HEPATOPROTECTIVE ACTIVITY OF SESAME MEAL ON HIGH FAT FED WISTAR RATS

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Abstract

Aim

Sesamum indicum have been widely used in tradition medicine for thousand of year, it improves liver functions and provides protection against high fat fed metabolic rats. Present investigations were carried out on the hepatoprotective role of sesame meal treatment to high fat fed wistar rat. Healthy adult male wistar rats were divided into five groups. Group I: rats were fed a standard laboratory diet (20g/rat/day), Group II: rats were fed a high-fat diet alone (20mg/rat/day), Group III: rats were fed with combined mixture of 70% of high-fat diet with 30% sesame meal (20mg/rat/day), Group IV: rats were fed a high fat diet was administered with pioglitazone (25mg/kg of body weight) via intravenous in each day, Group V: rats were fed combined mixture of 70% standard laboratory pellet and 30% sesame meal (20mg/rat/day). The rats were sacrificed at the end of the experimental (thirteen week) period. High fat fed rat registered significantly increase in body weight and liver weight at the end of experimental period. The high fat fed rat substantially elevated its serum and liver tissue AST, ALT, ALP, bilirubin with decreased in total protein levels. Whereas the levels of all parameters significantly restored towards normalization by the sesame meal treatment. The results obtained suggest that the sesame meal have potent hepatoprotective action on high fat fed rats. A comparison of the performance in both sesame meal and pioglitazone treatment on high fat fed rat in respect of hepato-protective role is clearly indicate that the sesame meal treatment was more or less very equal to the result of pioglitazone as well as to the normal level.

Key words

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

Introduction

Liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. Liver is involved in several vital functions, such as metabolism, secretion and storage. Hepatitis or inflammatory disorder involves inflammation and damage to the hepatocytes. Hepatitis is one of the most prevalent diseases in the world [1].Every year 18,000 people had been reported to die due to liver cirrhosis caused by viral hepatitis [2]. Ectopic fat storage occurs in obesity, particularly in the liver leading to a condition termed nonalcoholic fatty liver disease (NAFLD) characterized by varying degree of liver injury that progresses from steatosis to tratohepatitis, fibrosis and necrosis. Due to its prominent association with IR (insulin resistance)/obesity, NAFLD is regarded as the hepatic manifestation of metabolic syndrome [3]. Liver has great capacity to detoxicate toxic substances and synthesizes useful principles. Therefore, damage to liver inflicted by hepatotoxic agents is of grave consequences [4].

Experimental studies have reported that animals fed a high fat diet (HFD) for more than two months develop weight gain, dyslipidemia, hyperglycemia, oxidative stress and IR[5, 6]. Besides, consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines, and macrophage infiltration that favour the progression of liver disease [7]. In ayurveda many indigenous plants have been mentioned and well established as hepatoprotective agents [8].

Sesamum indicum (Pedaliaceae) is the traditional health food around the world, sesame meal is the residue after pressing the oil from the seed, and it is an excellent source of protein (47.1% [9] to 52.9% [10]) and has an amino acid composition similar to that of soybean meal (47.7%). Sesame seed is not only a good source of edible nutrients, but it is also widely considered to have medicinal value, including antiaging effects [11] antimutagenic [12] antioxidant [13, 14, 15], antihypertensive [14], anti-inflammatory [15], and inhibition of cholesterol absorption from the intestine and synthesis in the liver [16]. The seed is used as diuretic, emollient, galactogogue, lenitive [17] and acts as a tonic for the liver and kidneys [18].

Sesame being one of the important seed for medicinal treatment in the world. However, limited studies are available on the effect of sesame treatment on hepatoprotective effect in high fat diet fed animal. In this regard, the present study was aimed to investigate the effect of sesame meal on high-fat fed rats related to different hepatoprotective effect by comparing pioglitazone treatment.

MATERIALS AND METHODS

Experimental Animal

Healthy adult male albino rats (Wistar stain) were purchased from central animal house, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (27±2˚C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 170 to 200g. The animals were fed with a diet of standard pellets (Hindustan Lever Ltd, Bombay) and provided free access of water and libitum and food during the tenure of acclimatization to the environment for a minimum period of two weeks prior to the commencement of experiments, according to NH (New Hampshire) guidelines.

