



# DETERMINANT AND PROTECTOR OF HEPATOSIS IN HIGH-FAT-FED WISTAR RATS

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## Abstract

### Aim

*Sesamum indicum* has a long history of use in traditional medicine; it protects metabolic rats given a high-fat diet and enhances liver functioning. The current study examined the hepatoprotective effects of sesame meal on Wistar rats who were given a high-fat diet. A total of five groups of healthy adult male wistar rats were created. Rats in Group I received a standard laboratory diet (20g/rat/day), rats in Group II received a high-fat diet (20mg/rat/day), rats in Group III received a combination of 70% high-fat diet and 30% sesame meal (20mg/rat/day), rats in Group IV received pioglitazone (25mg/kg of body weight) intravenously administered daily, and rats in Group V received a combined mixture of 70% standard laboratory pellet and 30% sesame meal (20mg/rat/day). Once the trial period (thirteen weeks) came to a close, the rats were killed. At the conclusion of the trial, rats who were given a high-fat diet showed a considerable rise in both their body weight and liver weight. While total protein levels declined, AST, ALT, ALP, and bilirubin levels in the blood and liver tissues of the rats given a high-fat diet increased significantly. However, after being treated with sesame meal, the values of all parameters returned to a normal range. Findings indicate that sesame meal has a strong hepatoprotective effect on rats that are given a high-fat diet. In terms of hepato-protective function, comparing the effects of sesame meal therapy to those of pioglitazone on rats that were given a high-fat diet shows that the sesame meal treatment was almost as effective as the pioglitazone treatment, if not more so.

## Key words

Sesame meal, high fat diet, wistar rat, pioglitazone. hepatoprotective.

## Introduction

The liver is the body's biggest and most intricate internal organ. Through its many and varied functions, it contributes significantly to the upkeep of the interior environment. Among the many important roles played by the liver are metabolism, secretion, and storage. The inflammatory illness known as hepatitis causes harm to the hepatocytes by inflaming them. Among the world's most common illnesses, hepatitis ranks high [1]. Cirrhosis of the liver brought on by viral hepatitis is responsible for the deaths of 18,000 persons every year [2]. In obesity, there is an abnormal accumulation of fat in the liver, a condition known as nonalcoholic fatty liver disease (NAFLD). This illness is marked by a progression of liver injuries, starting with steatosis and progressing to tratrohepatitis, fibrosis, and necrosis. It is considered the liver symptom of metabolic syndrome [3] since NAFLD is strongly linked to IR (insulin resistance) and obesity. One of the liver's many functions is to synthesis beneficial principles and detoxicate harmful chemicals. Consequently, hepatotoxic agent injury to the liver is quite dangerous [4]. Animals subjected to a high-fat diet (HFD) for more than two months exhibit symptoms of insulin resistance, hyperglycemia, oxidative stress, and weight gain, according to experimental studies[5, 6]. Also, eating a lot of calories makes you more likely to have liver disease because it causes your body to produce more inflammatory cytokines, which leads to lipid buildup and macrophage infiltration [7]. As hepatoprotective agents, ayurveda makes reference to a wide variety of native plants [8].

The byproduct of extracting the oil from *sesamum indicum* seeds is known as sesame meal. This traditional health food is a great source of protein, with a protein content ranging from 47.1% [9] to 52.9% [10] and an amino acid composition comparable to that of soybean meal, at 47.7%. In addition to its nutritional value, sesame seed is thought to have medicinal properties, such as anti-inflammatory effects [15], antioxidant effects [13, 14, 15], antihypertensive effects [14], and the ability to inhibit the absorption of cholesterol from the intestine and its synthesis in the liver [16]. In addition to its tonic effects on the liver and kidneys, the seed has many other uses, including as a diuretic, emollient, galactagogue, and lenitive [17, 18]. When it comes to therapeutic uses, sesame seeds are among the most significant in the world. But there aren't a ton of research looking at the hepatoprotective effects of sesame therapy in animals on a high-fat diet. Here, we compared pioglitazone therapy to see how sesame meal affected rats given a high-fat diet in terms of hepatoprotective effects.

