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Effects of N-Acetylcysteine and Folic Acid on Hepatic Steatosis and Oxidative Stress Caused by Obesity in Rats

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Abstract

Aim: In this study, we investigated the effects of using N-acetylcysteine and/or folic acid on liver fattening in rats with high-fat diet and obesity.

Materials and methods: 35 Wistar albino rats used in the study were divided into four groups. Groups are as follows: FAG (N=8) high-fat diet+folic acid 10 mg/kg, NASG (N=8) high-fat diet+N-acetylcysteine 100 mg/kg, NFG (N=8) high-fat feed +N-acetylcysteine was regulated as 100 mg/kg+folic acid 10 mg/kg, animals fed with PCG (N=10) high-fat feed. Rats were fed high-fat diet for 12 weeks. Folic acid and N-acetylcysteine treatments were performed at the end of the feeding period after they were determined to be obese according to the Lee index. After 4 weeks of application, biochemistry, oxidant-antioxidant, histochemical comet assay and iNOS staining laboratory studies were performed by taking liver tissue and blood under anesthesia.

Results: It was observed that feeding with a high-fat diet caused obesity, fatty liver and oxidative stress. In the treatment of non-alcoholic steatohepatitis, N-acetylcysteine and folic acid were found to cause partial improvement in oxidative stress. The fact that in liver enzymes of total cholesterol averages were significantly high showed that using these two drugs together in NFG could increase for the glycolipid/phospholipid permeability and increase cholesterol levels. It was observed that folic acid protects the liver from oxidative stress by decreasing malondialdehyde values and increasing superoxide dismutase values, and statistically corrected the SOD values of N-acetyl cysteine. It was determined that folic acid repairs DNA fractures caused by oxitative stress and N-acetylcysteine application partially improves in DNA fractures. The most effective result in iNOS immunohistochemical staining showing damage to the fatty liver was found to be effective in the use of combinations in NFG. It was observed that the drug providing improvement in liver histochemical was N-acetylcysteine, although folic acid did not make a statistically significant difference even though it provided some improvement in liver histochemical.

Conclusion: As a result of the findings we obtained, folic acid was thought to be an effective chemical against the formation of damage in the fatty liver. We think that more research should be done about folic acid against liver fatty. We think that the use of N-Acetylcysteine and folic acid can increase cholesterol through the permeability of lipid mechanism due to folic acid.

Keywords: Obesity; Fatty liver injury; N-acetylcysteine; Folic acid; Biochemistry

Abreviations: FAG: Folic Acid Group, NASG: N-Acetylcysteine Group; NFG: Folic acid+N-Acetylcysteine Group; PCG: Positive Control Group; TK: Total Cholesterol; TG: Triglyceride; AST: Aspartate Aminotransferase; ALT: Alanin Aminotransferase; STV: Standard Deviation Values

Introduction

Overweight is perceived as a sign of wealth and health by all societies in the historical process. But obesity is increasingly prevelant as a result of excess energy intake and deterioration of energy balance in sedentary life of today's human being; preventable deaths after smoking are second among developed and developing societies [1].

Undoubtedly, although the most effective way of obesity treatment is seen as diet and sports, obese patients choose the way of slimming by using some chemical or herbal products without any help, without changing their daily life styles and eating habits. Free radical production in the body increases as a result of consumption of these chemical and herbal products. These products interact with biomolecules in cells such as DNA, protein, lipids, carbohydrates and resulting oxidative DNA damage leads to mutagenicity, carcinogenicity and aging. This mechanism of damage consists of the separation and coupling reactions of carbon-centered sugar radicals and free radicals that lead to the formation of OH-(Hydroxide) or H-(Hydrogen) bonded heterocyclic base radicals.

The excessive reaction of these radicals leads to the formation of many damaged products. Since DNA damage is important among the possible results, accurate and precise measurements should be made. The N-acetylcysteine we use in this study is the name given to the N-acetylated derivative of L-cysteine, a natural amino acid. N-acetylcysteine interacts with the electrophilic group of oxidant radicals through its glutathione mechanism directly through its nucleophilic free thiol (-SH) group. The folic acid we use in this study is the active form folate used in the body. Folate deficiency has been shown to cause an increase in liver lipid tissue, but the relationship between obesity and folate and folic acid it has not been fully explained. In this study, we aimed to show the effects of folic acid used in DNA making on antioxidant properties and lipid metabolism and its effects on fatty liver and oxidative stress together with N-acetyl cysteine [2].

