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Evaluation of the Protective Efficiency of Ethyl Gallate Against Non-Alcoholic Fatty Liver Disease Induced by a High-Fat Diet in Mice

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ABSTRACT

A significant portion of the population is affected by nonalcoholic fatty liver disease (NAFLD). Unfortunately, there are no particular pharmacological treatments that have been approved for it. This study aimed to investigate the protective effect of ethyl gallate against NAFLD induced by a high-fat diet in mice. By using a high-fat diet for 16 weeks, the NAFLD model was created in mice. Two oral doses of ethyl gallate (20 mg/kg and 40 mg/kg) were given. The liver injury was assessed by the liver index, along with serum evaluations of liver functions and lipid profiles. A histopathology study was also performed. Triglyceride (TG) and sterol regulatory element-binding protein 1C (SREBP-1C) levels in the liver were also measured, and the liver's oxidative stress and inflammation were assessed. The results demonstrated that ethyl gallate could mitigate the liver injury, particularly when it was administered at the high dose. It resulted in a reduction in both the liver index and NAFLD activity score. In addition, it improved significantly the liver function and reduced serum TG levels. Furthermore, by decreasing the liver levels of tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB), ethyl gallate showed an anti-inflammatory effect, and it also could reduce oxidative stress. Moreover, the high dose of ethyl gallate reduced the hepatic TG and SREBP-1C levels. In conclusion, by controlling lipid metabolism, lowering oxidative stress, and suppressing the NF-κB signaling pathway, ethyl gallate provides protection against NAFLD.

Keywords: High fat diet**,** Ethyl gallate, Lipid metabolism, Inflammation, Oxidative stress.

Introduction

Nonalcoholic fatty liver disease (NAFLD) poses an escalating challenge to worldwide public health.[1] Over the past ten years, the prevalence of NAFLD has increased by more than thirty percent globally.[2] Its main feature is the liver's high triglyceride buildup (more than 5%) without the presence of overconsumption of alcohol or other liver disease causes.[1] A portion of individuals with NAFLD may experience a progression to more serious liver conditions, characterized by hepatocyte injury (often described as ballooning), inflammation, and ultimately fibrosis, a condition known as non-alcoholic steatohepatitis (NASH).[3] NAFLD is acknowledged as the hepatic indication of metabolic syndrome and is frequently correlated to various metabolic risk factors, involving obesity, dyslipidemia, hypertension, and diabetes.[4] Traditionally, there are "two-hit hypothesis explain how NAFLD begins and develops to NASH. Nevertheless, current findings indicated other complicated mechanisms, known as the "multiple parallel hits hypothesis" theory. It includes various factors working together. It emphasizes that insulin resistance, along with endoplasmic reticulum stress, chronic low-grade inflammation, genetic reasons, issues with adipose tissue, microbiome, and mitochondrial dysfunction, all act as concurrent contributors to both the beginning and advancement of NAFLD.[5]

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NAFLD diagnosis relies on radiological or histopathological evidence that indicates the existence of fatty alterations in the liver.[6] Although several efforts have been made, an authorized medication for NAFLD has still not been developed. Currently, pharmacological treatments have demonstrated only limited efficacy and may come with many side effects.[7] Recent decades have seen a rise in study on the use of herbal medications to treat NAFLD due to their extensive availability, low risk of adverse effects, and indications of possible therapeutic advantages.[8] One of the phenolic compounds, ethyl gallate (EG), exists in several plants,[9] such as red wine, walnuts, Amla fruit pulp, and mango panicles, as well as logwood leaves and the gum arabic tree.[10] EG was shown to be non-toxic and safe.[11] EG serves as a food additive and has demonstrated antimicrobial properties, along with the capacity to neutralize free radicals. In addition, it has anti-diabetic, anti-obesity, and anti-inflammatory effects, along with potential cancer prevention benefits.[10,12] However, although phenolic compounds have enormous potential for use in various industries, they also face certain difficulties that necessitate the development of strategies aimed at enhancing bioavailability, creating sustainable extraction and refinement techniques, and implementing stability procedures to expand their range of applications.[13] Due to the ascending prevalence of NAFLD and the lack of approved therapies, this research was conducted to assess the protective benefits of ethyl gallate against NAFLD.