## MATERIALS AND METHODS

### Experimental Animal

In this work, we used mature male albino rats (Wistar stain) that were acquired from the central animal house at Rajah Muthiah Medical College, Annamalai University. Room temperature ( $27\pm 2^{\circ}\text{C}$ ) was maintained for the rats in polypropylene cages. Animals weighing between 170 and 200 g were randomly assigned to either the control or experimental groups. In accordance with NH (New Hampshire) guidelines, the animals were given free access to water, food, and standard pellets for at least two weeks before the experiments began, during which time they were allowed to acclimate to their new environment.

Table -1 Composition and energy content of the high-fat diet (HFD)

| Type of composition             |       |
|---------------------------------|-------|
| Casein [ $\geq 85\%$ deprotein] | 190   |
| Corn starch                     | 250.7 |
| Sucrose                         | 100   |
| Soybean oil                     | 40    |
| Lard                            | 320   |
| Fiber                           | 50    |
| Vitamin mix                     | 10    |
| Mineral mix                     | 35    |
| L-cystein                       | 1.8   |
| Choline                         | 1.5   |
| Antioxidant                     | 0.008 |
| Total grams                     | 1000  |
| Energy content                  |       |
| Total energy content [Kcal/kg]  | 5,404 |
| carbohydrate [%]                | 26    |
| protein [%]                     | 14    |
| Lipids [%]                      | 60    |

Table 1 shows the necessary level of composition and energy content of high fat diet, recommended by American Institute of Nutrients [19]. The American Institute of nutrients (AIN 93M) recommended the necessary of composition and energy content in high fat diet (table 1)

### Sesame cake

Sesame cake was purchased from local market from Chidambaram, Tamilnadu, India.

## Chemicals

Pioglitazone hydrochloride ( $C_{19}H_{21}ON_2O_3S.HCl$ ) was obtained as a gift sample from Flembic pharmaceuticals Pvt. Ltd. Baroda, India. All other chemicals used in the experiments were of analytical grade.

## Experimental design

The acclimatized animals were divided into five groups of six rats in each.

Group 1 (CONTROL): Rats were fed a commercially available standard laboratory diet (20mg/rat/day) and water ad libitum.

Group 2 (HF) : Rats were fed a High-fat diet (20g/rat/day) and water ad libitum.

Group 3 (HF+SM) : Rats were fed with combined mixture of 70% High-fat diet and 30% sesame meal (20g/rat/day)

Group 4 (HF+PIO) : Rats were fed a High-fat diet (20g/rat/day) and administrated Pioglitazone (25mg/kg of body weight) via intravenous in every day.

Group 5 (SM) : Rats were fed with combined mixture of 70% of standard laboratory pellet 30% Sesame meal (20 g/rat/day).

## Sample collection

Animals were subjected to overnight fasting and cervical dislocation before being slaughtered at the conclusion of thirteen weeks of therapy. After obtaining blood samples using retro-orbital puncture while under mild ether anesthesia, the serum was extracted by centrifuging the blood at 2000 rpm for 10 minutes. The serum was then stored at 20°C until the tests were completed. The rats used in the experiment had their livers removed and rinsed in distilled water after the dissection. Using the conventional approach, we assessed the total protein in serum and liver tissue, as well as the enzyme activity of ALP, AST, and ALT. We also measured the concentration of bilirubin in serum.

## Biochemical analysis

Total protein was estimated by a previously described method [20] method. The activities of serum AST ALT were estimated by using commercially available kits, by the method of [21]. The serum ALP was estimated by the method of [22] and total bilirubin was estimated by the method of [23].

## Statistical analysis

Data analyses were carried out using one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using the SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at  $P \leq 0.05$  and the values sharing a common superscript did not differ significantly.

