels of extracellular matrix components viz. hyaluronic acid, leptin and adiponectin compared to control group (group 1). On the other hand, rats fed with standard diet simultaneously with hydroxy citric acid (group 5; HCA control) does not show any significant effect on the levels of leptin and adiponectin but showed
significant increase in hyaluronic acid levels when compared to control group (group 1).

5.2.9. Inflammatory mediators have also been investigated as potential diagnostic tools. NASH was associated with an increase in tumor necrosis factor alpha (TNF-\(\alpha\)) and MPO levels and the imbalance may play an important role in the development of NASH. The pattern of inflammatory markers has been depicted in the Table 8 and Fig. 13(A-B). A significant increase in the levels of inflammatory markers such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and myeloperoxidase (MPO) was noticed in experimental NASH rats (group 2) as compared to control group (group 1). The experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone) and rats treated with quercetin (group 7; NASH+quercetin) showed significant decrease in the levels of TNF-\(\alpha\) and MPO. Whereas, the experimental NASH rats treated with hydroxy citric acid (group 8; NASH+HCA) does not show any significant effect on the levels of inflammatory markers viz. TNF-\(\alpha\) and MPO. Rats fed with standard diet simultaneously with pioglitazone (group 3; pioglitazone control), with quercetin (group 4; quercetin control) and with hydroxy citric acid (group 5; HCA control) does not show any significant effect on the inflammatory markers compared to control group (group 1).
5.2.10. Effect of pioglitazone, quercetin and hydroxy citric Acid on the levels of different phospholipids in experimental NASH was shown in Table 9. NASH induced group (group 2) showed significant decrease in the levels of phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol when compared to the control group (group 1) with significant increase in the phosphatidyl glycerol when compared to control group (group 1). The experimental NASH rats treated with pioglitazone (group 6; NASH+pioglitazone), with quercetin (group 7; NASH+quercetin) and with hydroxy citric acid (group 8; NASH+HCA) showed protective effect normalizing the levels levels of phosphotidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol back to normal, compared to the control group (group 1) as shown in Table 9.

5.2.11. The separation of phosphotidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol by thin layer chromatography (TLC) analysis was showed in Fig. 14. The changes in the levels of these phospholipids were distinct in the livers of the rats in the livers of the experimentally induced NASH group (group 2) when compared to the control group (group 1). Lane 1 represents the phospholipids standard. Lane 2 represents the different fractions of phospholipids viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol for control group (group 1), Lane 3 represents the
different fractions of phospholipids viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol for experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA). Lane 4 represents the different fractions of phospholipids viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol for experimental NASH treated with quercetin (group 7; NASH+quercetin). Lane 5 represents the different fractions of phospholipids viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol for experimentally induced NASH group (group 2) and Lane 6 represents the different fractions of phospholipids viz. phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol for experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone).

5.2.12. Quantitative real-time polymerase chain reaction (RT-PCR) analysis of vascular endothelial growth factor (VEGF) messenger RNA (VEGF mRNA) was analyzed in all the groups and was represented in Fig. 15. Lane 1 represents the expression of VEGF mRNA in control group (group 1). Lane 2 represents the expression of VEGF mRNA in experimentally induced NASH group (group 2). Lane 3 represents the expression of VEGF mRNA in experimental NASH treated with quercetin (group 7; NASH+quercetin). Lane 4 represents the expression of VEGF mRNA in experimental NASH treated with
pioglitazone (group 6; NASH+pioglitazone). Lane 5 represents the expression of VEGF mRNA in experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA). High expression of VEGF mRNA in hepatic cells was observed in experimentally induced NASH group (group 2) when compared to the expression of VEGF mRNA in control group (Lane 2; group 1). Very mild increase in the expression of VEGF mRNA in was observed in experimental NASH treated with quercetin (Lane 3; group 7; NASH+quercetin) and mild increase in experimental NASH treated with pioglitazone (Lane 4; group 6; NASH+pioglitazone) and in experimental NASH treated with hydroxy citric acid (Lane 5; group 8; NASH+HCA) were observed when compared with the NASH induced group (Lane 2; group 2). The drug quercetin showed an effective inhibition of VEGF mRNA expression as evidenced in the Fig. 15 (Lane 3) and perhaps only smaller inhibition of VEGF mRNA level seen in hydroxy citric acid and pioglitazone treated rats (Lane 4 and 5).