Materials and Methods

Chemicals and drugs

'Ethyl gallate was obtained from TCI - Tokyo Chemical Industry (Tokyo, Japan), Silymarin was obtained from Profoods Nutrition Pvt. Ltd (Kalher Bhiwandi, India. Carboxymethylcellulose sodium salt (CMC sodium), 2-Thiobarbituric acid (TBA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), Glutathione reduced (GSH), Tris hydrochloride (Tris HCL) and Sulfuric acid were obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Hydrogen peroxide and Trichloroacetic acid were obtained from Avra Synthesis Private Limited (Hyderabad, India). Pyrogallol and Triton X-100 were purchased from Ibuychemikals (Chennai, India). Potassium

Permanganate was purchased from Agrawal Drugs Pvt. Limited (Haridwar, India).'

Animals
Male C57/BL6 mice were bought from Vayas Labs in Hyderabad, India. The mice were grouped in cages of three and kept in a regulated setting at a temperature maintained at 23 ± 3 °C with no restriction to both food and tap water. This research received approval from 'the Institutional Animal Ethics Committee (IAEC) of Andhra University in India' (Approval no. 516/01/A/CPCSEA).

NAFLD induction
NAFLD was induced using "a high-fat diet, where 60% of the energy came from fats" (VRKHFD, Batch NO. 049/24-25). This specific high-fat diet was obtained from VRK Nutritional Solutions in Pune, India, and is comprised of starch, casein, and lard, along with minerals and vitamins. For a duration of 16 weeks, mice were fed this diet to create the NAFLD model.

Experimental design
Thirty C57/BL6 mice were housed for one week in the animal house to acclimatize to the housing conditions before they were separated into four groups, and in each group, there were 6 mice:
Group 1: Mice were provided with a standard diet for the duration of the experiment, and this group represents 'the normal control group'.
Group 2: The high-fat diet was given to mice for 16 weeks to create the NAFLD model.
Group 3: Silymarin was suspended in 1 % carboxymethylcellulose (CMC) and administered to the mice in this group at a dose (100 mg/kg, orally) daily,[14] while they were also provided with the high-fat diet for 16 weeks. This group served as 'the reference drug group'.
Group 4: Ethyl gallate was dissolved in 1 % CMC and administered to the mice in this group at a low dose (20 mg/kg, orally) daily,[15] while they were also provided with the high-fat diet for 16 weeks.
Group 5: Ethyl gallate was dissolved in 1% CMC and administered to the mice in this group at a high dose (40 mg/kg, orally) daily, while they were also provided with the high-fat diet for a period of 16 weeks.
The mice in both group 1 and group 2 were also given the drug vehicle (1% CMC) orally throughout the experiment.
Food intake was calculated daily, and body weight was documented weekly for a duration of 16 weeks. After 16 weeks, all the mice in all the groups were fasted overnight and then were humanely euthanized by decapitation performed under deep general anesthesia. Blood was drawn from the jugular vein, and the animals' body weights were recorded before they were sacrificed. The dissection was done immediately, and the liver was taken out, and the liver index was estimated. After that, the liver morphology was examined, and then a portion of it was taken and fixed in formalin (10%) for histopathology study. The liver's residual fraction was kept for further biochemical testing at -20 °C.

Biochemical tests in serum
An automated biochemistry analyzer and particular assay kits were used to determine the serum levels of "alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)".

Pathological evaluation
For pathological evaluation, the liver tissues that had been fixed in formalin were embedded in paraffin. Following this, hematoxylin and eosin (HE) staining was applied to slices that were 5 micrometers thick. A pathologist, who was fully blinded to the samples, assigned the NAFLD activity score, which takes into account:[16] 'Steatosis (0–3), Inflammation (0–3), Ballooning degeneration (0–2).'

Hepatic TG concentration determination
The liver was homogenized at room temperature with a chloroform/methanol solution in a 2:1 ratio. Following the addition of sulfuric acid, the mixture underwent centrifugation for 20 minutes at a speed of 2000 rpm. The resultant lower phase, which contains triglycerides (TG) and phospholipids, was moved to a new test tube and combined with a solution of Triton X-100 (1%) in chloroform. After that, it evaporated. The dried samples were reconstituted with water and analyzed for TG levels using a specific TG kit (TRUE chemie, Chennai, India).[17]

Hepatic lipid metabolism status determination
Using a specific mouse ELISA kit that was bought from Krishgen Biosystems (Mumbai, India), SREBP-1C levels in the liver were assessed.