Materials and Methods

This work it was carried out in suleyman demirel university experimental animals production and research laboratories. Ethics committee permission was obtained from suleyman demirel university animal experiments local ethics committee with the decision dated 03.12.2015 and numbered 21/10.

Experimental groups and obese making protocol

Lee index: Lee index is an index based on body weight and naso-anal height of animals, which is very similar to body mass index in human medicine and used in animal obesity studies. The formula is shown below visually.

$$\text{Lee's Index} : \frac{\sqrt[3]{W(gr)}}{L(mm)} \times 10^4$$

35 Wistar albino rats purchased Burdur Mehmet Akif Ersoy university laboratory of experimental animals were obtained for obesity and fatty liver (9 ± 11 weeks old, 250 ± 30 g weight). Suleyman Demirel university experimental animals laboratory was taken to care with 22°C ± 0.5°C and 12:12 hours day and night period. The weekly weight measurements of animals were made obese by the Lee index method at the end of 12 weeks. Obese rats were divided into groups by numbering from their tails [3].

Groups

Folic Acid Group (FAG) (N=8): High-fat diet+folic acid 10 mg/kg

N-Acetylcysteine Group (NASG) (N=8): High-fat diet+N-Acetylcysteine 100 mg/kg

N-Acetylcysteine+Folic acid Group (NFG) (N=8): High-fat diet +N-Acetylcysteine 100 mg/kg+folic acid 10 mg/kg

Positive Control Group (PCG) (N=10): It was organized as animals fed high-fat diet

Folic acid 10 mg/kg and N-acetylcysteine 100 mg/kg were administered intraperitoneally in proportion to their body weight. After 28 days (4 weeks) of application, the rats were sacrificed with ketamine and xyzlazine anesthesia. It is reserved for the procedures to be performed by taking the liver and blood.

Oily feed was specially prepared by Arden research experiment laboratory feed/Ankara firm. Feed content was prepared in accordance with Lieber Decarli model. The first 3 days of feeding were mixed with standard feed to get used to eating fat. With the gradual transition, the transition to fatty food was achieved.

Draper and Hadley double heating method was used for MDA measurement of lipid peroxide products. This method consists of evaluating the color formation caused by thiobarbituric acid reaction with MDA by spectrophotometric measurement. Calculated by the absorption coefficient of the MDA-thiobarbituric acid complex and the result is expressed as nanomoles in milligrams of protein. Microprotein levels in supernatants of homogenized samples were measured by bredford method by manual spectrophotometer. The absorbance of the colored solutions formed as a result of the binding of coomassie brilliant blue G-250 dye to proteins was measured at 595 nm. CAT activity was studied according to the Aebi method. The method is based on the principle of measuring the absorbance of H_2O_2 spent during conversion to water and molecular oxygen in the presence of H_2O_2 CAT spectrophotometrically at 240 nm. Results are expressed as k/mg protein.

SOD activity was measured by spectrophotometric method, using the appendix to the olympus AU 2700 (Japan) brand autoanalysis, using the randox brand commercial kit. The principle of the method is the formation of uric acid and the O_2-radical from the xanthine reaction, as a result of the reaction catalyzed by xanthine oxidase, followed by the formation of the red colored formazone compound (2-(4-iodophenyl)-3-(4-nitrophenol)-5 phenyl tetralium. SOD activity is measured by the degree of inhibition of this reaction.

Biochemical studies were done in Isparta private Davraz hospital laboratory. Architect alanine aminotransferase kit was studied with spectrophotometric method. Alanine aminotransferase (architect/aeroset aspartate aminotransferase reagent kit), architect aspartate aminotransferase kit and aspartate aminotransferase (architect/aeroset alanine aminotransferase reagent kit) were taken for study. The architect triglyceride kit takes the optical readings for a certain period of time and incubates at a certain time and the temperature required to incubate and mix the triglyceride (architect triglyceride kit). The architect cholesterol kit with the total cholesterol (architect cholesterol kit) samples and reagents and then calculates the relevant analysis result AST, ALT, TK and TG kits were taught.