5.2.13. Detection of Cytochrome P450 2E1 (CYP2E1) cenzyme levels in liver by immunoblot analysis was depicted in Fig. 16. Lane 1 represents the expression of CYP2E1 in control group (group 1). Lane 2 represents the expression of CYP2E1 in experimentally induced NASH group (group 2). Lane 3 represents the expression of CYP2E1 in experimental NASH...
treated with quercetin (group 7; NASH+quercetin). Lane 4 represents the expression of CYP2E1 in experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone). Lane 5 represents the expression of CYP2E1 in experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA). Lane 6 represents the CYP2E1 standard. CYP2E1 catalytic activity was increased in experimentally induced NASH group (group 2) compared to control group (group 1) as evidenced in Fig. 16. Immunoblot analysis revealed the low levels of CYP2E1 in the experimental NASH treated with pioglitazone, quercetin and hydroxy citric acid NASH animals (Fig. 16). An approximate 2-fold decrease in the level of CYP2E1 levels have been observed in experimental NASH treated with quercetin (group 7; NASH+quercetin) compared to NASH group (group 2) and mild decrease in the levels of CYP2E1 level was observed in experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone) compared to NASH group (group 2) and also in experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA). The quantification of CYP2E1 paralleled the results of immunoblots as evidenced in Fig. 17.

5.2.14. Fig. 18(A-F) depicts the immunohistochemistry of cytokeratin – 18 (CK-18) expression in - control group (group 1), experimentally induced NASH group (group 2), experimental NASH treated with pioglitazone (group 6; NASH+pioglitazone),
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Experimental NASH treated with quercetin (group 7; NASH+quercetin), experimental NASH treated with hydroxy citric acid (group 8; NASH+HCA) and in alcoholic liver disease (ALD). Liver tissue lysates were analyzed by immunohistochemistry for the expression of CK-18 (Fig. 18) and then quantification was carried out. The results of the quantification were depicted in Fig. 19. There observed no expression of CK-18 cells in hepatic tissue of the rats in control group (group 1) (Fig. 18A). Greater expression of CK-18 cells was observed in experimentally induced NASH group (group 2) (Fig. 18B) reflecting the liver damage. However, CK-18 levels were markedly reduced by treatment with pioglitazone, quercetin and HCA suggesting their role to prevent cell death. The liver tissues of the rats in experimental NASH treated with pioglitazone group (group 6; NASH+pioglitazone) showed less expression of CK-18 cells than that of the NASH group (group 2) (Fig. 18C). The liver tissues of the rats in experimental NASH treated with hydroxy citric acid group (group 8; NASH+hydroxy citric acid) showed moderate expression of CK-18 cells than that of the NASH group (group 2) (Fig. 18D). Whereas, the liver tissues of the rats in experimental NASH treated with quercetin (group 7; NASH+quercetin) showed very mild expression of CK-18 cells than that of the NASH group (group 2) (Fig. 18E), showing maximum protection against NASH. All three treatment
drugs provide favorable alterations in CK-18 levels in comparison with NASH group (Fig. 18 and Fig. 19). The over expression of CK-18 was also observed in ALD compared to the rats in control group (Fig. 18F) but the percentage of decrease was less compared to NASH as evidenced in Fig. 19.

