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Effect of Metformin, Pioglitazone and Rosuvastatin on Induced Non-alcoholic Fatty Liver in Rats

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ABSTRACT

Background: non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases affecting up to 30% of the general population worldwide**.**

Objective: the aim of this work was to study the effects of metformin, pioglitazone and rosuvastatin on serum lipids cholesterol and triglycerides (TG), liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), random blood sugar (RBS), oxidative stress malondialdehyde (MDA), and histopathological changes in induced non-alcoholic fatty liver in rats.

Patients and Methods: fifty male albino rats weighing 100-120 grams of local strain were used. Animals were purchased from Nile Pharmaceutical Company. They were kept in suitable cages at room temperature with the natural light-dark cycle. They were maintained on a standard diet of commercial rat chow and free tap water. They were kept for 10 days for adaptation to the new environment before the start of the experiment. **Results:** triglycerides decreased significantly in all treated groups as compared to hypercholesterolemic control group. ALT decreased significantly in all treated groups as compared to hypercholesterolemic control group. AST decreased significantly in all treated groups as compared to hypercholesterolemic control group. Blood glucose decreased significantly in Metformin and Rosuvastatin treated groups as compared to hypercholesterolemic control group.

Conclusion: the three drugs could be utilized as a treatment option to guard against fat deposition in the liver or progression of steatosis to fibrosis and cirrhosis. However, further studies are required to examine the molecular mechanisms underlying the beneficial effect of the insulin sensitizers in NAFLD patients.

Keywords: Metformin, Pioglitazone, Rosuvastatin, Non-alcoholic, Fatty Liver in Rats.

INTRODUCTION

Non-alcoholic fatty liver disease is defined as a chronic liver condition characterized by hepatic fat accumulation in the absence of other identifiable cause such as alcohol abuse, viral or autoimmune hepatitis, alpha-1 anti-trypsin deficiency, medications like corticosteroids and estrogens, and other conditions[(1)].

Non-alcoholic fatty liver disease encompasses a spectrum of hepatic pathologies, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease [(2)]**.**

Non-alcoholic fatty liver disease is highly associated with several components of metabolic syndrome, particularly obesity, increased plasma lipid levels (primarily triglycerides), glucose intolerance, and type 2 diabetes mellitus (T2DM) with insulin resistance (IR)[(3)]**.**

High-fat diet (HFD) accounts for the largest incidence of obesity in the world. Diet-induced weight loss and life style modifications, including physical exercise and qualitative changes in the diet, have beneficial effects on NAFLD and help manage IR. However, only a small percentage of patients with NAFLD can implement these measures efficiently [(4)]**.**

Despite the abundance of clinical trials, NAFLD therapy remains a challenge for the scientific community, and there are no licensed therapies. Moreover, lifestyle modifications, such as diet and physical exercise, may be proven to be effective, but they are challenging to implement[(5)]**.**

Various studies have shown that statins may have additional effects that improve hepatic insulin sensitization in animal models and humans [(6)]**.** Considering the close relationship between IR and the pathogenesis of fatty liver disease, insulin sensitizers could be regarded as the treatment of choice. Metformin and Pioglitazone are the most popular drugs tested against NAFLD [(7)]**.**

AIM OF THE WORK

The aim of this work is to study the effects of metformin, pioglitazone and rosuvastatin on serum lipids cholesterol and triglycerides (TG), liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), random blood sugar (RBS), oxidative stress malondialdehyde (MDA), and histopathological changes in induced non-alcoholic fatty liver in rats.

This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of Medicine, Al-Azhar University, Cairo, Egypt. The study was approved by the Ethics Board of Al-Azhar University.

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MATERIALS AND METHODS

❖ **Chemicals and animal groups design:**

- Rosuvastatin tablets (20 mg tab. obtained from local market, Egypt).
- Pioglitazone tablets (45 mg tab. obtained from local market, Egypt).
- Metformin tablets (500 mg tab. obtained from local market, Egypt).
- Normal saline (500 ml NaCl 0.9%).