Comet assay analysis was done with visual scoring (AU) technique. 100 cells were evaluated per sample (50 cells per slide). A fluorescent microscope for ethidium bromide was scored at 40X at 488 nm wavelength. DNA fractures were evaluated in 5 categories: 0=undisturbed cells, 1=least damaged cell, 2=moderately damaged cell, 3=less damaged cell at maximum, 4=cells with maximum tail length and maximum damaged cells. For this, while counting the cells in each sample (100 cells were counted in total), the cells were scored between 0-4 according to the degree of damage and the number of cells for each score in the sample was recorded. Histochemical staining; changes observed in liver tissue stained with H-E belonging to the groups were evaluated using the binocular microscope according to the scoring made.

For the iNOS immunohistochemical staining tissue reaction, positive cells were determined by counting every 100 cells, including 20 cells in 5 randomly selected areas. The obtained results were statistically evaluated by oneway anova bonferronni test and tabulated [4].

Results

When evaluated in terms of body weight, a significant difference was found in all groups (between VA1 and VA10) compared to weeks ($p<0.05$).

Lee index results

Lee index values of all rats were calculated after the feeding period. All groups were obese according to the Lee index method. The calculation was made with the formula as excelle=$((x^{(1/3)})/y)^*(10^4)$.

Sample; for a rat weighing 356 g;/200*10000=7.0873/200= 0.035436*10000=354,365

When evaluated according to the Lee index, the rats in all groups were diagnosed as obese since they exceed 300. There was no statistically significant difference between the groups. When group averages were evaluated, it was seen that PCG and FAG averages showed high values while the lowest average was in NASG [5].

Biochemical results

At the end of sixteen weeks, the values of ALT, AST, TG, TK were measured in all rat sera in the experiment and statistical evaluation was made (Table 1).

Gruplar	AST Ort ± Stv	ALT Ort ± Stv	TK Ort ± Stv	TG Ort ± Stv
FAG	115.88 ± 32.51	48.48 ± 14.76	55.50[a] ± 20.26	91.37 ± 33.69
NASG	137.40 ± 28.04	54.51 ± 10.38	50.87[b] ± 5.69	63.25 ± 24.50
NFG	162.28 ± 42.83	53.47 ± 9.55	69.87[a] ± 11.03	83.12 ± 17.47
PCG	157.70 ± 7.52	68.76 ± 18.58	51.50[a] ± 8.21	72.00 ± 34.16
p	0.031	0.250	0.025	0.226

Note: [a,b]There is no statistically significant difference between groups bearing the same letter for each feature (p>0.05). Statistical findings were evaluated with Kruskal Wallis and ANOVA test. Comparison between groups was evaluated by performing post-hoc test via SPSS 22.0

Table 1: Comparison of biochemical values of all groups.

At the end of the feeding period, no significant difference was found between the groups for AST values (p>0.05). When the averages of the groups are analyzed, although FAG shows low values, no significant results were found in its comparison with PCG. There was no significant difference for ALT values between the groups at the end of the feeding period (p>0.05). When the average of the groups is analyzed, although FAG shows low values, no significant results were found in comparison with PCG [6].

At the end of the feeding period, a significant difference was found between the groups for TK values (p<0.05). Comparing NFG with PCG and other groups, TK values were found to be significantly higher. There was no significant difference for TG values between the groups at the end of the feeding period (p>0.05). When the averages of the groups are analyzed, although FAG shows high values, it did not give any significant results in comparison with PCG. The comparison results of PCG and other groups are shown in Table 2.

Gruplar	MDA Ort±Stv	SOD Ort±Stv	CAT Ort±Stv
FAG	0.50[a] ±0.11	2.76[a] ± 1.7	1.39 ±0.58
NASG	0.57[b] ±0.59	2.72[a] ± 1.19	3.02 ±2.51
NFG	0.64[b] ±0.10	2.02[a] ± 1.07	2.93 ±3.18
PCG	0.64b-±0.14	0.93[b]- ± 0.39	3.04- ± 1.65
p	0.036	0.020	0.170

Note: [a,b]There is no statistically significant difference between groups bearing the same letter for each feature (p>0.05). Statistical findings were evaluated with Kruskal Wallis and ANOVA test. Comparison between groups was evaluated by performing post-hoc test *via* SPSS 22.0

Table 2: Comparison of antiocsidant values of all groups.

