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Possible Amelioration of the Severity of Nutritional Steatohepatitis by Guggulsterone in Mice

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases worldwide, which is characterized by steatosis, inflammation, and fibrosis. The aim of this designed study is to evaluate the ability of guggulsterone to prevent high fat diet induced steatohepatitis in mice. Five groups of male mice were selected and treated as the following: group I, mice had free access to standard commercial diet and considered as control group, group II, mice were fed a specially formulated high-fat diet for 12 weeks to induce non-alcoholic liver disease, while groups III, IV and V the mice were administered high fat diet containing guggulsterone at 500, 1000 and 2000 ppm concentration respectively for 12 weeks. Maintaining mice on fat rich diet only resulted in inducing the metabolic and histological changes related to NAFLD. While the treatment with guggulsterone significantly improves the evaluated markers. These results demonstrate guggulsterone may be useful in preventing the development of steatohepatitis.

Keywords: Guggulsterone, Steatohepatitis.

امكانية التقليل من شدة التهاب الكبد الدهني التغذوي باستخدام الكيكولستيرون في الفئران

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الخلاصة

أصبح مرض الكبد الدهني واحد من أكثر أمراض الكبد المزمنة شيوعا حول العالم, الذي يتميز بالتشحم مصاحبا له الالتهاب والتليف في الكبد. الهدف من هذه الدراسة هو تقييم قدرة المركب الكيكولستيرون على الوقاية من التهاب الكبد الدهني المستحث باستخدام حمية غذائية عالية الدهون على الفئران. خمس مجاميع من الفئران الذكور تم اتخاذها ومعالجتها كالتالي: المجموعه الاولى الفئران فيها غذيت على نظام غذائي اعتيادي واعتبرت كمجموعه السيطره, المجموعه الثانية تم تغذية الفئران على نظام غذائي عالي الدهون فقط لمدة ١٢ اسبوع لاحداث مرض الكبد الدهني, اما المجموعة الثالثة والرابعة والخامسة تم تغذيه الفئران فيها بنظام غذائي عالي الدهون ويحتوي على الكيكولستيرون وبتراكيز ٥٠٠, ١٠٠٠, ٢٠٠٠ جزء من المليون على التوالي. ابقاء الفئران على حمية عالية الدهون ادى الى احداث تغيرات ايضية ونسيجية متعلقه بالمرض .اما العلاج بالكيكولستيرون ادى الى تحسن المعاييرالمقاسة.بالنتيجة قد يكون استخدام الكيكولستيرون مفيد في منع تطور التهاب الكبد الدهني.

الكلمات المفتاحيه: كيكولستيرون ، التهاب الكبد الدهني .

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide; it is a multi-factorial disorder with the contribution of a variety of environmental and genetic factors[1].

The progression of NAFLD was introduced originally as a disease of two consecutive hits which suggest the accumulation of fat in the hepatocytes sensitize the liver hepatocytes to a second metabolic insult associated with oxidative stress and proinflammatory cytokines (TNF-α, IL-6, IL-8,IL-1β) release, that result in cellular damage by inflammation, steatonecrosis, subsequently fibrosis[2]. The primary mechanism underlying hepatocyte dysfunction that leads to disease progression is lipotoxicity. Lipotoxic injury occurs due to excessive accumulation of lipids especially free fatty acid (FFA) lead to oxidative stress through the generation of bioactive lipotoxic metabolites and reactive oxygen species (ROS), proinflammatory cytokines expression upregulation, and β-oxidation inhibition[3][4]. Guggulsterone [4,17(20)-pregnadiene-3,16-dione] is the active ingredient obtained from Oleogum resin, known as gum guggul of Commiphora Mukul plant; it has been used for a long time to treat many disease states such as hyperlipidemia, atherosclerosis, arthritis, obesity, and other inflammatory disorders in Ayurvedic medicine. Guggulsterone is an farnesoid x receptor (FXR) antagonist, guggulsterone inhibits FXR induction of ileum bile acid-binding protein IBABP, bile salt export pump BSEP and the small heterodimer partner (SHP) expression [5]. Guggulsterone also found to be able to inhibit nuclear factor-kappa B (NF-κB) activation by suppressing the activity of Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IkBa) kinase in Hepatic stellate cells (HSCs) thus guggulsterone act as an antifibrotic agent[6].