❖ **Kits:**

- Kits for estimation of serum lipids, liver enzymes, random blood sugar, and oxidative stress enzymes (Diamond Diagnostic Company, Egypt).

❖ **Stains:**

- Hematoxylin and eosin stain: It was used for histopathological examination using the electric light microscope and purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA.

❖ **Animal design:**

- Fifty male albino rats weighing 100-120 grams of local strain were used. Animals were purchased from Nile Pharmaceutical Company. They were kept in suitable cages at room temperature with the natural light-dark cycle. They were maintained on a standard diet of commercial rat chow and free tap water. They were kept for 10 days for adaptation to the new environment before the start of the experiment.

➢ **Rats were randomized into two groups:**

Group 1: Consisted of (10) rats, were fed standard diet and received normal saline at a dose of 1ml/ rat by gastric tube daily throughout the study and served as normal control group.

Group 2: Consisted of (40) rats and were fed a high fat diet (HFD) containing 80.5% basal feedstuff, 2% cholesterol, 7% lard, 10% yolk flour and 0.5% bile salt for 8 weeks to induce NAFLD and NASH changes [(8)].

➢ **After about 8 weeks of HFD feeding they were further subdivided into four subgroups (10) rats for each as follow:**

Group 2a: rats were fed HFD and normal saline by gastric tube daily and serving as NAFLD control group.

Group 2b: rats were fed HFD with rosuvastatin suspension, at daily dose of 20mg/kg by gastric tube for 7 weeks [(9)].

Group 2c: rats were fed HFD with pioglitazone suspension, at daily dose of 5mg/kg by gastric tube for 7 weeks [(8)].

Group 2d: rats were fed HFD with metformin suspension, at daily dose of 100mg/kg by gastric tube for 7 weeks [(10)].

- **Oral administration of the preparations:**

Calculated doses of drugs were prepared and preserved in the refrigerator. Drugs were dissolved in distilled water with a concentration of (10 mg/ml) for facilitation of dose calculation and administration. All preparations were administered orallyusing gastric gavage (a smooth stainless steel tube), connected to 3ml syringe. The tube has a wide bore and smooth beveled tip to avoid esophageal perforation. It was introduced into the esophagus during administration to ensure adequate drug delivery and to avoid regurgitation.

- **Obtaining blood samples:**

At the end of the study and on the morning, blood samples (without heparin) were taken from the epicanthus of fasting rats (14hours) by hypodermic needles into centrifugation tubes. The tubes put in the refrigerator for 1 hour for clotting, and then centrifuged at 2000 rpm for 10 minutes. Serum was taken by Pasteur pipette into Ependorph tubes that were kept at -20°C till analysis within one week [(11)]. The serum was collected for estimation of lipid profile and liver enzymes.

- **Preparation Liver homogenate:**

After animals were sacrificed, livers were immediately excised, washed from blood in ice cold saline, blotted dry by filter papers.Small piece of each liver right lobe was fixed in 10% phosphate-buffered formalin for histological examination. About 0.5 gm of each liver was homogenized by ultrasonic homogenizer in 5ml ice-cold phosphate buffered saline (PBS) to obtain ultimately10% (w/v) whole liver homogenate. The homogenate was centrifuged at 15000 rpm for 15 min and the supernatant was stored at - 20°C until used for determination of malondialdhyde (MDA)[(12)].

- **Histopathological examination:**

Preparation:

At the end of the study period, rats were fasted overnight and sacrificed by cervical decapitation and liver were excised immediately from the rats and fixed in 4% buffered isotonicformalin solutionfor 24 hours and embedded in paraffin.Paraffin blocks were then made for the tissue samples and different sections were obtained. Sections of 5-mm thickness were cut and stained with Mayer's hematoxyline and eosin and examined by light microscope. The morphological changes were photo'd using digital camera–aided computer system (Nikon digital camera DXM 1200, Japan)[(13)].