As a result of comparing groups in terms of MDA values at the end of the feeding period, MDA values were found to be significantly lower in FAG compared to PCG and NFG (p<0.05). When Super Oxide Dismutase (SOD) values were examined between the groups at the end of the feeding period, it was observed that NASG and FAG had higher values according to PCG data (p<0.05). NFG values are also statistically significantly higher [7].

There was no significant difference for CAT values between the groups at the end of the feeding period (p>0.05). Although FAG CAT values were found lower than all other groups, they were not statistically significant. Comparisons of PCG and other groups are shown in Table 2.

Comet assay analysis findings

At the end of the feeding period, a significant difference was found for the comet assay values between the groups (p<0.05). The most significant low result compared to PCG is in FAG. Folic acid has been shown to be a strong protective chemical against DNA fracture formation. Pairwise comparison results of PCG and other groups are shown in Figure 1 [8].

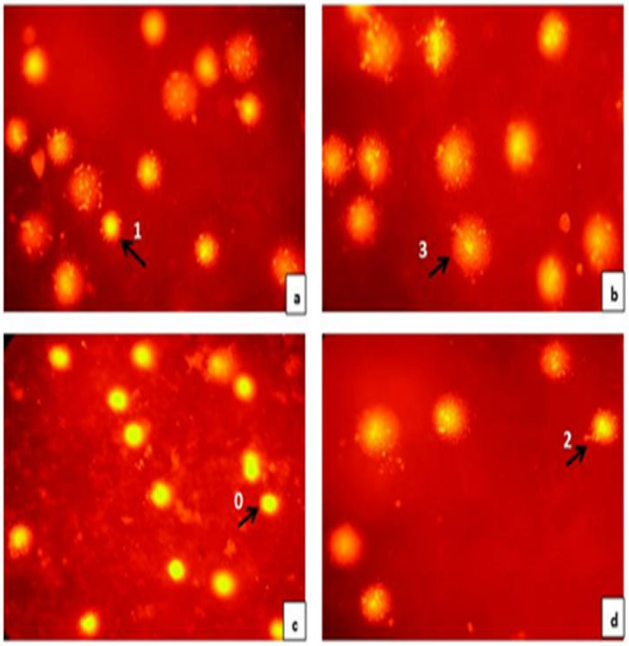

Figure 1: Comparison of comet assay results of all groups. a) Comet assay image of PCG; b) comet assay image of NASG; c) comet assay image of FAG; d) comet assay image of NFG. According to the degree of damage, the cells were classified into five cathogaries: Undamaged (not migrated) and severely damaged (DNA migration).

Histochemical findings

At the end of the feeding period, it was observed that the most damaged group was PCG as a result of histochemical evaluation in liver tissues of all groups. In this group, fatty degeneration in the liver, mononuclear cell infiltrations in the portal area and parenchyma, sinusoidal dilation and vascular congestion were observed quite frequently.

According to the histochemical findings of the liver, it was determined that the group closest to the normal liver was the NFG group. The results of the groups are shown in Figure 2 [9].

(-) score (negative score): No structural changes, (+) score (1 positive score): Mild, (++) score (2 positive scores): Moderate, (+++) score (3 positive score): Refers to a serious structural change. In structural evaluations in sections stained with hematoxylin-Eosin.

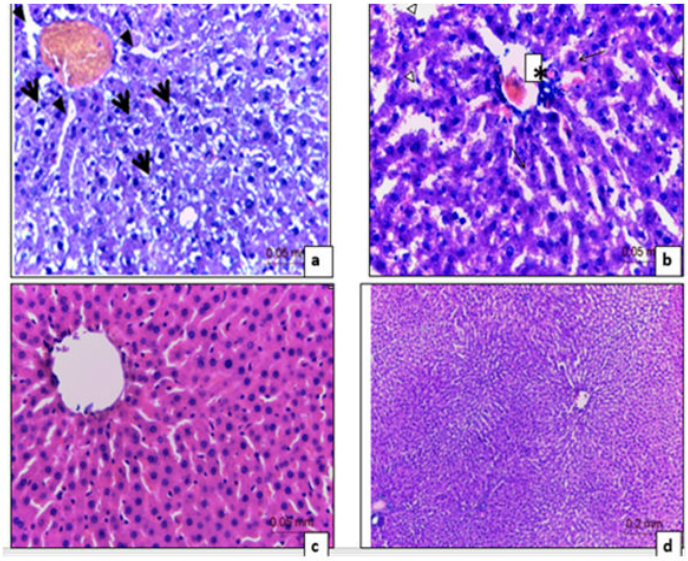

Figure 2: Liver histochemical evaluation images.