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Materials and Methods

Experimental protocol

Thirty-five white male mice of six weeks' old were provided by the animal house of the College of Pharmacy/ University of Baghdad. Their weights ranged between 20-30 gm. The animals were housed in well-ventilated plastic cages, and were maintained under conditions of relatively controlled temperature, humidity, and 12 hrs. light / dark cycle. With a free access to a standard commercial diet that was purchased from the local market and tap water *ad libitum*. The animals randomly were divided into five groups of 7 animals in each group, as the following:

Group I: mice utilized in this group were fed standard commercial diet and considered to be the control group.

Group II: mice utilized in this group were fed a specially formulated high-fat diet (HFD) for 12 weeks to induce non-alcoholic liver disease.

Group III: mice utilized in this group were fed a high-fat diet that contains guggulsterone at a concentration of 500 ppm for 12 weeks.

Group IV: mice utilized in this group were fed a high-fat diet that contains guggulsterone at a concentration of 1000 ppm for 12 weeks.

Group V: mice utilized in this group were fed a high-fat diet that contains guggulsterone at a concentration of 2000 ppm for 12 weeks.

The animal's weight of all groups was measured routinely once weekly and at zero time. The animals of each group at the end of the experiment after overnight fasting were anesthetized using diethyl ether, and blood was collected from the heart by cardiac puncture, then the animals were sacrificed and the liver were obtained and weighed for calculating liver index (by dividing liver weight in mg by the last body weight in gram was recorded), then stored for analysis.

Samples preparation and analysis

Collected blood from the anesthetized animal was placed in a plain Eppendorf tube and then centrifuged at 3000 (rpm) for 15 minutes. The supernatant was collected and used in the estimation of liver enzymes activity, total bilirubin (TB), tumor necrosis factor- alpha (TNF- α), and Lipid profile, by using ready-made kits mice specific for each parameter.

Histological examination

Various sections of liver tissues obtained from mice utilized in this study were histologically examined according to the Junqueira et al. 1995method [7]. Liver specimens were collected from the tissue and kept in 10% formalin solution pH 7.4 and then undergo a serial of dehydration in different grades of alcohol. Cleaning of tissues was done by means of xylol, and then the tissues were embedded in paraffin wax. The blocks were cut by microtome into 5µm slices, and then washed in a water bath and left in an oven for dewaxing. Then, the blocks sectioned and stained with Hematoxylin and Eosin (H&E) stain. Light microscopic examinations were performed, and photomicrographs were taken, this was done in a blind fashion by a senior pathologist.

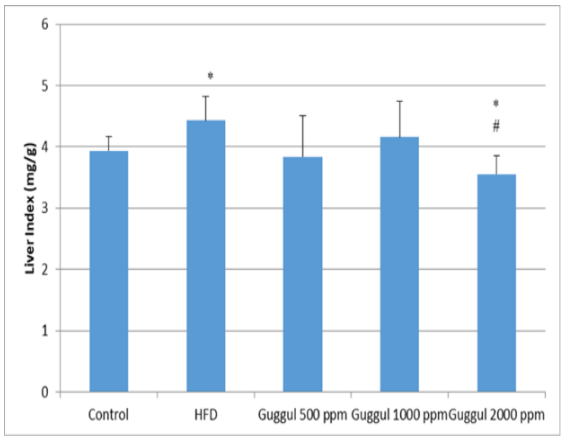
Statistical analysis

All the comparisons between groups were conducted by using student's t-test. A level of $p < 0.05$ was considered statistically significant. All data are represented as a (mean ± standard deviation) for the continuous variables.

Results

Effects of guggulsterone on liver index

In figure-1, the liver index (measured by dividing the liver weight by the last record of the total body weight) of high-fat diet only mice diet showed significant increase in comparison with the control group ($P<0.05$). Treatment with guggulsterone at 2000 ppm caused a significant decrease in liver index ($P<0.05$) in comparison with HFD groups, while the treatment with guggulsterone of (500 and 1000) ppm concentration caused non-significant difference when compared to HFD groups($P>0.05$).