Microscopic study:

All slides were studied by computerized light microscope in Pathology Department, Faculty of Medicine, Al-Azhar University. Liver sections were observed for fatty changes of liver cells, necrosis, and disarrangement of liver cords.

Statistical analysis of results:

The variability of results was expressed as the mean ± standard error (X ± SE). Statistical analysis of the difference between groups was performed by using the one-way analysis of variance (ANOVA) followed by Tukey's test as a post hocanalysis. Charts were done using Excel program, Microsoft Office xp 2007.

Degree of significance:

$P > 0.05$ = insignificant difference.

$P < 0.05$ = significant difference.

Significance:

- **In conditions of comparison between normal and hypercholesterolemic control groups:**

  $P < 0.05$ = significant difference ( ).
  $P < 0.01$ = high significant difference **( ).**
  $P < 0.001$ = very high significant difference ( )

- **In conditions of comparison between hypercholesterolemic control and hypercholesterolemic treated groups:**

  $P < 0.05$ = significant difference **( ).** ▲
  $P < 0.01$ = high significant difference **( ).** ★
  $P < 0.001$ = very high significant difference **( ).** ★★

Values will be expressed as mean ±standard error of the mean (SEM).

RESULTS

***In the normal control group (Group 1)**

- The mean of total serum cholesterol level was 138±10.2 mg/dl.
- The mean of serum TAGs level was 70.75±8.14 mg/dl.
- The mean of serum ALT level was 27±4.6 mg/dl.
- The mean of serum AST level was 18.6±3 mg/dl.
- The mean of blood glucose level was 98.8±7.35 mg/dl.
- The mean of Malondialdehyde (MDA)level was 1.05 ± 0.7 nmol/g.

***In hypercholesterolemic group (Group 2a)**

- The total serum cholesterol level was 206±9 mg/dl, this value showed significant increase compared to control group.
- The serum TAGs level was 97.50±7.14 mg/dl, this value showed significant increase compared to control group.
- The serum ALT level was 90±9.17 mg/dl, this value showed significant increase compared to control group.
- The serum AST level was 64.5±7.23 mg/dl, this value showed significant increase compared to control group.
- Theblood glucoselevel was 148.8±34 mg/dl, this value showed significant increase compared to control group.
- The Malondialdehyde (MDA)level was 13.4±2.20 nmol/g, this value showed significant increase compared to control group.

Table (1): Showing changes in lipid levels in group2a

Group / Parameter	Group 1	Group 2a NAFLD-control group
T. CH Mean± SD	138±10.2	206±26*
T.A.G Mean± SD	70.75±8.14	97.5±16.14*

***:** Significantly different from –ve control group 1

Table (2): Showing changes in liver enzymes levels in group2a

Group / Parameter	Group 1	Group 2a NAFLD-control group
ALT (Mean± SD)	27±4.6	90±14.17 *
AST (Mean± SD)	18.6±3	64.5±7.23 *

*: Significantly different from –ve control group 1 .

Table (3): Showing changes in blood glucose levels in group2a

Group / Parameter	Group 1	Group 2a
Blood glucose	98.8±7.35	148.8±4*

*: Significantly different from –ve control group 1.

Table (4): Showing changes in Melondialdehyde (MDA) level in group2a

Group / Parameter	Group 1	Group 2a NAFLD-control group
MDA Mean± SD	1.05 ± 0.07	13.4±2.20 *

*: Significantly different from –ve control group 1.

***In hypercholesterolemic Rosuvastatin treated group(Group 2b)**

- the mean of total serum cholesterol level was 156.13 ±9.14 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- the mean of serum TAGs level was 77.13 ±9.4 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- the mean of serum ALT level was 33 ±5.33 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- the mean of serum AST level was 30±5.5 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- the mean of blood glucoselevel was 97.88±10.71 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- the mean of Malondialdehyde (MDA)level was 6.4 ±2.5 nmol/g. There is significant decrease compared to hypercholesterolemic group.