According to oily degeneration in hepatocytes, mononuclear cell infiltration in the portal area and parenchyma, sinusoidal dilatation and vascular congestion were evident in PCG (+++) hepatocytes, oily degeneration (arrow), sinusoidal dilatation (arrowhead), vascular congestion (asterisk) were evident. According to PCG, minimal improvement was observed in hepatocytes in fatty degeneration and mononuclear cell infiltration in the portal area and parenchyma (++), but no improvement in sinusoidal dilatation and vascular congestion (+++). Oily degeneration (arrow), mononuclear cell infiltration (thick arrow), sinusoidal dilation (arrowhead) and vascular congestion (asterisk) are observed in hepatocytes. PCG, hepatocytes showed significant improvement in fatty degeneration and mononuclear cell infiltration in the portal area and parenchyma, while minimal improvement in sinusoidal dilation and vascular congestion (++). According to PCG, in NFG; a close-up view to normal liver histology was observed. While fatty degeneration and portal area and mononuclear cell infiltration in the parenchyma were not observed in hepatocytes (-), sinusoidal dilatation and vascular congestion were very rare (+) [10].

Inos results

The most severe iNOS reaction was observed in PCG, in this group, iNOS positive immunoreation was observed in the bile ducts and vascular endothelium, especially hepatocytes. In the FAG, NASG and NFG groups, the iNOS positive reaction was significantly reduced and statistically significant. Statistical analysis of iNOS immunoreaction is shown in Table 3 and Figure 3.

iNOS	Ort. ± Stv	p
FAG	7.00 ± 0.77^{b}	0.000
NASG	7.37 ± 0.49^{b}	0.00
NFG	3.75 ± 0.97^{c}	0.000
PCG	16.37 ± 1.17^{a}	0.05

Note: ^{a,b}There is no statistically significant difference between groups bearing the same letter for each feature ($p>0.05$). Statistical findings were evaluated with Kruskal Wallis and ANOVA test. Comparison between groups was evaluated by performing post-hoc test *via* SPSS 22.0

Table 3: Statistical results of INOS reaction.

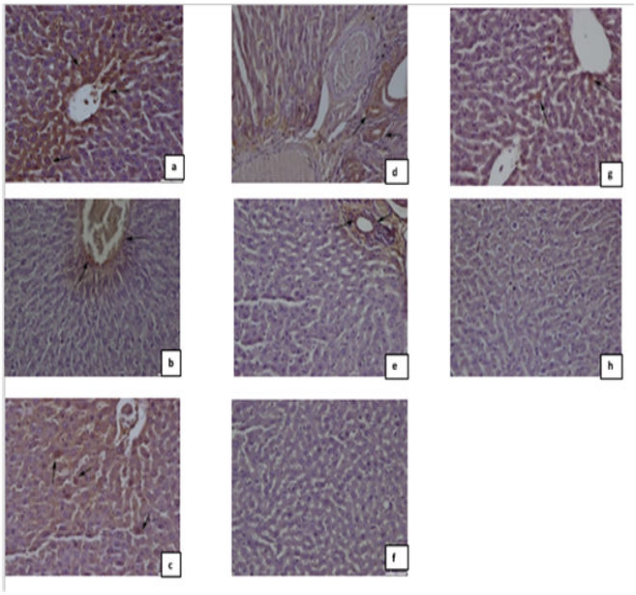

Figure 3: Immunohistochemical staining images. a) Intense iNOS positive immunoreaction in PCG (arrows); b) positive iNOS immunoreaction in the vascular endothelium in the PCG (arrows); c) decreased iNOS immunoreaction in hepatocytes in FAG, few positive cells (arrows); d) mild iNOS positive reaction (arrows) in bile duct epithelium of a rat in FAG; e) mild iNOS positive immunoreaction in the bile ducts of a rat in NASG (arrows); f) NASG negative INOS reaction; g) very mild INOS positive immunoreaction in a small number of hepatocytes in a rat in NFG; h) NFG negative iNOS reaction.