Figure(1) Effects of guggulsterone on the liver index of mice models with high-fat diet induced NAFLD,
*** is a significantly different compared to the control group (P<0.05). # is significantly different compared to the HFD group (P<0.05).**

Effects of guggulsterone on the serum lipid profile

In figure 2, mice fed with high-fat diet showed a significant increase in cholesterol level in comparison with the control ($p<0.05$). Treatment with guggulsterone at (500, 1000 and 2000) ppm showed a significant decrease in cholesterol level compared to the HFD group ($P<0.05$). In Figure 3, the treatment with guggulsterone showed non-significant ($p>0.05$) effect on TG level in comparison with HFD.

In Figure 4, treatment with guggulsterone at (500 and 1000) ppm showed non-significant (p>0.05) difference in LDL level in comparison with HFD while treatment with guggulsterone at (2000) ppm showed significant decrease in LDL level in comparison with HFD(P<0.05).

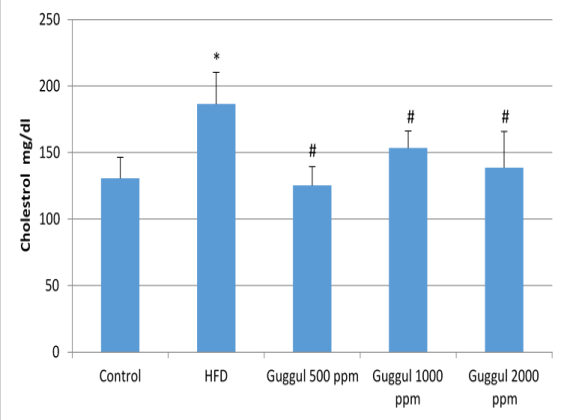
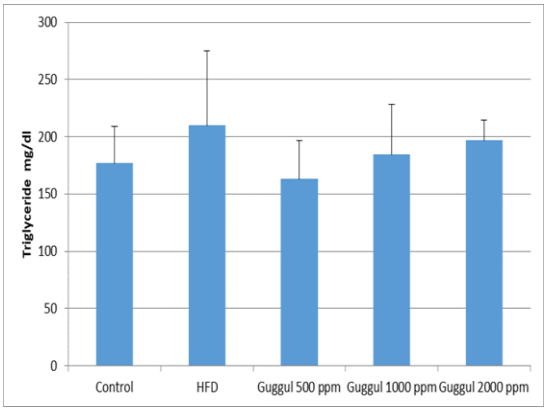


Figure (2) Effects of guggulsterone on cholesterol level of mice models with high-fat diet induced NAFLD.

*is a significantly different compared to the control group (P<0.05).

#is significantly different compared to the HFD group (P<0.05).



Figure(3) Effects of guggulsterone on TG level of mice models with high-fat diet induced NAFLD.

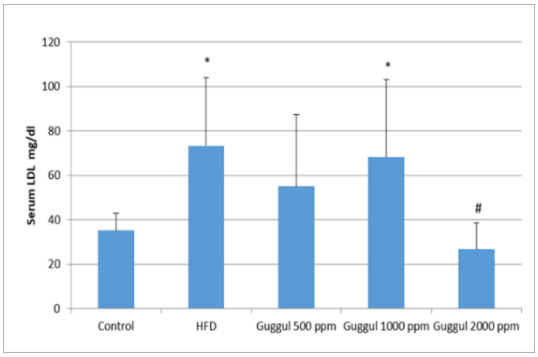


Figure 4. Effects of guggulsterone on the LDL level of mice models with high-fat diet induced NAFLD.

*is a significantly different compared to the control group (P<0.05).

#is significantly different compared with the HFD group (P<0.05).

Effects of guggulsterone on the serum levels of liver enzymes activity

In figure 5, the treatment at (1000 and 2000) ppm concentration of guggulsterone resulted in a significant decrease in AST level compared to the HFD group (*P*<0.05). There was a marked increase in AST level in HFD group compared to control group, as the control group showed a higher standard deviation value, the increase in AST level appeared to be statistically Non-significant. In figure 6, only the treatment with concentration of (2000) ppm of guggulsterone caused a significant decrease (P<0.05) in ALT level compared to HFD group. There was a marked increase in AST level in HFD group compared to control group, as the control group showed a higher standard deviation value, the increase in AST level appeared to be statistically Non-significant. In figure 7, high-fat diet only group showed a significant increase in serum alkaline phosphatase (ALP) level compared to control group (P<0.05), The administration of guggulsterone at (1000 and 2000) ppm caused a significant decrease in ALP serum level compared to HFD group (P<0.05).