Table (5): Showing changes in lipid levels in group2b

Group / Parameter	Group 2b	Group 2a NAFLD-control gro
T.CH(Mean±SD)	156.13 ±9.1	206±26
T.A.G Mean±SD	77.13 ±9.4*	97.5±16.1

*: Significantly different from +ve control group 2a .

Table (6): Showing changes in liver enzymes levels in group 2b

Group / Parameter	Group 2b	Group 2a
ALT Mean±SD	33 ±5.33*	90±14.17
AST Mean±SD	30±5.5*	64.5±7.23

*: Significantly different from +ve control group 2a.

Table (7): Showing changes in blood glucose levels in group 2b

Group / Parameter	Group 1	Group 2a NAFLD-control group
Blood glucos Mean±SD	97.88±10.71*	148.8±34

*: Significantly different from +ve control group 2a.

Table (8): Showing changes in Melondialdehyde (MDA) level in group2b

Group / Parameter	Group 1	Group 2a NAFLD-contr group
MDA Mean±S	6.4 ±1.5*	13.4±2.20

* : Significantly different from +ve control group 2a.

***In hypercholesterolemic Pioglitazone treated group(Group 2c)**

- The mean of total serum cholesterol level was 172 ±11.9 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum TAGs level was 81.38 ±9.85 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum ALT level was 46 ±6.43 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum AST level was 36.8 ±5.9 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of blood glucoselevel was 138.25±24 mg/dl. There is no significant difference compared to hypercholesterolemic group.
- The mean of Malondialdehyde (MDA)level was 9.2 ±1.9 nmol/g.There is significant decrease compared to hypercholesterolemic group.

Table (9): Showing changes in lipid levels in group2c

Group / Parameter	Group 2c	Group 2a NAFLD-contr group
T.CH Mean±SD	172 ±11.9 *	206±26
T.A.G Mean±SD	81.38 ±9.85 *	97.5±16.14

* : Significantly different from +ve control group 2a .

Table (10): Showing changes in liver enzymes levels in group2c

Group / Parameter	Group 2c	Group 2a NAFLD-contr group
ALT Mean±SD	46 ±6.43 *	90±14.17
AST Mean±SD	36.8 ±5.9 *	64.5±7.23

* : Significantly different from +ve control group 2a.

Table (11): Showing changes in blood glucose levels in group2c

Group / Parameter	Group 2c	Group 2a NAFLD-contr group
Blood glucose Mean±SD	138.25±24	148.8±34

* : Significantly different from +ve control group 2a.

Table (12): Showing changes in Malondialdehyde (MDA) level in group2c

Group / Parameter	Group 2c	Group 2a NAFLD-contr group
MDA Mean±S	9.2 ±1.9 *	13.4±2.20

*: Significantly different from +ve control group 2a.

***In hypercholesterolemic Metformin treated group(Group 2d)**

- Serum cholesterol level was 170.38 ±13 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum TAGs level was 78.88 ±2.58 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum ALT level was 41 ±9.38 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of serum AST level was 27 ±4 mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of blood glucoselevel was 106.6 ±9.95mg/dl. There is significant decrease compared to hypercholesterolemic group.
- The mean of Melondialdehyde (MDA)level was 7.2 ±2.1 nmol/g. There is significant decrease compared to hypercholesterolemic group.

Table (13): Changes in lipid levels in group2d

Group / Parameter	Group 2d	Group 2a NAFLD-control group
T.CH Mean±SD	170.38 ±13 *	206±26
T.A.G Mean±SD	78.88 ±2.58 *	97.5±16.14 *

*: Significantly different from +ve control group 2a.

Table (14): Showing changes in liver enzymes levels in group2d

Group / Parameter	Group 2d	Group 2a NAFLD-contr group
ALT Mean±SD	41 ±9.38 *	90±14.17
AST Mean±SD	27 ±4 *	64.5±7.23

*: Significantly different from +ve control group 2a.

Table (15): Changes in blood glucose levels in group2d

Group / Parameter	Group 2d	Group 2a NAFLD-contr group
Blood glucose Mean±SD	106.6 ±9.95 *	148.8±34

*: Significantly different from +ve control group 2a .