Discussion

Increased excess fatty tissue in the body is shown to increase levels of intracellular fatty acid metabolites such as diacylglycerol and fatty Acyl CoA and plasma free fatty acids. Obesity, which occurs as a result of the increase of lipogenesis and triglyceride accumulation in the cell at the end of this process, is seen as an important health problem in developed and developing societies today. In many studies, insulin resistance of obesity, type 2 diabetes and positive correlation with fatty liver have been revealed. As a result of these triggering systems, obesity is becoming widespread worldwide.

Although N-Acetylcysteine which is known as mucolytic in this study has been investigated many times against steatohepatitis, the results have been reported differently. There is little information in the literature about folic acid. As a result of our study, we found that the application with N-acetylcysteine is not effective on the biochemical values (AST, ALT, TK, TG) in liver fatty, but it is effective on the antioxidant SOD parameter. We thought that less studied folic acid in terms of literature protects the liver from damage by preventing DNA fractures and the formation of oxidant MDA. In this study, we found that the use of these two drugs together could increase the TK and TG values due to the mechanisms related to increasing the phospholipid/lipid permeability of the folic acid in the membranes, even if the liver damage improves antioxidant, iNOS levels and histopathological structure [11].

Studies have shown that rats fed with High-Fat Diet (HFD) (100 mg/kg fat, 20 mg/kg cholesterol) for four to eight weeks, enzyme levels in the liver increase and Non Alcholic Faty Liver Diesase (NAFLD) occurs in histopathologies. Reported that liver rats increased and NAFLD developed in rats fed with 70% fat diet for 3 weeks. In this study, adapting this model, similar results were obtained only after the 12th week. As a result of our study, an increase in PCG liver enzyme levels was consistent with the studies of Lieber et al. As a result of our measurements, body weight and Lee index values in all groups show compatibility with the obesity model. The findings we obtained in this study were observed that obesity increased body weight and fattening associated with fatty liver.

Body weight, Lee index, adipose tissue weight and plasma lipid levels have been reported to be high in rats fed with HFD. In a study, it was reported that in the group using NADPH oxidase inhibitor against adiposity, reactive oxygen derivatives in adipose tissue and irregularity in adipokines decreased, development of diabetes, hyperlipidemia and hepatic steatosis were prevented. In another study, it was reported that body weight, hepatic lubrication, insulin resistance, lipid accumulation, oxidative stress and inflammatory response were high in mice fed with HFD. It has been shown that vitamin deficiencies such as folic acid and B12, which are micronutrients, may also be associated with an increase in adiposity. In this study, the results of Lee index values and obesity findings are consistent with the literature.

Liver enzymes AST, ALT, TK and TG are important criteria in determining liver damage. In folic acid deficiency, gene expression including TG deposition and fatty acid synthesis in the liver has been shown to increase. However, considering that folic acid is correlated with lipid metabolism, folic acid supplementation has been shown to suppress de novo lipogenesis and reduce TG accumulation by coordinating TG hydrolysis and export in the polymer hepatocytes of newborn chickens. It has been reported that folic acid can be considered as a preventive strategy for abdominal fat accumulation in chickens or fatty liver. There are different results in the literature.

In a study by applying folic acid supplements similar to our study in rats, it was reported that lipid metabolism disorder and phospholipid/lipid permeability in membranes increased. Combination treatment of Curcumin (CMN) and Ursodeoxycholic Acid (UDCA) has been shown to be associated with the restoration of serum TG levels rising with HFD.

In this study, TK values of FAG, where we applied folic acid for 4 weeks, were found to be significantly higher than PCG. It is thought that this increase in TK levels in FAG may increase the phospholipid/lipid permeability of folic acid and cause an increase in cholesterol levels. It has been shown that the highest TK values are present in the folic acid group, potentialization may be caused by folic acid, the need to investigate the mechanism of folic acid and the increase of TK levels of folic acid to be supplemented. It is thought that folic acid supplementation may cause TG and TK accumulation as well [12].