## Results

Table 2 Effect of sesame meal on serum total protein, ALP (Alkaline phosphatase), AST (Aspartate transaminase), ALT (Alanine transaminase), Bilirubin in control and experimental rats.

| Experimental groups | Total protein (mg/dl)  | ALP (IU/L)               | AST (IU/L)               | ALT (IU/L)              | Bilirubin (mg/dl)      |
|---------------------|------------------------|--------------------------|--------------------------|-------------------------|------------------------|
| CONTROL             | 6.45±0.47 <sup>a</sup> | 75.36±3.86 <sup>a</sup>  | 47.50±2.52 <sup>a</sup>  | 25.17±1.24 <sup>a</sup> | 0.83±0.03 <sup>a</sup> |
| HF                  | 5.34±0.12 <sup>a</sup> | 139.82±3.67 <sup>a</sup> | 81.52±2.72 <sup>a</sup>  | 62.29±2.44 <sup>a</sup> | 1.30±0.04 <sup>a</sup> |
| HF+SM               | 6.07±0.14 <sup>b</sup> | 82.03±3.92 <sup>c</sup>  | 52.36±4.01 <sup>c</sup>  | 28.90±1.35 <sup>c</sup> | 0.71±0.02 <sup>c</sup> |
| HF+PIO              | 6.15±0.16 <sup>a</sup> | 89.75±4.26 <sup>b</sup>  | 50.14±5.46 <sup>b</sup>  | 32.92±3.61 <sup>b</sup> | 0.87±0.04 <sup>b</sup> |
| SM                  | 6.35±0.09 <sup>a</sup> | 72.03±3.56 <sup>b</sup>  | 45.44±4.68 <sup>ab</sup> | 23.42±1.55 <sup>b</sup> | 0.66±0.06 <sup>b</sup> |

Values are expressed as mean of six individuals in each group  $\pm$  SD.

HF-High Fat diet; PIO-Pioglitazone; SM-Sesame meal.

<sup>a,b,c,d,ab</sup> Values sharing a common superscript (a, b, c, d) do not differ significantly at  $p < 0.05$  (DMRT).

Table 2 shows the estimated levels of serum total protein, AST, ALT, ALP, bilirubins in control and various experimental group of rat. An elevation in serum AST, ALT, ALP, bilirubin and a declined total protein level in high fat fed rats were highly significant when compared with the corresponding control rats. Administration of sesame meal and pioglitazone to high fat fed rats caused marked reduction in the elevated activities of AST, ALT, ALP, bilirubin with corresponding treatment of total protein towards the normal level.

Table 3 Effect of sesame meal on liver total protein, ALP (Alkaline phosphatase), AST (Aspartate transaminase), ALT (Alanine transaminase) of control and experimental rats

| Experimental group | Total protein (mg/g)          | ALP (IU/L)                    | AST (IU/L)                     | ALT (IU/L)                       |
|--------------------|-------------------------------|-------------------------------|--------------------------------|----------------------------------|
| CONTROL            | 8.62 $\pm$ 0.22 <sup>c</sup>  | 0.21 $\pm$ 0.04 <sup>a</sup>  | 626.44 $\pm$ 6.45 <sup>a</sup> | 946.63 $\pm$ 16.44 <sup>b</sup>  |
| HF                 | 4.44 $\pm$ 0.51 <sup>c</sup>  | 0.29 $\pm$ 0.02 <sup>a</sup>  | 776.66 $\pm$ 8.77 <sup>a</sup> | 1105.03 $\pm$ 31.06 <sup>c</sup> |
| HF+SM              | 7.35 $\pm$ 0.50 <sup>a</sup>  | 0.26 $\pm$ 0.04 <sup>c</sup>  | 656.74 $\pm$ 7.55 <sup>b</sup> | 953.75 $\pm$ 14.77 <sup>a</sup>  |
| HF+PIO             | 7.75 $\pm$ 0.42 <sup>b</sup>  | 0.23 $\pm$ 0.03 <sup>b</sup>  | 653.74 $\pm$ 7.99 <sup>a</sup> | 963.78 $\pm$ 66.42 <sup>b</sup>  |
| SM                 | 8.42 $\pm$ 0.51 <sup>bc</sup> | 0.20 $\pm$ 0.04 <sup>ab</sup> | 622.57 $\pm$ 9.88 <sup>a</sup> | 942.63 $\pm$ 12.55 <sup>bc</sup> |

Values are expressed as mean of six individuals in each group  $\pm$  SD.