Hepatic oxidative stress status determination
To assess the hepatic oxidative stress status, firstly the liver tissue was homogenized with a cold phosphate-buffered saline solution using a homogenizer to form a homogenate (10 w/v). A section of this mixture was employed to assess malondialdehyde (MDA) concentrations. The remaining homogenate was centrifuged. The supernatant was carefully taken and utilized to evaluate 'catalase (CAT) activity, superoxide dismutase (SOD) activity, and the levels of reduced glutathione (GSH)'.

MDA levels determination
The thiobarbituric acid test is a widely used test for assessing lipid peroxidation by identifying malondialdehyde, a significant aldehyde produced during this process. The assay is based on quantifying the concentration of a pink product formed from the reaction between TBA and MDA. This is accomplished using a spectrophotometer at 532 nm.[18] In brief, liver homogenate was mixed with the TBA reagent, boiled, cooled, and centrifugated. At 532 nm, the resultant supernatant's absorbance was recorded.[19]

GSH levels determination
This assay relies on the interaction between GSH and Ellman's reagent, leading to the creation of the TNB chromophore.[20] The sample cuvette includes the supernatant combined with Ellman's reagent solution in sodium phosphate buffer. A spectrophotometer was used to detect the absorbance at 412 nm.[21] The GSH levels in an unknown sample were determined using the regression curve or linear equation obtained from standard GSH solutions.[20]

SOD activity determination
The assay employs the capacity of SOD to prevent the autoxidation of pyrogallol. Initially, a specific volume of pyrogallol solution was thoroughly combined with a Tris-HCl buffer that contains EDTA disodium salt to remove any metal ions that could promote the reaction. Every 30 seconds, the absorbance at 325 nm was measured for 5 minutes, both in the absence of the sample and in its presence.[22]

CAT activity determination
In vitro assessment of catalase activity was performed following the steps:[23] Initially, the supernatant was placed in test tubes. Then, hydrogen peroxide was added, and after a duration of 3 minutes, sulfuric acid was added to end the reaction. After that, potassium permanganate was added. Finally, a spectrophotometer was used to detect the absorbance at 480 nm. Hydrogen peroxide was added.

Hepatic pro-inflammatory status determination
Specific mouse ELISA kits from Krishgen Biosystems (Mumbai, India) were used to assess the amounts of NF-κB and TNF-α in the liver.

Statistical analysis
The data were shown as 'mean ± standard error of the mean (SEM)'. A one-way ANOVA was conducted to examine the data with the help of GraphPad Prism 9. For parametric data, Tukey's multiple range test was utilized. In instances of non-parametric data, the Kruskal-Wallis test was employed, followed by Dunnett's test. Statistical significance was defined as a *p*-value of under 0.05.

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Results and Discussion

NAFLD is characterized by irregular lipid processing within the liver, and it is the most prevalent liver disease globally. NAFLD is strongly correlated to cardiovascular diseases, which are a leading factor in fatalities related to NAFLD. The lack of effective drugs for the management NAFLD is an obstacle in its therapy.[24] Thus, the current study has been conducted to find a new treatment for NAFLD. In this study, ethyl gallate efficacy has been investigated in the protection against NAFLD.

This study revealed that providing C57/BL6 mice with a diet consisting of 60 kcal % fat for a duration of 16 weeks effectively created a model of NAFLD.

Food consumption was much lower in the high-fat diet group than in the normal control group ($p < 0.0001$). Compared to the NAFLD model group, the groups treated with ethyl gallate or silymarin did not show a prominent difference in food intake ($p > 0.05$). However, the dietary intake of these groups differed significantly from that of the normal control group ($p < 0.0001$) (Figure 1A). The group of NAFLD model revealed a notable rise in body weight compared to the normal control ($p < 0.05$). However, the groups that received ethyl gallate or silymarin revealed a considerable decrease in body weight compared to the NAFLD group ($p < 0.05$) (Figure 1B). It has been demonstrated in a previous study that ethyl gallate suppressed adipogenesis in 3T3-L1 cells by lowering the expression levels of early adipogenic markers, such as C/EBPα and PPARγ.[25]