5.2.15. The levels of tissue polypeptide specific antigen (TPSA) estimated by ELISA in control group (group 1), experimentally induced NASH (group 2) and also compared with alcoholic liver disease (ALD) was depicted in Fig. 20. TPSA has recently been proposed as diagnostic marker of apoptosis in NASH and significantly increased levels of TPSA were observed in experimentally induced NASH group (group 2) when compared to the control group (group 1). Whereas, TPSA was not expressed in alcoholic liver disease (ALD) giving a hope that it may be useful as a marker to diagnose NASH and more studies to be conducted on usefulness of TPSA as a maker to diagnose NASH.
Fig. 5 (A-H): PHASE II: HISTOPATHOLOGICAL STUDIES (COMPARATIVE STUDY OF ROLE OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID EXPERIMENTAL MODEL OF NASH) (20x MAGNIFICATION)

Fig. 5A: Group 1; Control - Showed normal hepatocytes
Fig. 5B: Group 2; NASH (High fat diet) - Showed diffused fatty infiltration of hepatocytes with mono nuclear inflammatory infiltrate
Fig. 5C: Group 3 (Pioglitazone Control) - Shows normal hepatocytes; no pathological changes observed;
Fig. 5D: Group 4 (Quercetin Control) - No pathological changes, shows normal hepatocytes
Fig. 5E: Group 5 (Hydroxy Citric Acid Control) - No pathological changes, shows normal hepatocytes
Fig. 5F: Group 6 (NASH + Pioglitazone) - No fatty degeneration is seen
Fig. 5G: Group 7 (NASH + Quercetin) - Hepatocytes appear normal, no obvious fatty & inflammatory changes are seen
Fig. 5H: Group 8 (NASH + Hydroxy Citric Acid) - Local hepatocyte necrosis was observed.
Fig. 6 (A-H): PHASE II: SCANNING ELECTRON MICROSCOPY (SEM) STUDIES (COMPARATIVE STUDY OF ROLE OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID IN EXPERIMENTAL MODEL OF NASH)

Fig. 6A: Group 1 (Control) - Liver section of rat showing epithelium and normal layers with normal architecture

Fig. 6B: Group 2 (NASH induced with High fat diet) - Liver section of NASH rat showing accumulation of fat on liver tissues, with cellular destruction showing pathological changes

Fig. 6C: Group 3 (Pioglitazone Control) - Liver section of rat showing epithelium and normal layers with normal architecture

Fig. 6D: Group 4 (Quercetin Control) - No pathological changes observed and showed normal layers with normal architecture
Fig. 6E: Group 5 (Hydroxy Citric Acid Control) - No pathological changes observed and showed normal layers with normal architecture.

Fig. 6F: Group 6 (NASH + Pioglitazone) - Liver section of NASH rat showing marked reduction in the size of fat accumulation.

Fig. 6G: Group 7 (NASH + Quercetin) - Hepatocytes appear normal & no obvious fatty & inflammatory changes are seen.

Fig. 6H: Group 8 (NASH + Hydroxy Citric Acid) - Local hepatocyte necrosis with inflammatory collections is seen but showed reduction in the size of fat accumulation.
**Results**

**TABLE – 2: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM LIVER MARKER ENZYMES IN EXPERIMENTAL NASH.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH + HCA</th>
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<tr>
<td>ALP (IU/L)</td>
<td>119.1±8.4</td>
<td>121.6±10.1</td>
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<td>ALT (IU/L)</td>
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<td>51.3±3.1</td>
<td>55.2±4.1</td>
<td>59.9±4.9</td>
<td>97.1±8.7</td>
<td>84.2±7.2</td>
<td>130.3±10.8</td>
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<tr>
<td>AST (IU/L)</td>
<td>68.9±3.8</td>
<td>107.4±8.9</td>
<td>66.4±2.4</td>
<td>63.9±3.6</td>
<td>71.6±5.3</td>
<td>80.9±7.3</td>
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<td>ALT:AST</td>
<td>0.80±0.07</td>
<td>1.7±0.02</td>
<td>0.77±0.06</td>
<td>0.86±0.08</td>
<td>0.83±0.06</td>
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<td>LDH (IU/L)</td>
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<td>449.9±32.8</td>
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Values are expressed as Mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; 
^aP<0.001 compared to NASH group; ^bP<0.01 compared to NASH group; ^cP<0.05 compared to NASH group
Fig. 7A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM ALKALINE PHOSPHATASE (ALP) IN EXPERIMENTAL NASH.
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Fig. 7B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM ALANINE TRANSAMINASE (ALT) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 7C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM ASPARTATE TRANSAMINASE (AST) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
**Results**