Table (16): Changes in Malondialdehyde (MDA) level in group2d

Group / Parameter	Group 2d	Group 2a NAFLD-contr group
MDA Mean±S	7.2 ±1.1 *	13.4±2.20

*: Significantly different from +ve control group 2a.

Histopathology:

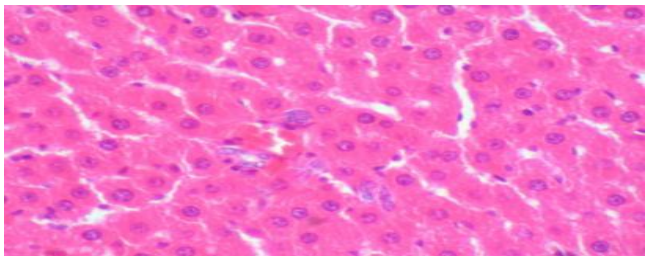

Figure (1): Group 1 –ve control: No fatty changes: Hepatocytes show amphophylic cytoplasm with no fat vacuoles. (Haematoxylin and Eosin stain).

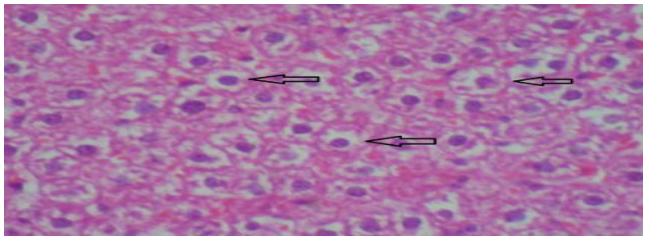

Figure (2): Group 2a +ve control: Severe fatty changes: Hepatocytes showing large amount of cytoplasmic fat vacuoles arranged perinuclear in microvascular pattern with many cells showing large clear cytoplasmic area.

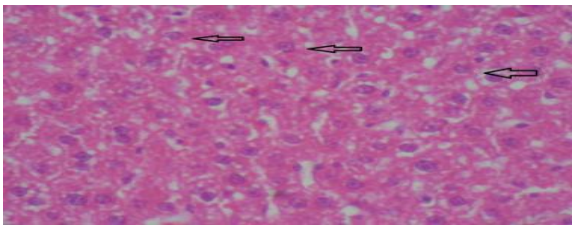

Figure (3): Group 2b Rosuvastatin treated group: Mild to moderate fatty changes. Hepatocytes showing modeate amount of cytoplasmic small fat vacuoles arranged perinuclear in microvesicular pattern with few of them starting to coaese forming large clear areas.

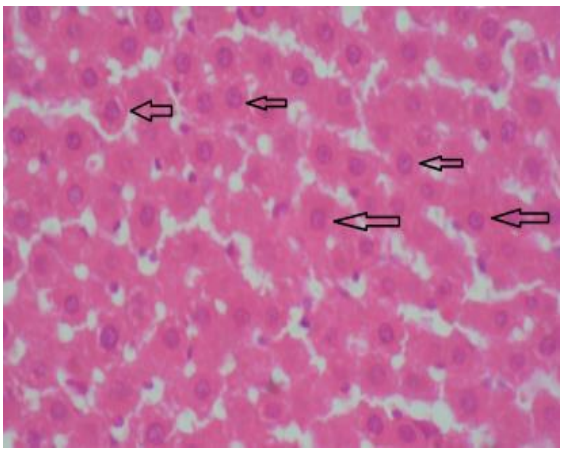

Figure (4): Group 2c Pioglitazone treated group: Mild fatty changes. Hepatocytes showing few peinuclear small fat vacuoles in microvesicular pattern.