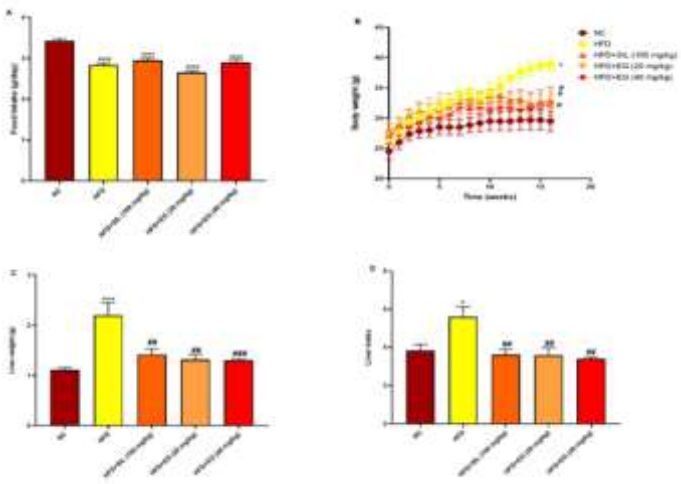

Figure 1: Effects of ethyl gallate on ‘‘(A) food intake, (B) body weight, (C) liver weight, and (D) liver index. “Results are shown as the mean±SEM (n = 6). $^{*}p < 0.05$, $^{****}p < 0.0001$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ when compared to the HFD group. NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg); HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg).’’

Mice fed a high-fat diet showed considerably greater liver weight and liver index than the normal control group ($p < 0.0001$, $p < 0.05$, respectively). Administration of silymarin or ethyl gallate caused a notable reduction in both liver weight ($p < 0.01$ for silymarin and the low dose of ethyl gallate, $p < 0.001$ for the high dose of ethyl gallate) and liver index ($p < 0.01$) when compared to the NAFLD model group (Figure 1C) (Figure 1D). Serum levels of ‘ALT, AST, ALP, and TG’ were considerably raised in the NAFLD model group than in the normal control group ($p < 0.001$, $p < 0.001$, $p < 0.05$, $p < 0.01$, respectively). In contrast, there was no considerable elevation in serum levels of ‘TC, LDL-C, HDL-C’, and bilirubin when compared to the normal control group ($p > 0.05$) (Table 1). Compared to the NAFLD model group, low-dose administration of ethyl gallate showed a considerable reduction in serum ‘ALT and AST’ levels ($p < 0.05$) , but there was no marked decrease in serum ALP and TG levels ($p > 0.05$). Additionally, administration of silymarin caused only a notable decrease in serum ‘ALT, AST, and TG’ levels compared to the NAFLD model group ($p < 0.05$). However, the group that received the high dose of ethyl gallate exhibited a further decrease in serum ‘ALT, AST, and TG’ as well as a notable decrease in serum ALP levels in comparison to the NAFLD model group ($p < 0.01$) (Table 1). These findings are consistent with a previous study which showed that ethyl gallate prevented mice with acute acetaminophen-induced liver injury from experiencing an increase in serum AST and ALT.[15]

The normal control group exhibited no morphological or histological alterations in the liver specimens. On the other hand, the liver specimens were taken from mice in the group of NAFLD model demonstrated many morphological and histological alterations. The morphological changes included color changes and enlargement in the liver (Figure 2), and the histological changes included moderate steatosis and a rise in inflammatory cell infiltration in the liver (Figure 3), and the NAFLD activity score was notably increased compared to the normal control ($p < 0.001$) (Figure 4). Conversely, administering a high dose of ethyl gallate (40 mg/kg) or silymarin revealed a reduction in these morphological and histological changes (Figure 2) (Figure 3), and the NAFLD score in the group treated with silymarin was remarkably lower compared to the NAFLD model group ($p < 0.05$). Moreover, the high dose of ethyl gallate caused a further reduction in the NAFLD activity score ($p < 0.01$) (Figure 4). However, the lower dose of ethyl gallate revealed some reduction in the liver inflammation (Figure 2) (Figure 3), but the NAFLD activity score was not significantly decreased when compared to the NAFLD model group ($p > 0.05$) (Figure 4).