Fig. 7D: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE RATIO OF SERUM ALANINE TRANSAMINASE (ALT) AND ASPARTATE TRANSAMINASE (AST) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 7E: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM LACTATE DEHYDROGENASE (LDH) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Results

Fig. 7F: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM GAMMA GLUTAMYL TRANSFERASE (GGT) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
## TABLE – 3: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF VARIOUS BIOCHEMICAL PARAMETERS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH + HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>2.5±0.2</td>
<td>4.58±0.3 *</td>
<td>2.41±0.19</td>
<td>2.59±0.22</td>
<td>2.43±0.25</td>
<td>3.6±0.3 c</td>
<td>3.8±0.1 c</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.9±0.07</td>
<td>10.6±0.89 *</td>
<td>0.81±0.07</td>
<td>0.67±0.05</td>
<td>0.64±0.04</td>
<td>2.6±0.01 a</td>
<td>1.1±0.02 a</td>
<td>8.5±0.41 c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.06 ±0.09</td>
<td>2.9±0.01 *</td>
<td>1.89±0.17</td>
<td>1.7±0.19</td>
<td>1.4±0.13</td>
<td>1.4±0.01 a</td>
<td>1.5±0.01 a</td>
<td>1.7±0.01 a</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>13.5±1.6</td>
<td>28.7±1.5</td>
<td>17.6±1.6</td>
<td>16.4±1.3</td>
<td>14.3±1.2</td>
<td>24.1±1.4 c</td>
<td>19.4±1.6 a</td>
<td>20.6±1.9 b</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.36±0.29</td>
<td>9.01±0.58</td>
<td>3.45±0.31</td>
<td>3.67±0.32</td>
<td>3.81±0.33</td>
<td>5.18±0.43 a</td>
<td>5.13±0.38 a</td>
<td>4.35±0.25 a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>107.8±8.1</td>
<td>160.5±9.2</td>
<td>112.4±7.5</td>
<td>103.6±8.1</td>
<td>114.2±9.3</td>
<td>110.5±10.4 b</td>
<td>120.4±11.4 c</td>
<td>128.8±10.8 c</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 8A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM ALBUMIN IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
**Fig. 8B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM TOTAL BILIRUBIN IN EXPERIMENTAL NASH.**

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 8C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM CREATININE IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 8D: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM UREA IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group
Fig. 8E: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SERUM URIC ACID IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; ^aP<0.001 compared to NASH group; ^bP<0.01 compared to NASH group; ^cP<0.05 compared to NASH group
Fig. 8F: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF BLOOD GLUCOSE IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; \( ^a \)P<0.001 compared to NASH group; \( ^b \)P<0.01 compared to NASH group; \( ^c \)P<0.05 compared to NASH group
Results

**TABLE – 4: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF LIPID PROFILE PARAMETERS IN EXPERIMENTAL NASH.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH + HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>140.5±5.3</td>
<td>457.3±21.5</td>
<td>135.2±9.9</td>
<td>142.6±10.6</td>
<td>133.9±9.9</td>
<td>248.2±18.3</td>
<td>213.9±14.9</td>
<td>174.5± 12.9</td>
</tr>
<tr>
<td>Free cholesterol (nmole/g)</td>
<td>7546±289.5</td>
<td>9485±213.9</td>
<td>7432±305.8*</td>
<td>7645±204.8</td>
<td>7469±177.6*</td>
<td>8446±195.4</td>
<td>8016±200.2</td>
<td>7823±187.3</td>
</tr>
<tr>
<td>Esterified cholesterol (nmole/g)</td>
<td>7580±189.0</td>
<td>19489±345.6</td>
<td>7513±256.4</td>
<td>7546±189.5</td>
<td>7560±304.8</td>
<td>8915±207.6</td>
<td>8638±301.2</td>
<td>8461±265.4</td>
</tr>
<tr>
<td>Phospholipids (mmole/100g liver)</td>
<td>4.61±0.28</td>
<td>3.75±0.19</td>
<td>4.31±0.38</td>
<td>4.56±0.41</td>
<td>3.61±0.21*</td>
<td>3.93±0.23</td>
<td>4.82±0.35</td>
<td>4.68±0.27</td>
</tr>
<tr>
<td>Triglycerides (mg/g)</td>
<td>74.2±5.3</td>
<td>185.3±11.2</td>
<td>80.1±7.3*</td>
<td>73.5±6.1</td>
<td>69.4±4.1</td>
<td>141.6±10.9</td>
<td>104.8±8.8</td>
<td>116.4±9.6</td>
</tr>
<tr>
<td>FFA (mg/g)</td>
<td>27.18±1.6</td>
<td>79.6±5.7</td>
<td>26.4±1.9</td>
<td>27.6±2.4</td>
<td>31.7±1.8</td>
<td>40.8±3.5*</td>
<td>35.9±2.3</td>
<td>52.3±4.6</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; #P<0.001 as compared to control group.
Results