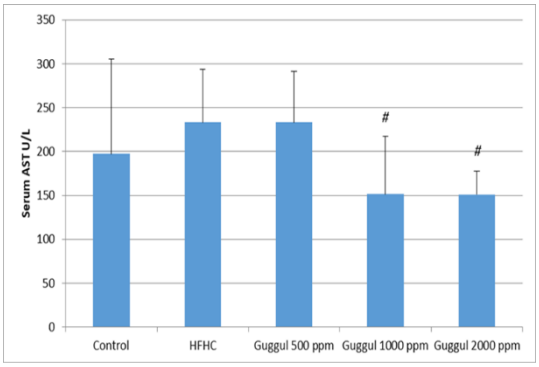


Figure (5) Effects of guggulsterone on serum AST level of mice models with high-fat diet induced NAFLD.

is significantly different compared to HFD group (P<0.05).

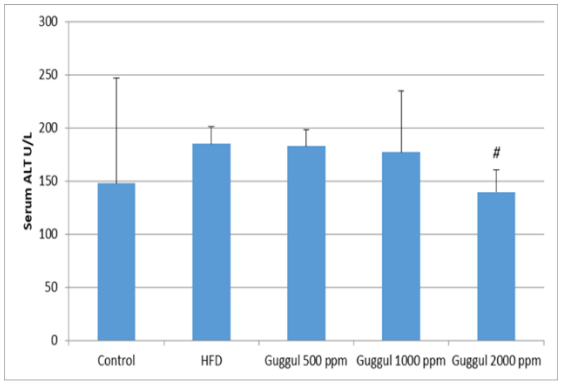
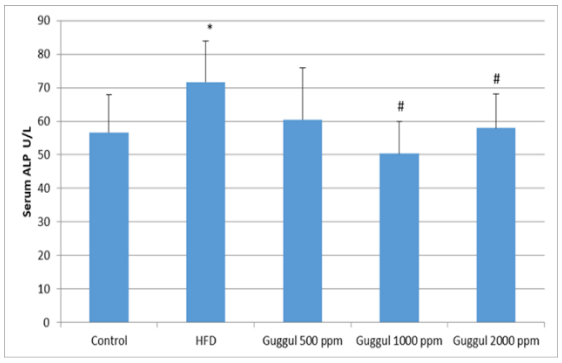


Figure (6) Effects of guggulsterone on serum ALT level of mice models with high-fat diet induced NAFLD.

is significantly different compared with HFD group (P<0.05).



Figure(7) Effects of guggulsterone on serum ALP level of mice models with high-fat diet induced NAFLD.

* is a significantly different compared to control group (P<0.05). # is significantly different compared to HFD group (P <0.05).

Effects of guggulsterone on the serum levels of total bilirubin (TB)

In figure 8, there is a significant increase in the level of TB in mice maintained on high-fat diet compared to the control group (P<0.05). While all guggulsterone treated groups showed a significant decrease in the levels of serum TB in comparison with HFD group (P<0.05).

Effects of guggulsterone on the serum levels of creatinine kinase (CK)

In figure 9, mice fed with high-fat diet showed a significant increase in CK level compared to the control group (P<0.05). While groups treated with (1000 and 2000) ppm concentration of guggulsterone showed a significant decrease in the levels of CK compared to HFD group (P<0.05).

Effects of guggulsterone on the serum TNF-α level

In figure 10, mice fed high-fat diet only showed a significant increase in the level of serum TNF-α compared to the control group (*P*<0.05). The treatment with 2000 ppm guggulsterone showed a significant decrease in the levels of TNF-α (*P*<0.05) compared to the HFD group, while the treatment with 500 & 1000 ppm concentration of guggulsterone result in non-significant effect in comparison with HFD group.

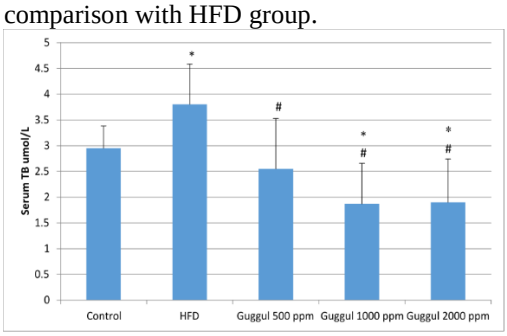


Figure (8) Effects of guggulsterone on serum TB level of mice models with high-fat diet induced NAFLD.