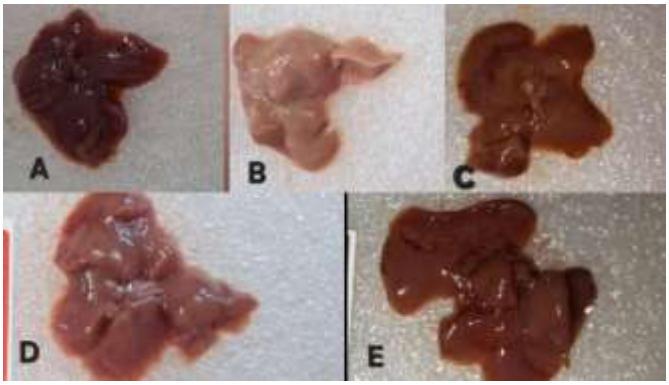

Figure 2: Effects of ethyl gallate on the morphology of the liver. ‘‘(A) Normal control group, (B) High-fat diet group, (C) High-fat diet with silymarin (100 mg/kg), (D) High-fat diet with ethyl gallate (20 mg/kg), (E) High-fat diet with ethyl gallate (40 mg/kg)’’.

In several studies, it has been revealed that lipids are crucial in the advancement of NAFLD. Disruptions in production, export, and absorption of lipids lead to the buildup of triglycerides (TG) and other neutral lipids within liver cells, which is a defining characteristic of NAFLD. Larger lipid droplets produced by hepatocytes accumulate TG, which can compress surrounding organelles and cause damage and inflammation in the liver cells.[26] SREBPs are transcription factors that manage the process of lipogenesis, and changes in this process have been linked to the onset of NAFLD.[27] SREBP-1c plays a vital role in preserving lipid balance in the liver.[28] Activation of SREBP-1c boosts the expression of genes that promote lipogenesis, leading to increased fatty acids production and increasing the risk of hypertriglyceridemia.[29] Substantial evidence suggests that a diet rich in fats triggers SREBP-1c, which significantly contributes to the onset and advancement of NAFLD. As a result, inhibiting SREBP-1c is considered a promising approach for preventing and treating NAFLD.[30]

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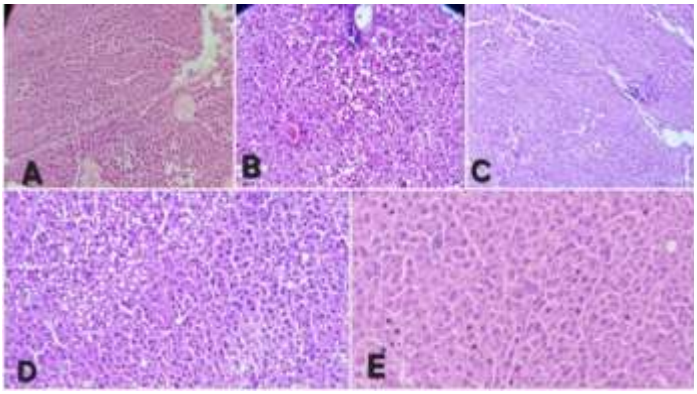

Figure 3: Effects of ethyl gallate on H&E staining of the liver. "(A) Normal control group, (B) High-fat diet group, (C) High-fat diet with silymarin (100 mg/kg), (D) High-fat diet with ethyl gallate (20 mg/kg), (E) High-fat diet with ethyl gallate (40 mg/kg)". (Magnification, 10 ×)

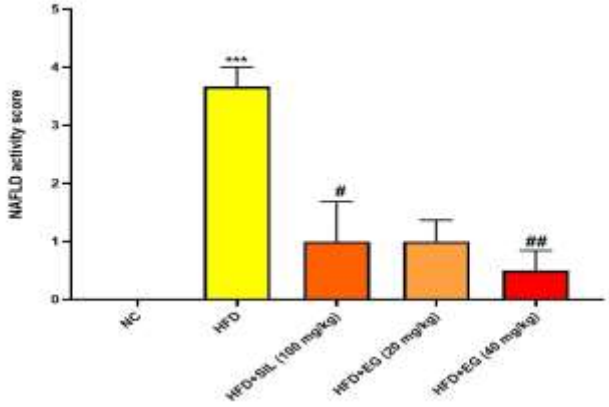

Figure 4: Effects of ethyl gallate on NAFLD activity score. "Results are shown as the mean±SEM (n = 6). $^{***}p < 0.001$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. NC: normal control; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg); HFD: high-fat diet; HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg)".