Fig. 9A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF TOTAL CHOLESTEROL IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 9B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF FREE CHOLESTEROL IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; \(^a\)P<0.001 compared to NASH group; \(^b\)P<0.01 compared to NASH group; \(^c\)P<0.05 compared to NASH group; \(^d\)P<0.001 as compared to control group
Fig. 9C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF ESTERIFIED CHOLESTEROL IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; ¹P<0.001 compared to NASH group; ²P<0.01 compared to NASH group; ³P<0.05 compared to NASH group; ⁴P<0.001 as compared to control group
Fig. 9D: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF PHOSPHO
LIPIDS IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; a P<0.001 compared to NASH group; b P<0.01 compared to NASH group; c P<0.05 compared to NASH group; d P<0.001 as compared to control group
Fig. 9E: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF TRIGLYCERIDES IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; \(^{a}\)P<0.001 compared to NASH group; \(^{b}\)P<0.01 compared to NASH group; \(^{c}\)P<0.05 compared to NASH group; \(^{d}\)P<0.001 as compared to control group
Fig. 9F: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF FREE FATTY ACIDS (FFA) IN EXPERIMENTAL NASH.

Results

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
### TABLE – 5: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF LIPOPROTEINS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH + HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mg/dl)</td>
<td>47.6±3.1</td>
<td>45.3±2.6</td>
<td>53.1±5.7</td>
<td>49.8±4.2</td>
<td>54.0±4.9</td>
<td>48.9±3.1</td>
<td>58.5±3.1</td>
<td>65.4±3.5</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>38.6±1.9</td>
<td>108.4±9.3</td>
<td>37.1±3.2</td>
<td>37.8±2.9</td>
<td>36.9±2.1</td>
<td>60.4±3.8</td>
<td>68.6±4.9</td>
<td>58.9±4.1</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>106.5±8.4</td>
<td>382.4±17.5</td>
<td>104.9±9.4</td>
<td>105.8±8.3</td>
<td>102.9±9.4</td>
<td>206.1±14.6</td>
<td>244.4±18.2</td>
<td>163.7±11.6</td>
</tr>
<tr>
<td>HDL:LDL</td>
<td>1.23±0.1</td>
<td>2.39±0.19</td>
<td>1.43±0.12</td>
<td>1.31±0.11</td>
<td>1.46±0.14</td>
<td>1.34±0.09</td>
<td>1.22±0.11</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>TC:HDL</td>
<td>2.95±0.16</td>
<td>10.09±0.99</td>
<td>2.54±0.18</td>
<td>2.86±0.19</td>
<td>2.47±0.22</td>
<td>5.07±0.38</td>
<td>4.32±0.31</td>
<td>3.46±0.24</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 animals in each group. *P<0.001 and †P<0.05 compared to control group; ‡P<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; #P<0.001 as compared to control group.
Fig. 10A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF HIGH DENSITY LIPO PROTEIN (HDL) IN EXPERIMENTAL NASH.