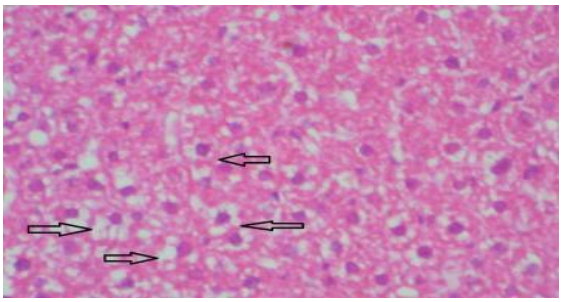

Figure (5): Group 2d Metformin treated group: Moderate fatty changes: Hepatocytes showing large amount of cytoplasmic fat vacuoles arrangedperinuclear in microvascular pattern with many cells showing clear cytoplasmic areas.

DISCUSSION

Due to limitations of human studies on NAFLD/NASH (such as ethical limitations in administering drugs, use of liver biopsy and the long period of development and progression of disease), animal models can give definitive information, not only in clarifying the pathogenesis of NAFLD but also in examining therapeutic effects of numerous agents [(14)].

The present study investigated the therapeutic effect of rosuvastatin, pioglitazone and metformin against high fat diet induced NAFLD.

Statins are commonly used in the treatment of hypercholesterolaemia and coronary artery disease. Several studies have already shown that statins can decrease serum lipid and aminotransferase levels[(15)].

Pioglitazone is an orally administered insulin-sensitizing thiazolidinedione agent developed for the treatment of type two diabetes mellitus. Pioglitazone activates nuclear PPAR-γ, which leads to increased transcription of genes encoding various proteins regulating glucose and lipid metabolisms [16].

Metformin is attributed to biguanide drugs cluster and pioglitazone is attributed to thiazolidinedione drugs cluster. These 2 medications are used widely for diabetic diseases. The conception of using these drugs for NAFLD was triggered during the past decade [17].

Induction of NAFLD was done by a high fat diet (HFD) containing 80.5% basal feedstuff, 2% cholesterol, 7% lard, 10% yolk flour and 0.5% bile salt for 8 weeks to induce NAFLD and NASH changes [8].

By comparing results of the used three drugs:

Effect on total cholesterol level, Rosuvastatin showed significant decrease compared to NAFLD control group and pioglitazone treated group with no significant decrease compared to metformin treated group. Also no significant difference between pioglitazone and metformin treated group, in agreement with **Sahar *et al.*** [18].

Effect on TAGs level, all treated groups showed significant decrease compared to NAFLD control group, with no significant increase compared to normal control group except pioglitazone treated group which showed significant increase compared to normal control group.

Effect on ALT level, all treated groups showed significant decrease compared to NAFLD control group. Rosuvastatin treated group showed no significant increase compared to normal control group while as metformin and pioglitazone treated groups showed significant increase compare to normal control group.

Effect on AST level, all treated groups showed significant decrease compared to NAFLD control group, with significant increase compared to normal control group. Pioglitazone treated groups showed significant increase compare to metformin and rosuvastatin treated groups.

Effect on Blood glucose level, Rosuvastatin and metformin treated groups showed significant decrease compared to NAFLD control group. Pioglitazone treated group showed no significant change compared to normal control group or NAFLD control group.

Effect on Melondialdehyde (MDA) level, all treated groups showed significant decrease compared to NAFLD control group, with significant increase compared to normal control group. Rosuvastatin and metformin treated groups showed significant decrease compared to pioglitazone treated group.

Measurement of triglyceride and cholesterol levels and liver enzymes are the best parameters to evaluate the severity of fatty liver [17].

Various studies have shown that increased serum LDL, cholesterol, and triglycerides and reduced HDL are involved in pathogenesis of many diseases such as fatty liver disease [19].

Various studies have shown that serum cholesterol and triglyceride levels increase in patients with fatty liver, which is associated with prevalence of the disease. The results of another study showed that the risk of fatty liver disease increases in the people with high cholesterol, triglycerides levels [20].

Other studies have shown that mean activity of AST enzyme increases since this enzyme leaks into the blood serum [21].