In this research, The NAFLD model group exhibited a greater buildup of lipids in the liver, as indicated by significantly elevated hepatic triglyceride (TG) levels, which were considerably elevated than those in the normal control group ($p < 0.01$). Nevertheless, when treated with silymarin, the liver's lipid accumulation was significantly reduced compared to the NAFLD group ($p < 0.05$). Furthermore, the group treated with the high dose of ethyl gallate demonstrated a further reduction in the liver's lipids accumulation ($p < 0.01$), suggesting that ethyl gallate has a beneficial effect on lipid metabolism. On the other hand, the low dose of ethyl gallate did not result in any considerable reduction in the hepatic TG levels when compared to the NAFLD group (Figure 5). Additionally, the results of hepatic SREBP-1C levels in the NAFLD group showed an increase in these levels, indicating an increase in fatty acid synthesis in the liver. This increase was considerable in comparison to the normal control ($p < 0.0001$). However, compared to the NAFLD model group, silymarin administration demonstrated a reduction in the levels of SREBP-1C ($p < 0.05$), and administration of ethyl gallate at the high dose caused a further reduction in the levels of SREBP-1C ($p < 0.01$). In contrast, when compared to the NAFLD model group, the lower dose of ethyl gallate had no discernible impact on the hepatic SREBP-1C levels ($p > 0.05$) (Figure 6).

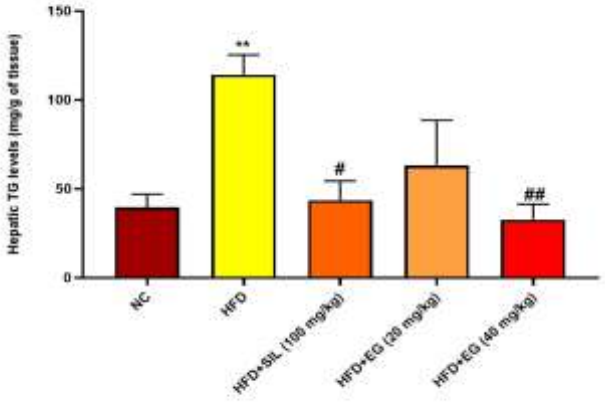

Figure 5: Effects of ethyl gallate on the levels of TG in the liver. "Results are shown as the mean±SEM (n = 6). $^{**}p < 0.01$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg); HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg)".

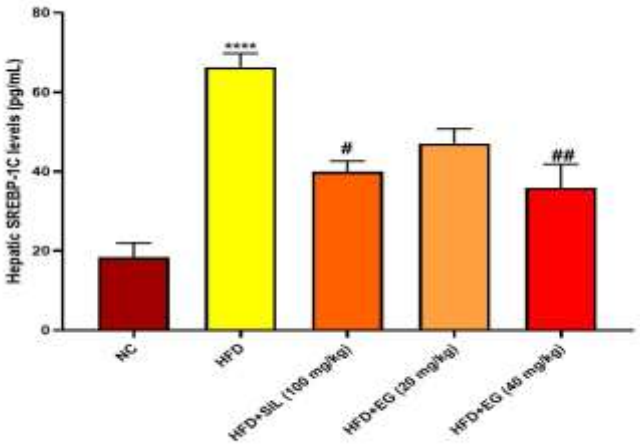

Figure 6: Effects of ethyl gallate on the levels of SREBP-1C in the liver. "Results are shown as the mean ± SEM (n = 6). $^{****}p < 0.001$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg); HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg)".

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Table 1: Effects of ethyl gallate on the serum biochemical parameters

Parameter	Groups				
	NC	**HFD**	**HFD + SIL (100 mg/kg)**	**HFD + EG (20 mg/kg)**	**HFD + EG (40 mg/kg)**
ALT (IU/L)	24.00±6.03	102.30±28.33***	48.50±3.45$^{\#}$	48.17±5.76$^{\#}$	39.33±2.35$^{\#\#}$
AST (IU/L)	130.50±27.67	317.70±21.42***	173.70± 23.00$^{\#}$	173.50±33.76$^{\#}$	140.80±19.03$^{\#\#}$
ALP (U/L)	55.67±9.49	97.33 ± 13.59*	65.00±10.01	61.83 ± 4.18	51.83±7.99$^{\#\#}$
Bilirubin (mg/dL)	0.14±0.02	0.18±0.05	0.11±0.01	0.12±0.004	0.11±0.01
TG (mg/dL)	76.83±2.18	113.80±6.35**	82.67±5.18$^{\#}$	93.67±11.04	76.83±2.40$^{\#\#}$
TC (mg/dL)	95.50±4.82	114.30±6.89	109.30±4.84	106.00±7.51	101.30±6.31
LDL-C (mg/dL)	47.73±4.29	68.90±8.52	62.23±4.11	60.60±4.63	56.50±4.93
HDL-C (mg/dL)	28.17±2.15	28.08±0.37	28.50±0.76	29.92±0.71	30.50±0.56

"Results are shown as the mean±SEM (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg); HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg); ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol."