HF-High Fat diet; PIO-Pioglitazone; SM-Sesame meal.

<sup>a,b,c,d,ab,bc</sup> Values sharing a common superscript (a, b, c, d) do not differ significantly at  $p < 0.05$  (DMRT).

Table 3 shows the levels of estimated total protein, AST, ALT, ALP in liver tissue of control and various experimental groups of rat. Significant elevation in liver AST, ALT, ALP and a decreased total protein level in high fat fed rat were observed when compared with the corresponding control rats. Sesame meal and pioglitazone treatments to high fat fed rats caused a marked reduction in the elevated activities of AST, ALT, and ALP and also increased total protein level towards normal in liver tissue.

## Discussion

A calorie-rich diet promotes the development of liver disease by leading to fat buildup [24], an overabundance of inflammatory cytokines [25], and the infiltration of macrophages [7]. Hyperlipidemia may change the associated marker enzyme profiles in blood and liver tissue and lead to cirrhosis of the liver, as seen in rats by a high-fat diet [25]. has shown that a diet rich in fat increases the risk of hyperglycemia. [27] and its impact on endothelial functions[28,29], musculoskeletal system, and liver physiology. There are a lot of diseases that affect the liver, which is essential for metabolism, detoxification, and secretory function, but so far, there have been no successful treatments; still, research into potential new medications is continuing [30]. The therapy of liver problems has a long history of using several folk medicines that originate from plants [31]. It is still a struggle for contemporary medicine to manage liver problems. When it comes to liver problems, Ayurveda relies heavily on herbal and herbomineral remedies [32]. After reading this, you should have no trouble believing that rats on a high-fat diet may serve as a reliable model to study the pathogenesis of hyperlipidemia [33]. Thus, this research aimed to compare the hepatoprotective effects of sesame meal and pioglitazone with those of a high-fat diet in a rat model for the effects of induced changes on marker enzymes in blood and liver tissue. Serum AST, ALT, and ALP activities are indicators of liver function; these enzymes were initially more concentrated in the cytoplasm [33], but they leak into the bloodstream in proportion to the degree of liver damage [34, 35]. The levels of bilirubin, as well as the enzymes AST and ALT, are the most often used indicators of hepatic integrity in clinical toxicological research [36]. In order to evaluate hepatic damage, ALT is often included in biochemical profiles [37].

Tables 2 and 3 show that the hepatotoxic effects of the high-fat diet on the rats in this study are evident from the elevated levels of AST, ALT, and ALP in their blood and liver when compared to the control groups. One of the most sensitive indicators of liver injury is an increase in blood marker enzyme levels [38]. As stated in [39], ALP mostly enters the liver via bone, and in cases of hepatobiliary disorders, its concentration in blood is increased by biliary excretion. Consistent with other research, this study found that rats given a high-fat diet had an increase in ALP in their liver tissue, suggesting