* is a significantly different compared to the control group (P<0.05). # is significantly different compared to the HFD group (P<0.05).

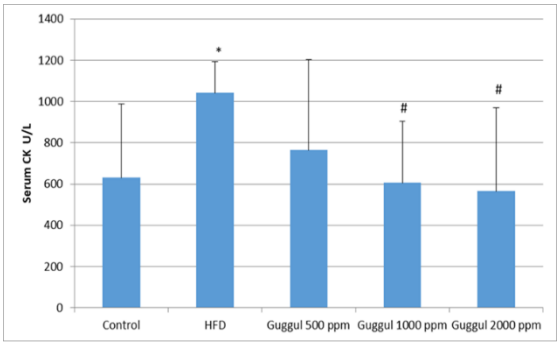


Figure (9) Effects of guggulsterone on serum CK level of mice models with high-fat diet induced NAFLD.

* is a significantly different compared to the control group (P<0.05). # is significantly different compared to the HFD group (P<0.05).

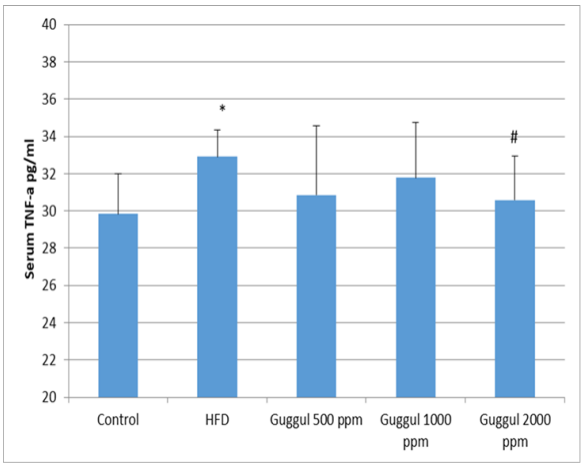


Figure (10) Effects of guggulsterone on serum TNF-α level of mice models with high-fat diet induced NAFLD.

* is a significantly different compared to the control group (P<0.05). # is significantly different compared to the HFD group (P<0.05).

Effects of guggulsterone on the liver tissue histology

Blinded histological assessment was performed on hematoxylin and eosin stained hepatic tissue to detect the presences of steatosis, ballooning degeneration and inflammation (Figure 11), there was no evidence of steatosis, ballooning degeneration or inflammation observed in control group. The H and E stained HFD liver tissue section showed the presences of steatosis in the form of pronounced micro-vesicular fatty change (small, clear, optically empty lipid vacuoles), and macro-vesicular fatty change in which the size of the vacuoles increases pushing the nucleus to the periphery of the cell, giving the cell an empty ring-like appearance, that with the development of inflammation and ballooning degeneration. The H and E stained liver tissue section of guggulsterone treated group at 500 ppm showed mild steatosis in the form of small lipid droplets, while the treatment with guggulsterone at 1000 and 2000 ppm reduced the liver tissue to look like that of the control group with no sign of steatosis, ballooning degeneration or inflammation.