It is thought that oxidative stress significantly contributes to liver damage in NAFLD, helping the condition progress from basic steatosis to NASH. Research indicates that mitochondrial dysfunction during NAFLD can disrupt the balance of lipids in liver and increase the production of reactive oxygen species.[31] In this study, the NAFLD model group exhibited an imbalance in the relationship between oxidants and antioxidants within the liver tissue. The levels of MDA significantly increased, while GSH levels significantly decreased compared to the normal group ($p < 0.001$, $p < 0.01$, respectively). Moreover, CAT and SOD activity was lower than in the normal control group ($p < 0.05$). However, administration of silymarin caused a notable decrease in the hepatic MDA levels and a notable increase in the hepatic GSH levels and SOD activity compared to the NAFLD model group ($p < 0.05$, $p < 0.01$, $p < 0.05$, respectively). Moreover, oxidative stress in the liver of groups treated with ethyl gallate, especially at the high dose, was observed to be lower than those in the NAFLD model group. The hepatic MDA levels significantly decreased with the high dose of ethyl gallate ($p < 0.05$), although GSH levels did not significantly rise compared to the NAFLD model group ($p > 0.05$). Additionally, this high dose of ethyl gallate caused a notable increase in the activities of SOD and CAT compared to the NAFLD model group ($p < 0.05$). However, the low dose of ethyl gallate did not lead to a noteworthy decrease in the hepatic MDA levels, nor did it show a significant increase in GSH levels when compared to the NAFLD model group ($p > 0.05$). In addition, it did not result in a meaningful decrease in CAT activity compared to the NAFLD model group ($p > 0.05$). Nonetheless, compared to the NAFLD model group, a rise in SOD activity was noted ($p < 0.05$) (Table 2). Numerous earlier investigations have demonstrated ethyl gallate's antioxidant potential. It has been shown in a previous study that ethyl gallate, which is extracted from the leaves of *Acacia nilotica (L.)*, is one of the effective antioxidants evaluated using the DPPH assay.[32] It also has been indicated in another previous study that the protective role of ethyl gallate in the H_2O_2-induced oxidative stress-damaged PC12 cell model is correlated with the activation of the Nrf2 signaling pathway.[33]

Table 2: Effects of ethyl gallate on the oxidative stress status in the liver

Parameter	Groups				
	NC	**HFD**	**HFD + SIL (100 mg/kg)**	**HFD + EG (20mg/kg)**	**HFD + EG (40 mg/kg)**
MDA (µmol/100g tissue)	2.23±0.43	5.12±0.36***	3.02±0.40$^{\#}$	4.01±0.43	2.98±0.59$^{\#}$
GSH (µmol/g tissue)	0.51±0.04	0.32±0.02**	0.52±0.03$^{\#\#}$	0.46±0.01	0.40±0.02
SOD (U/mL)	1.43±0.05	1.04±0.06*	1.41± 0.10$^{\#}$	1.45±0.09$^{\#}$	1.44±0.10$^{\#}$
Catalase (U/mL*10^{-2})	46.23±0.51	33.60±1.78*	37.43±2.97	40.67±1.39	45.28±0.77$^{\#}$

"Results are shown as the mean±SEM (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. 'NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg), HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg); MDA: malondialdehyde; GSH: reduced glutathione; SOD: Superoxide

Furthermore, it has been found in another previous study that the positive impacts of ethyl gallate in acetaminophen-induced acute liver injury can be linked to its antioxidant abilities, capacity to neutralize free radicals, and anti-inflammatory characteristics.[15] Moreover, it has been highlighted in another previous study the antioxidant properties of ethyl gallate. This study demonstrated that administration of ethyl gallate showed a reduction in lipid peroxidation (LPO) levels, an increase in superoxide dismutase (SOD) levels, a decrease in nitric oxide (NO) levels, and a restoration of glutathione S-transferase (GST) levels in the model of ulcerative colitis induced by dextran sulfate sodium (DSS) in mice.[10] Additionally, it has been indicated in another previous study the significant rise in both enzymatic and non-enzymatic antioxidant levels, along with a reduction in lipid peroxidation, a marker of oxidative stress, suggests that 1% ethyl gallate effectively protects skin tissues from oxidative damage due to its granular antioxidant properties.[34]