that their liver's excretory function was disrupted [40]. Hepatic and biliary cells contain the enzymes ALT and AST, as stated in the literature [41]. These enzymes were found to be higher in blood and liver tissue of rats that were given a high-fat diet. This suggests that the elevation might be caused by hepatocellular damage induced by the toxicity of the food. This closely resembles the assertion made in [42]. When the biliary system is inflamed or there is hepatic damage, the enzymes produced by the injured cells are discharged into the bloodstream, which raises their serum levels [41]. A similar increase in AST and ALT levels in the blood and liver was seen by Recknagel as a result of hepatic injury in animals given a high-fat diet [43]. The rats in this research had lower serum and liver protein levels after being administered a high-fat diet. Protein depletion in rats given a high-fat diet may be attributable to the release of metabolic energy during poisoning. It seems plausible that localized injury in the endoplasmic reticulum causes the protein loss in rats given a high-fat diet, which is in agreement with the claim made in [44]. The evaluation of hepatic function may be done by determining blood bilirubin levels; an abnormal rise in these levels indicates hepatic dysfunction and severe jaundice [45]. Compared to the control rats, the rats given a high-fat diet in this study had far higher bilirubin levels. This pattern of findings is in agreement with the claim that elevated blood bilirubin levels are diagnostic of induced hepatitis [46, 47]. The results revealed that the rats given pioglitazone and sesame meal individually mixed with a high-fat diet had bilirubin in their blood, total protein in their liver tissue, and a noticeable normal level of enzyme profiles (AST, ALP, and ALT). It is believed that pioglitazone regulated enzyme metabolism, which may explain why it was beneficial in treating rats on a high-fat diet. Researchers found that pioglitazone improved early-phase fibrogenesis in the liver by inhibiting hepatic inflammation, collagen synthesis, and hepatic stellate cell activation in rats with liver fibrosis caused by a high-fat diet [48] or bile duct ligation [49]. The results show that pioglitazone acts as a hepato-protector against high-fat toxicity in rats, as the group treated with the drug showed a significant reduction in the modification of biochemical alterations. On the other hand, pioglitazone caused liver damage in rats given a high-fat diet over an extended length of time. The number 48. [49] Patients administered pioglitazone have shown signs of liver inflammation and increased liver enzymes, according to Galli. Pioglitazone has a negative impact on bone, leading to bone loss and an increased risk of fractures, according to recent studies [49]. Additionally, cardiac outcomes, such as recurrent heart attacks, are reduced with pioglitazone treatment [50]. In contrast to the control group, rats given either a sesame meal-high-fat diet or only sesame meal shown a considerable decrease in biochemical components (enzymatic levels) in blood and liver tissue, suggesting that sesame meal has an antihepatotoxic effect. In rats that were given a high-fat diet, the sesame therapy helped improve enzyme resistance. Rats given a high-fat diet together with pioglitazone and sesame seed mixture had lower bilirubin levels than rats given the high-fat diet alone. Extract potential for biliary dysfunction stabilization is supported by pioglitazone and sesame-mediated decrease of elevated bilirubin levels in combination with a high-fat diet. As time goes by with pioglitazone medication, some studies have shown that lesser adverse effects, such liver damage, may become more noticeable [51]. This study shown that sesame meal may reduce cellular levels of increased ALT, ALP, and AST, making it a significant modulator of enzyme resistance in obesity and diabetes caused by high-fat diets. Sesame treatment findings showed that compared to pioglitazone, sesame meal was better at treating high-fat diet toxicity. It is believed that eating sesame seeds may inhibit cellular vaculation and a rise in plasma gamma-tocopherol [52], which in turn boosts vitamin E activities [54]. Consistent with the findings of [55], which found that sesame seed lecithine acts as an antioxidant and hepatoprotective function at the cellular level, especially in animals given a high-fat diet, the current results on the sesame treated group are also encouraging. There is evidence that sesamin and sesamol, which are found in high concentrations in sesame [56], may enhance the rate of lipid oxidation in the liver's mitochondria and peroxisomal organelles [57]. In addition to their health-promoting and antioxidant properties, sesame lignans may block the absorption and synthesis of chlorophyll in liver [5-9]. Before Buzzelli discovered that hepatocellular necrosis and/or increased membrane permeability were improved by herbal therapy in liver function tests. [61] Sesamum seed flavonoids reportedly have antioxidant and free radical scavenging capabilities, which explain their beneficial effects.

## Conclusion

The results of this research show that sesame meal (*Sesamum indicum*) protects the liver from the harmful effects of a high-fat diet by lowering serum bilirubin levels, hepatic marker enzyme activities, and hepatotoxic effects on the liver. Flavonoids and lecithine are two phytochemical components found in sesame seed that have antioxidant and hepatoprotective effects. After a longer length of time, sesame therapy outperformed pioglitazone treatment in terms of its hepatoprotective activity, restoring biochemical components in rats given a high-fat diet to a normal tendency without adverse effects.

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