*P<0.001 and @ P<0.05 compared to control group; *P<0.001 compared to NASH group; b P<0.01 compared to NASH group; c P<0.05 compared to NASH group; d P<0.001 as compared to control group
Results

Fig. 10B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF LOW DENSITY LIPO PROTEIN (LDL) IN EXPERIMENTAL NASH.

*P<0.001 and @P<0.05 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 10C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF VERY LOW DENSITY LIPO PROTEIN (VLDL) IN EXPERIMENTAL NASH.

*P<0.001 and †P<0.05 compared to control group; ‡P<0.001 compared to NASH group; §P<0.01 compared to NASH group; ¶P<0.05 compared to NASH group; ‡‡P<0.001 as compared to control group
Fig. 10D: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE RATIO OF HIGH DENSITY LIPOPROTEIN (HDL) AND LOW DENSITY LIPOPROTEIN (LDL) IN EXPERIMENTAL NASH.

*P<0.001 and @ P<0.05 compared to control group; †P<0.001 compared to NASH group; ‡P<0.01 compared to NASH group; §P<0.05 compared to NASH group; ¶P<0.001 as compared to control group
Fig. 10E: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE RATIO OF TOTAL CHOLESTEROL (TC) AND HIGH DENSITY LIPOPROTEIN (HDL) IN EXPERIMENTAL NASH.

*P<0.001 and Ξ P<0.05 compared to control group; *P<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
TABLE – 6: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF LIPID PEROXIDATION, NONENZYMATIC AND ENZYMATIC ANTIOXIDANTS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH + Pioglitazone</th>
<th>Group 7: NASH + Quercetin</th>
<th>Group 8: NASH + HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>5.1±0.36</td>
<td>9.9±0.11</td>
<td>4.8±0.31</td>
<td>4.7±0.32</td>
<td>5.3±0.45</td>
<td>6.9±0.51</td>
<td>5.3±0.48</td>
<td>6.3±0.57</td>
</tr>
<tr>
<td>GSH</td>
<td>74.3±4.8</td>
<td>33.7±1.7</td>
<td>74.5±0.68</td>
<td>76.1±0.71</td>
<td>69.1±0.63</td>
<td>68.2±3.1</td>
<td>64.8±4.7</td>
<td>43.9±3.8</td>
</tr>
<tr>
<td>CAT</td>
<td>5896±215.8</td>
<td>1994±144.3</td>
<td>5879±489</td>
<td>5895±502</td>
<td>5742±501</td>
<td>3895±201.7</td>
<td>4997±175.2</td>
<td>2589±174.8</td>
</tr>
<tr>
<td>SOD</td>
<td>125.4±7.1</td>
<td>40.6±5.7</td>
<td>86.4±7.1</td>
<td>129.3±9.5</td>
<td>108.9±9.3</td>
<td>79.8±6.2</td>
<td>96.9±8.4</td>
<td>44.8±4.8</td>
</tr>
<tr>
<td>GPx</td>
<td>638.1±34.0</td>
<td>304.5±19.8</td>
<td>628.4±57.6</td>
<td>642.8±61.4</td>
<td>604.2±59.8</td>
<td>364.4±25.7</td>
<td>529.1±30.6</td>
<td>317.8±29.4</td>
</tr>
<tr>
<td>GR</td>
<td>109.4±7.4</td>
<td>82.6±5.9</td>
<td>104.5±9.4</td>
<td>114.3±10.1</td>
<td>101.9±9.6</td>
<td>90.8±8.1</td>
<td>105.3±7.2</td>
<td>86.9±7.4</td>
</tr>
<tr>
<td>GST</td>
<td>272.1±13.6</td>
<td>103.8±8.4</td>
<td>261.9±20.4</td>
<td>283.4±15.6</td>
<td>269.7±12.4</td>
<td>119.6±17.5</td>
<td>194.5±11.1</td>
<td>108.6±9.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; #P<0.001 as compared to control group
Fig. 11A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF MALONDIALDEHYDE (MDA), AN INDEX OF LIPID PEROXIDATION IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; *P<0.001 compared to NASH group; *P<0.01 compared to NASH group; *P<0.05 compared to NASH group; *P<0.001 as compared to control group
Results