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

Type of composition	
Casein [≥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 Institue 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 hydrocholoride ($C_{19}H_2ON_2O_3S.HCl$) 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/dat) and administrated Pioglitazone (25mg/kg of body weight) via inrtravenous 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

At the end of thirteen weeks treatment, animals were kept starved overnight fasting and sacrificed by cervical dislocation. Blood samples were collected by retro-orbital puncture under light ether anesthesia and the serum was obtained by blood centrifugation at 2000 rpm for 10 minutes and kept at 20°C until analyses were done. The animals were dissected and the liver was taken from experimental rat and the tissue was washed in distilled water. The enzyme activity of ALP, AST, ALT and total protein in both the serum and liver tissue and also bilirubin in serum were estimated by using standard method.

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^{a}	75.36 ± 3.86^{a}	47.50 ± 2.52^{a}	25.17 ± 1.24^{a}	0.83 ± 0.03^{a}
HF	5.34 ± 0.12^{a}	139.82 ± 3.67^{a}	81.52 ± 2.72^{a}	62.29 ± 2.44^{a}	1.30 ± 0.04^{a}
HF+SM	6.07 ± 0.14^{b}	82.03 ± 3.92^{c}	52.36 ± 4.01^{c}	28.90 ± 1.35^{c}	0.71 ± 0.02^{c}
HF+PIO	6.15 ± 0.16^{a}	89.75 ± 4.26^{b}	50.14 ± 5.46^{b}	32.92 ± 3.61^{b}	0.87 ± 0.04^{b}
SM	6.35 ± 0.09^{a}	72.03 ± 3.56^{b}	45.44 ± 4.68^{ab}	23.42 ± 1.55^{b}	0.66 ± 0.06^{b}

Values are expressed as mean of six individuals in each group ± SD.

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

[a,b,c,d,ab] 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)	ALP (IU/L)
CONTROL	8.62±0.22[c]	0.21±0.04[a]	626.44±6.45[a]	946.63±16.44b[c]
HF	4.44±0.51[c]	0.29±0.02[a]	776.66±8.77[a]	1105.03±31.06[c]
HF+SM	7.35±0.50[a]	0.26±0.04[c]	656.74±7.55[b]	953.75±14.77[a]
HF+PIO	7.75±0.42[b]	0.23±0.03[b]	653.74±7.99[a]	963.78±66.42[b]
SM	8.42±0.51[bc]	0.20±0.04[ab]	622.57±9.88[a]	942.63±12.55[bc]

Values are expressed as mean of six individuals in each group ± SD.

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

[a,b,c,d,ab,bc] 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

Consumption of a calorie-rich diet results in lipid accumulation [24] excess production of inflammatory cytokines, and macrophage infiltration that favour the progression of liver disease [7]. [25] showed that high-fat diet could induce the hyperlipidemia in rats, and hyperlipidemia could alter the related marker enzyme profiles in serum and liver tissue and progress to liver cirrhosis [26, 27] have revealed that high fat diet promote hyperglycemia. [27] and its effect on muscle and liver physiology as well as endothelial functions [28, 29].

Liver is the key organ in the metabolism, detoxification and secretory function in the body and its disorders are numerous with no effective remedies, however, the search for new medicines still ongoing [30].Many folk remedies from plant origin have been long used for treatment of liver diseases [31].Management of liver diseases is still challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders [32]. From the above statement it is generally accepted that high-fat diets can be used to generate a valid rat model for the analysis of pathophysiology of hyperlipidemia [33]. Therefore, in this study high-fat diet fed to rat model for the induced alterations on marker enzymes in serum and liver tissue to examine the comparative effect of sesame meal and pioglitazone combination with dietary (high-fat diet) administration for their hepatoprotective role.

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP which are enzymes originally present in higher concentration in cytoplasm [33], when there is hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage [34, 35] Indication of hepatocellular integrity most commonly measured in clinical toxicology studies are the enzymes AST, ALT and bilirubin levels [36]. ALT is frequently included in biochemical profile for the purpose of assessing hepatic injury [37].

In the present investigation, the increased levels of AST, ALT and ALP have been observed in both liver and serum of high-fat fed rat compared to control groups (Table 2&3) indicating the hepatotoxic role of high-fat diet. An elevation in the levels of serum marker enzymes is generally regarded as one of the most sensitive index of the hepatic damage [38]. According to [39] ALP reaches the liver mainly from bone and its elevation in serum occurs through biliary excretion during hepatobiliary diseases. The elevation of ALP in liver tissue of high-fat fed rat indicates the disturbed excretory function of liver, which is co inside with the finding of previous study [40].

As indicated in literature, the enzymes ALT and AST, are present in the hepatic and biliary cells [41]. Observed elevated level of these enzymes in serum and liver tissue of high-fat diet fed rat indicates that these elevation might be due to hepatocellular damage caused by high-fat diet toxicity. This is closely resemblance to statement of [42]. These elevated enzymes in hepatocytes usually released into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells [41].Similarly, Recknagel reported that the hepatic damage induced in high-fat diet fed animal which could be rise in the level of AST and ALT in liver and blood [43].