The inflammatory response is a crucial factor in the development of NAFLD. Previous research has demonstrated that reducing inflammation enhances insulin sensitivity and lowers the index of the liver.[35] The transcription factor "NF-κB" is essential for regulating inflammation and is associated with the advancement of NAFLD.[36] The phosphorylation-dependent activation of the IKKs (IκB kinases) complex is an essential step in triggering the classical NF-κB pathway.[37] Ultimately, p-NF-κB initiates the release of pro-inflammatory cytokines, resulting in an inflammatory reaction.[35] Cytokines that promote inflammation, like IL-1 and TNF-α are pivotal at various stages of liver diseases, affecting key processes including the production of acute phase proteins, lipid metabolism, cholestasis, and the extent of fibrosis.[38] In this study, alongside the pathology examination for assessing inflammation in the liver, the hepatic levels of NF-κB and TNF-α were measured as well. These findings revealed that mice on only a high-fat diet for 16 weeks led to a notable elevation of these inflammatory markers compared to the normal control group ($p < 0.01$). Conversely, the group treated with silymarin revealed a notable decrease in these inflammatory parameters compared to the NAFLD model group ($p < 0.05$). However, the groups treated with ethyl gallate revealed a further effect on the liver's inflammatory condition decrease. The high dose of ethyl gallate led to a notable decrease in these inflammatory parameters compared to the NAFLD model group ($p < 0.01$). Likewise, the low dose of ethyl gallate also revealed a notable decrease in these inflammatory parameters compared to the NAFLD group ($p < 0.01$) (Figure 7A) (Figure 7B).

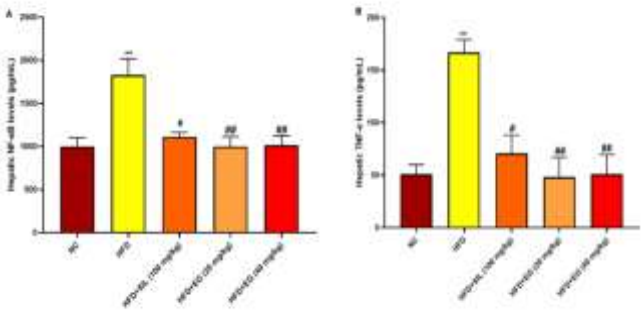

Figure 7: Effects of ethyl gallate on the hepatic NF-κB and TNF-α levels. "Results are shown as the mean ± SEM (n = 6). $^{**}p < 0.01$ when compared to the NC group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared to the HFD group. NC: normal control; HFD: high-fat diet; HFD + SIL (100 mg/kg): high-fat diet with silymarin (100 mg/kg), HFD + EG (20 mg/kg): high-fat diet with ethyl gallate (20 mg/kg); HFD + EG (40 mg/kg): high-fat diet with ethyl gallate (40 mg/kg)."

The findings of this study are compatible with many previous studies that showed the anti-inflammatory properties of ethyl gallate. It has been demonstrated in a previous study the potential of ethyl gallate in reducing proinflammatory cytokines levels (TNF-α, IL-6, IL-1β, and IFN-γ) in colonic tissue of C57BL/6 J mice with ulcerative colitis induced by dextran sulfate sodium (DSS).[10] It also has been revealed in another previous study that all the abnormalities in nuclear factor κ B, tumor necrosis factor-α, interleukin-1, p65, and p52 proteins were significantly inhibited by administration of ethyl gallate for acute liver injury induced by acetaminophen in mice.[15] It also has been reported in another previous study that ethyl gallate significantly inhibited the expression of NF-κB-p65 in the intestinal tissue of IIRI mice and protected against intestinal injury induced by ischemia-reperfusion.[39]

Conclusion

In conclusion, ethyl gallate demonstrated the ability to influence lipid metabolism in liver tissue and inhibit the NF-κB signaling pathway, thereby decreasing inflammatory cytokines such as TNF-α. Furthermore, it was discovered that ethyl gallate reduces oxidative stress in liver tissue. Altogether, this research indicates that ethyl gallate may offer protection against a model of NAFLD induced by a high-fat diet in mice.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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