ALP and AST enzymes leak into blood serum from different tissues in case of muscular and liver damage. Alanine aminotransferase is known as specific marker of liver damage since there is high level of this enzyme in cytoplasm of liver cells, which leaks into the blood serum through cellular membrane in case of liver damage[22].

The results of the present study showed a significant increase in liver enzymes in the groups with HFD compared to control. This indicates liver tissue destruction in the group with HFD. Histological studies confirm this finding. Changes in liver enzyme are associated with liver tissue damage caused by HFD.

Various studies have shown that the most important hypothesis in etiology of fatty liver disease claims oxidative damage, which leads to inflammation and progression of the disease [23].

In normal conditions, aerobic metabolism of the liver produces peroxidants (e.g., reactive oxygen species) at a constant rate, which is balanced with constant production of antioxidants. Peroxidant/antioxidant imbalance for peroxidant substitution (peroxidation) raises the hypothesis of oxidative stress in the liver (these conditions cause pathological changes in the liver).Reactive oxygen species with toxic effects leads to membrane lipid peroxidation [24].

Therefore, fat accumulation leads to membrane lipid peroxidation, oxidative stress, and consequently leakage of liver enzymes in the patients with fatty liver disease. Various studies have shown that reducing these risk factors (blood lipids and liver enzymes) can improve the patients [25].

Rosuvastatin significantly decreases liver mRNA of TNF-α, interleukin-6 (IL-6), and transforming growth factor-β1 (TGF-β1) (leading to hepatic inflammation reduction), hepatic and connective tissue growth factor (leading to liver fibrosis reduction) and serum alanine aminotransferase (ALT) levels in a diet-induced NASH rat model within 12 weeks treatment . Overall, rosuvastatin improved hepatic steatosis, inflammation and fibrosis [26].

Regarding the efficacy of rosuvastatin for the liver diseases, **Antonopoulos *et al.*** [27] reported that treatment with rosuvastatin (10 mg/day) normalized transaminases and lipid profiles in NAFLD patients with hyperlipidemia.

The reduction of plasma FFA by pioglitazone could be explained by that pioglitazone binds to PPAR-γ and stimulates peripheral adipocytes to increase their uptake of free fatty acids, which leads to reduction in the fat stored in muscle, liver and visceral fat deposits. Pioglitazone attenuates liver fibrosis by suppressing the activation of hepatic stellate cells into activated myofibroblasts suggesting an additional direct hepatoprotective effect[28].

Pioglitazone also decreased serum tumor necrosis factor alpha (TNF-α), thereby it could prevent inflammation and necrosis which have been implicated in the pathophysiology of NASH [29].

Metformin has anti-lipolytic effect, reducing plasma FFA concentration[30]. Previous study showed a significant reduction of fasting plasma FFA in nineteen patients with insulin resistance by 850mg (b.i.d) of metformin for six weeks[31].

This can be explained by the effect of metformin through the activation of liver kinase B1, leading to up regulation of adenosine monophosphate-activated protein kinase (AMPK)[32].

Activation of AMPK by metformin, not only inhibits gluconeogenesis, but also inhibits the sterol regulatory element-binding protein-1c (SREBP-1c), which is a transcription factor for genes involved in fatty acid synthesis and is inappropriately increased in liver of NAFLD patients [33].

AMPK also inactivates acetyl-CoA carboxylase and HMG CoA reductase reducing fatty acid and cholesterol synthesis capabilities [34].

CONCLUSION

Metformin, pioglitazone and rosuvastatin exhibited large effects on TG and cholesterol and moderate effects on FBS and MAD. All drugs had large effects on ALT and AST. Besides acting as insulin sensitizers, pioglitazone and metformin exhibited a hepatoprotective effect in nondiabetic NAFLD patients. Both drugs decreased liver fat content, modified lipid metabolism and managed liver transaminases with no evident hypoglycemia. So, they could be utilized as a treatment option to guard against fat deposition in the liver or progression of steatosis to fibrosis and cirrhosis. However, further studies are required to examine the molecular mechanisms underlying the beneficial effect of the insulin sensitizers in NAFLD patients.

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