Fig. 11B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF REDUCED GLUTATHIONE (GSH) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 11C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF CATALASE (CAT) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group;  aP<0.001 compared to NASH group;  bP<0.01 compared to NASH group;  cP<0.05 compared to NASH group;  dP<0.001 as compared to control group
Fig. 11D: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF SUPEROXIDE DISMUTASE (SOD) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 11E: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF GLUTATHIONE PEROXIDASE (GPx) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 11F: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF GLUTATHIONE REDUCTASE (GR) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 11G: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF GLUTATHIONE-S-TRANSFERASE (GST) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; a P<0.001 compared to NASH group; b P<0.01 compared to NASH group; c P<0.05 compared to NASH group; d P<0.001 as compared to control group.
TABLE – 7: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF EXTRA CELLULAR MATRIX (ECM) COMPONENTS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH + HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid (ng/ml)</td>
<td>43.6±2.9</td>
<td>82.8±7.1</td>
<td>49.5±3.4</td>
<td>42.1±2.6</td>
<td>70.4±5.8</td>
<td>60.9±4.4</td>
<td>57.4±2.8</td>
<td>80.4±6.7</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>34.6±2.2</td>
<td>48.5±3.7</td>
<td>40.8±2.9</td>
<td>33.9±3.1</td>
<td>34.1±2.4</td>
<td>47.1±2.9</td>
<td>37.3±3.3</td>
<td>40.3±2.6</td>
</tr>
<tr>
<td>Adiponectin (mg/L)</td>
<td>9.77±0.55</td>
<td>4.13±0.23</td>
<td>10.1±0.91</td>
<td>10.5±0.85</td>
<td>9.6±0.74</td>
<td>7.9±0.71</td>
<td>8.3±0.63</td>
<td>4.37±0.39</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; #P<0.001 as compared to control group.
Results

Fig. 12A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF HYALURONIC ACID IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; aP<0.001 compared to NASH group; bP<0.01 compared to NASH group; cP<0.05 compared to NASH group; dP<0.001 as compared to control group
Fig. 12B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF LEPTIN IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; †P<0.001 compared to NASH group; ‡P<0.01 compared to NASH group; §P<0.05 compared to NASH group; ¶P<0.001 as compared to control group
Fig. 12C: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF ADIPONECTIN IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; ^P<0.001 compared to NASH group; 7P<0.01 compared to NASH group; 6P<0.05 compared to NASH group; 8P<0.001 as compared to control group
TABLE – 8: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF INFLAMMATORY MARKERS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: Control</th>
<th>Group 2: NASH</th>
<th>Group 3: Pioglitazone Control</th>
<th>Group 4: Quercetin Control</th>
<th>Group 5: HCA Control</th>
<th>Group 6: NASH+ Pioglitazone</th>
<th>Group 7: NASH+ Quercetin</th>
<th>Group 8: NASH+ HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-alpha (ng/ml)</td>
<td>8.75±0.51</td>
<td>12.14±1.0 *</td>
<td>8.83±0.71</td>
<td>8.51±0.69</td>
<td>9.01±0.82</td>
<td>9.14±0.8 c</td>
<td>8.02±0.5 b</td>
<td>9.64±0.7</td>
</tr>
<tr>
<td>Plasma MPO (ng/ml)</td>
<td>40.6±3.8</td>
<td>59.3±4.3 v</td>
<td>39.2±2.6</td>
<td>41.5±3.8</td>
<td>43.6±4.1</td>
<td>46.9±3.5 c</td>
<td>44.3±3.2 b</td>
<td>53.2±4.9</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 animals in each group. *P<0.001 compared to control group; ^P<0.001 compared to NASH group; _P<0.01 compared to NASH group; "P<0.05 compared to NASH group
Fig. 13A: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF TUMOR NECROSIS FACTOR-ALPHA (TNF-α) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; †P<0.001 compared to NASH group; ‡P<0.01 compared to NASH group; §P<0.05 compared to NASH group
Results

Fig. 13B: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF PLASMA MYELOPEROXIDASE (MPO) IN EXPERIMENTAL NASH.