In the present study, serum as well as liver protein level were decreased in high-fat diet fed rats. The depletion in the protein levels might be due to liberate energy of their metabolism during the toxicity of high-fat fed rats. The reduction of protein in high-fat fed rat is possible due to localized damage in the endoplasmic reticulum which is agreed with the statement of [44].

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary diseases and severe disturbance of hepatocellular function [45]. In the present investigation, the rats fed with high-fat diet showed significantly increased levels of bilirubin as compared to control rats. This trend of result coincide with the statement of induced hepatitis is characterized by increased levels of bilirubin in serum [46, 47].

Findings on the rat fed with of pioglitazone and sesame meal in separately mixing with high fat diet showed the appreciable normal level of enzyme profile (ALT, AST and ALP) and total protein in serum and liver tissue as well as bilirubin in serum of wistar rat. The results on pioglitazone group indicated that pioglitazone was effective in the treatment high-fat diet fed rats possibly through the mechanism of regulating the enzyme metabolism. Research on piogltiazone for the treatment of liver fibrosis induced in rats by either high-fat diet administration [48] or bile duct ligation [49] indicated that pioglitazone inhibit both hepatic inflammation, collagen synthesis and hepatic stellate cell activation, there by ameliorating early-phase fibrogenesis in the liver. In this study the pioglitazone treated group with great prevention on the alteration of biochemical changes, which indicates the pioglitazone act as a hepato-protecter from high-fat toxicity in rat, However the piogltiazone induced the liver disease after prolonged period in high-fat diet fed rat.[48]. [49] Galli reported that inflammation of liver and elevated liver enzymes have surfaced in patients given pioglitazone. Recent research also points to an unfavourable effect of pioglitazone on bone (bone loss) resulting in an increased risk of fractures [49]. Further, the pioglitazone therapy is associated with a decrease in cardiac outcomes, including recurrent heart attack [50].

In the groups of sesame meal mixing with high-fat diet and sesame meal alone fed to rat showed the perfect tendency of biochemical component (enzymatic level) in both serum and liver tissue compared with control groups, indicating the antihepatotoxic role of sesame diet. The sesame treatment exerted beneficial effect on ameliorating the enzyme resistance in high fat diet fed rat. Administration of pioglitazone and sesame mixing with high-fat diet fed to rats showed decreased bilirubin level when compared to high-fat diet alone fed rats. The piogltiazone and sesame mediated reduction of the increased bilirubin level mixing with high fat diet suggests the possibility of the extract being able to stabilize biliary disfunction. Some studies showed that the pioglitazone treatment may exert milder side effect such as liver damage after longer period [51].

The present investigation showed that the sesame meal act as an important mediator of enzyme resistance in high-fat diet induced obesity and diabetes through its ability to decrease the elevated activity of ALT, ALP and AST at the cellular level. The results on sesame treatment indicated that sesame meal was more effective for the treatment of high-fat diet toxicity than the treatment of pioglitazone. Sesame seed consumption appears to increase plasma gamma-tocopherol and enhanced vitamin E activities [52] which are belived to prevent the fatty accumulation and vaculation at the cellular level [54]. The present finding on sesame treated group is well agreed with the report of [55] who has stated that the lecithine of sesame seed act as antioxidant and hepatoprotective role in cellular level, particularly in high-fat fed animal. High amount of sesamin and sesamolin have been identified in sesame [56] and are reported to increase the hepatic mitochondrial and the peroxisomal fatty oxidation rate [57]. Also, sesame lignans have antioxidant and health promoting activities [58] which can inhibit the absorbtion and the production of cholesterol in the liver [59]. [60] Buzzelli

reported that herbal treatment improved liver function test related to hepatocellular necrosis and/or increases membrane permeability. [61] Reported that the protective effect of flavanoid of sesamum seed was attributed to its antioxidant and free radical scavenging properties.

Conclusion

This study conclusively stated that sesame meal (*Sesamum indicum*) has antihepatotoxic effects on serum, hepatic marker enzyme activities and blood bilirubin level as well as improving protein level from high fat diet induced hepatotoxicity. The presence of phytochemical constituents like flavonoids and lecithine in sesame seed possessing antioxidant and hepatoprotective properties. Sesame treatment was more effective in hepatoprotective role by improving biochemical components in high-fat diet fed rat towards normal tendency without any side effect after longer period in comparison to pioglitazone treatment.

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