Rising oxidative stress is an important marker of adipose tissue increase in obesity associated with metabolic syndrome. Rising MDA values are an important indicator of the damage caused by feeding

with HFD in the liver. MDA levels are significantly higher in rat groups fed with HFD. N-acetylcysteine application has been shown in many studies to increase the level of MDA in HFD nutrition to normal limits. N-acetylcysteine is thought to prevent NASH development by increasing lipid peroxidation. In the study in which combination treatment of Curcumin (CMN) and Ursodeoxycholic Acid (UDCA) was applied, SOD improvement and a decrease in MDA level were reported.

SOD enzyme activity decreases significantly in the livers of animals fed with HFD. In a study examining the effect of myricetin, which is induced by HFD and used as an antioxidant from the flavonoid family to the pre-existing hepatic lubrication, it has been reported that the application alleviates HFD-induced lubrication and increases SOD activities. According to the results we obtained in this study, the lowest SOD values were determined in PCG. Consistent with the studies, it was observed that SOD values were low in HFD feeding. According to PCG animals, it was thought that antioxidant effects might be high due to the fact that N-acetylcysteine and folic acid increase SOD antioxidant enzyme activity against oxidative stress in all of NASG, FAG and NFG. It has been noted that the antioxidant capsule called Qiwei Tiexie, which is used as a medical product in the Tibet, significantly reduces the SOD levels in HFD-induced liver tissue and increased MDA values in NAFLD. In this study, NASG and NFG where N-acetylcysteine application was performed; It showed lower values compared to PCG. However, contrary to many studies, it has been shown that it has no statistically significant effect. The MDA values of FAG applied with folic acid were significantly lower than that of PCG, suggesting that folic acid may have an antioxidant effect on liver tissue [13].

Replication and repair mechanisms also deteriorate under oxidative stress, especially the DNA polymerase enzyme is affected by H_2O_2. A positive correlation has been demonstrated between fatty liver and DNA methylation, and an imbalance in single carbon metabolism has been reported to reduce DNA methylation in the liver cell of a methyl-free diet. Methyl donor foods such as folate, methionine, serine, betaine and choline are evaluated in the S-adenosylmethionine. SAM-SAH cycle in a single carbon metabolism. Imbalance in single carbon metabolism has been shown to be effective in fatty liver development by affecting SAM and SAH at the cellular level. In a study where quercetin used against NASH was evaluated against liver enzymes, oxidative stress and DNA damage, rats were fed rich in methionine and choline and steatohepatitis was formed in their livers. It has been reported that 100% NASH develops and a significant increase in DNA damage occurs in rats fed with methionine and choline diet. In this study, the comet assay scores, which revealed the DNA damage of the group who received folic acid for 4 weeks, were significantly lower and showed the protective effect of folic acid on DNA. The folic acid application group showed a significant improvement in comet assay tests compared to NFG and showed that folic acid is a strong DNA preservative substance.

Nutrition with HFD has been shown to improve fibrosis by disrupting liver function and histopathology. Following the development of non-alcoholic fatty liver (NASH) in rats, N-acetylcysteine treatment at a dose of 20 mg/kg/day for 6 weeks has been reported to improve liver histopathology. In a human study in 83 NASH patients, low levels of folate (B9) and vitamin B12 have been reported to be used as histological markers in monitoring NASH. Qiwei Tiexie capsule (QWTX), which is used as a medical product against lubrication in the tibet, has been shown to weaken hepatitis caused by liver steatosis and fat vacuoles by HE staining and electron micrograph tests in NAFLD. It has been shown to improve overall, partially correcting fatty degeneration in hepatocytes in the liver in NASG, portal area and mononuclear cell infiltration in the parenchyma, synozoidal dilatation and vascular congestion. A statistically significant improvement in liver degeneration, infiltration, dilation and vascular congestion was observed in NFG. The results we obtained are similar to the studies conducted.

NO is a highly used cell signaling molecule that regulates processes in the intracellular mechanism in tissues. Nitric oxide is a well-known pleiotropic agent that affects many aspects of hepatic physiology and pathophysiology and is produced through the activation of inducible and endothelial nitric oxide synthase isoforms. iNOS is an enzyme that increases NO production in tissues. The nitric oxide synthase enzyme has been accepted as a marker of damaged tissue. NoS forms (eNOS, iNOS) catalyze reactions such as providing superoxide source for NADPH.