*P<0.001 compared to control group; *P<0.001 compared to NASH group; ^P<0.01 compared to NASH group; cP<0.05 compared to NASH group
TABLE - 9: EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON THE LEVELS OF PHOSPHOLIPIDS IN EXPERIMENTAL NASH.

<table>
<thead>
<tr>
<th>Phospholipid standard</th>
<th>Aliquot spotted &amp; scrapped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Phosphatidyl Glycerol</td>
<td>4.06±0.03</td>
</tr>
<tr>
<td>Phosphatidyl Ethanolamine</td>
<td>4.09±0.02</td>
</tr>
<tr>
<td>Phosphatidyl Choline</td>
<td>2.2±0.01</td>
</tr>
<tr>
<td>Phosphatidyl Inositol</td>
<td>4.09±0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.0001 compared to Control Group;  *p < 0.001 compared to NASH group
Fig 14: SEPARATION OF PHOSPHOLIPIDS BY THIN LAYER CHROMATOGRAPHY (TLC)

Lane 1 - Phospholipid standard
Lane 2 - Control
Lane 3 - NASH+Hydroxy Citric Acid
Lane 4 - NASH+Quercetin
Lane 5 - NASH
Lane 6 - NASH+Pioglitazone
Fig. 15: RT-PCR ANALYSIS: EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR MESSENGER RNA (VEGF mRNA)

Lane 1 – Control
Lane 2 – NASH
Lane 3 – NASH+Quercetin
Lane 4 – NASH+Pioglitazone
Lane 5 – NASH+Hydroxy Citric Acid
Lane M - Marker
Fig. 16: DETECTION OF CYTOCHROME P450 2E1 (CYP2E1) ENZYME LEVELS IN LIVER BY IMMUNOBLOT ANALYSIS

Lane 1 – Control
Lane 2 – NASH
Lane 3 – NASH+Quercetin
Lane 4 – NASH+Pioglitazone
Lane 5 – NASH+Hydroxy Citric Acid
Lane M - Marker
Protein levels from individual animals were analyzed by densitometry, and the data is presented as mean intensity in relative arbitrary densitometric units (ADU) ± SEM with n = 6. Values bearing different letters are statistically different with \(^a\)p < 0.05 and \(^b\)p < 0.01 compared to NASH group.
Results

Fig. 18 (A-F): IMMUNOHISTOCHEMISTRY OF CYTOKERATIN – 18 (CK-18) IN NASH, DRUG TREATED GROUPS AND COMPARISON WITH ALCOHOLIC LIVER DISEASE (ALD)
Fig. 18A: Group 1 (Control) - There is no expression of CK-18 cells in normal hepatic tissue

Fig. 18B: Group 2 (NASH induced with High fat diet) - Arrows indicates the presence of CK-18 positive cells and shows greater expression of CK-18 cells than those of normal cells

Fig. 18C: Group 6 (NASH + Pioglitazone) - Shows less expression of CK-18 cells than those of NASH group

Fig. 18D: Group 8 (NASH + Hydroxy Citric Acid) - Shows moderate expression of CK-18 cells than those of NASH group;

Fig. 18E: Group 7 (NASH + Quercetin) - Shows very mild expression of CK-18 cells than those of NASH group;

Fig. 18F: ALD - Shows over expression of CK-18 cells in the liver tissue.
Results

Fig. 19: QUANTIFICATION OF CYTOKERATIN – 18 (CK-18) AND COMPARISON WITH ALCOHOLIC LIVER DISEASE (ALD)

The stained areas were analyzed and compared using NIH image software (Version 1.61). Values are expressed as mean ± SD.

*P<0.001 compared to control group; a P<0.01 and b P<0.001 compared to NASH group
Fig. 20: ESTIMATION OF TISSUE POLYPEPTIDE SPECIFIC ANTIGEN (TPSA) BY ELISA AND COMPARISON WITH ALCOHOLIC LIVER DISEASE (ALD)

Values are expressed as mean ± SD; *P<0.001 compared to control group