NO levels have been shown to be decreased in rabbits fed HFD. In a study, it was reported that NO levels decrease in rats fed with high cholesterol diet and NO levels close to the control group when N-acetylcysteine is used together with high cholesterol diet. In another study that looked at an increase in the level of cholesterol-induced NO, it was reported that NAS blocked NO up to a certain dose, but was insufficient as the dose increased [14].

It has been shown that folate may be associated with endogenous NO production in the liver that ischemia is damaged by high cholesterol diet. Cystoin Beta Synthase (CBS)+/-mice supplemented with 0.03 g/L folic acid showed a marked decrease in iNOS and an increase in eNOS protein level. The results have been interpreted that folic acid plays an antagonizing role in homocysteine mechanism and reduces NO bioavailability. In a study, an opinion was reported that folic acid and vitamin B12 prevent the increased expression of TNF-α, iNOS and mRNA content produced by nicotine.

The role of iNOS in the formation of fibrosis in the liver is not clearly understood. It has been reported that iNOS has both beneficial and harmful effects in animal models with NASH-related liver fibrosis. On the other hand, in a clinical study, low dose (0.4 mg/day) folic acid has been shown to have a beneficial effect on blood lipids by decreasing TK and LDL concentrations from liver enzymes and increasing apo AI concentrations. It has been reported that the decrease in eNOS production, which is one of the NOS forms, may show damage earlier than the height of TG, which is used as a marker of damage in the liver. In iNOS knockout mice where iNOS is not available, low or no iNOS has been linked to the elevation of TK values.

In another study in which folic acid supplementation was made, male mice received an HFD supplemented with folic acid (26 mg/kg diet) for 8 weeks. Folic acid supplementation has been shown to reduce hepatic lipid accumulation and fat formation in infected foci caused by HFD and this has been associated with a marked reduction in inflammatory cytokine expression. These results showed that the hepatoprotective effect of folic acid in NAFLD may be due in part to its anti-inflammatory effect. In this study, it was thought that folic acid may have increased the iNOS results by decreasing NO bioavailability with antagonist effect by the homocysteine mechanism. Our histopathological findings are compatible with our iNOS results [15].

Conclusion

In this study, the fact that PCG values showed a higher iNOS positive immunreaction compared to all other groups suggested that feeding on fatty diet may decrease NO values. Application of N-acetylcysteine and folic acid for 4 weeks significantly reduced the iNOS reaction in all groups. It has been shown that our treatment can correct iNOS values. The NFG iNOS results were found to be quite low compared to PCG and were associated with folic acid. It has been thought that the use of folic acid may have an agonist effect in N-acetylcysteine bioavailability, increase cholesterol through the methyl cycle and cause a decrease in iNOS level due to high cholesterol level.

The N-acetylcysteine and folic acid we use in the study we have done, on liver tissue and blood; when we consider biochemical parameters, antioxidant enzymes, histological structure, comet assay and iNOS immunreaction analysis results; we concluded that N-acetylcysteine may improve the liver histochemical structure, antioxidant enzyme activities and iNOS immunoassay analysis. We came to the conclusion that there is no effective chemical on DNA fractures and biochemical parameters AST, ALT, TK and TG levels that we examined with comet assay. When liver tissue and blood samples of folic acid, another chemical substance we use in this study are examined; according to the results of biochemical parameters, antioxidant enzymes, histological structure, comet assay and iNOS immunoassay analysis, we concluded that it may be protective against the liver in antioxidant enzyme activities, iNOS immunreaction and comet assay analysis. In biochemical and histological examinations, we concluded that there is no protective chemical substance.

In this study, when we examined the effects of using N-acetylcysteine and folic acid on blood biochemistry, comet assay, antioxidant enzyme, liver histology and iNOS immunreaction results; we thought that the use of two chemicals together could increase TK values from biochemical values with antagonist effect and protect the liver from damage by agonist effect according to histochemical and iNOS immunochemical staining analysis. It has been deemed necessary to investigate the mechanism of folic acid in detail that folic acid supplement may have negative results due to permeability mechanisms on total cholesterol biochemistry. It has been deemed necessary to continue to investigate the mechanisms in which folic acid supplements taken together with N-acetylcysteine can be used as effective drugs, considering liver histochemistry and iNOS reactions.

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