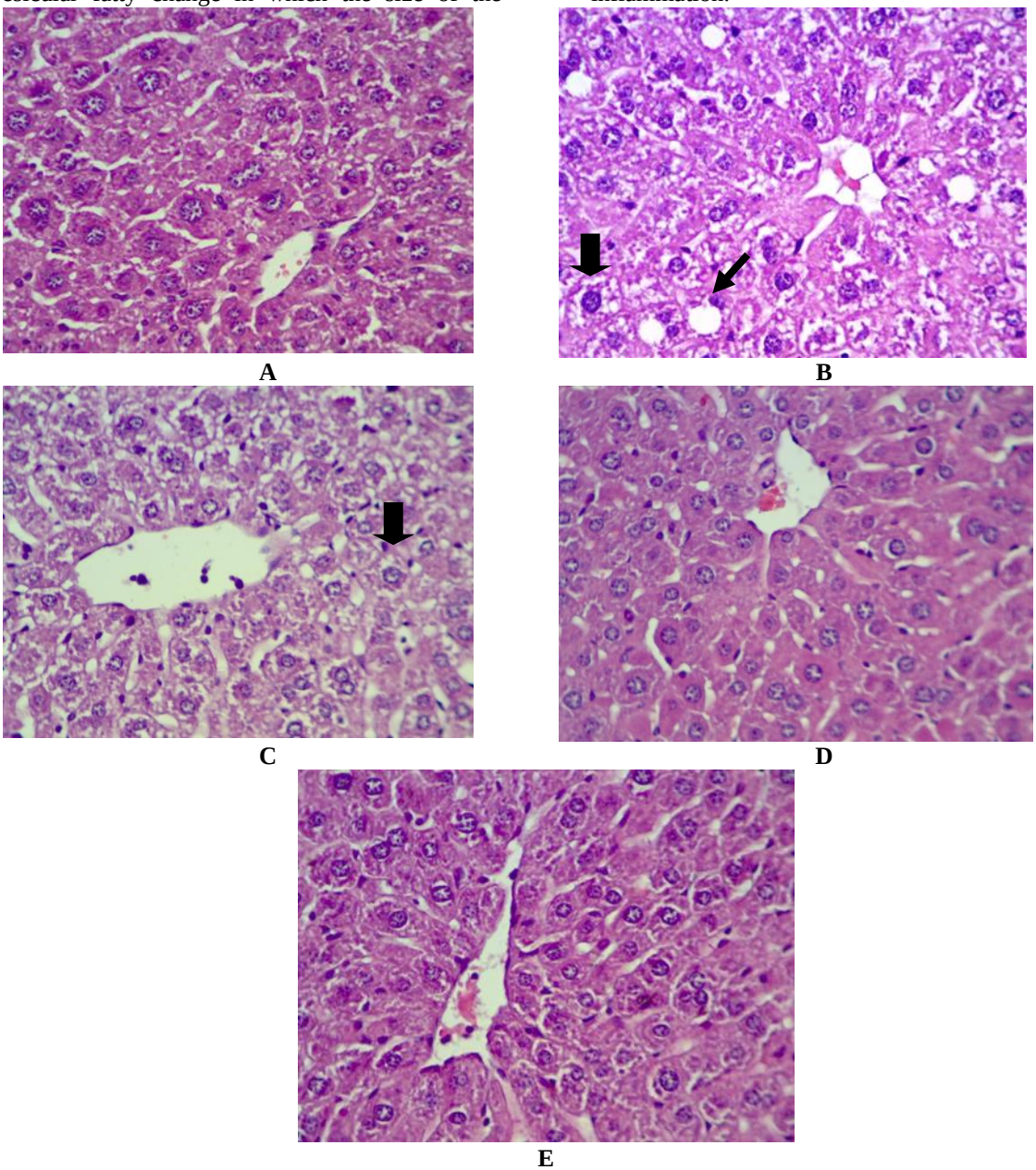


Figure (11) Histological assessment of NASH activity score (steatosis, inflammation and ballooning degeneration). Representative H and E stained sections from; control (A), HFD (B), GS 500ppm (C), GS 1000ppm (D) and GS 2000ppm (B), mouse models.A variable size of clear, optically empty,unstained fat vacuoles microvesicular (Thick arrow) and macrovesicular (Thin arrow) fatty changes.

Discussion

Non-alcoholic fatty liver disease (NAFLD) has turned to be one of the most common chronic liver disease worldwide; it is a multifactorial disorder with the contribution of a variety of environmental and genetic factors[1]. These results came in tune with the previous investigations that guggulsterone suppressed lipid accumulation in a dose-dependent manner[8], via inhibiting farnesoid X receptor (FXR) that consider as a major coordinators of adipocyte gene expression and differentiation, that result eventually in reduced liver index[9][5]. liver has a primary role in controlling plasma levels of LDL cholesterol because most of the LDL receptors are located in the liver. Guggul-lipid extracts have been used widely as a lipid-lowering agent; it has been well documented for its hypolipidemic activity[10]. Guggulsterone inhibits lipoproteins formation and lower the rate of intestinal fat and cholesterol absorption that result in an increase in the rate of fecal excretion of bile acids and cholesterol. Guggulsterone lipid lowering effect is due to farnesoid X receptor (FXR) antagonistic activity, studies showed it inhibit expression of FXR agonist-induced genes and BSEP expression induction[11][12]. The results from the present study have further proved the efficacy of the treatment with purified guggulsterone in lowering the serum total cholesterol and serum LDL levels compared to mice fed with high fat diet only. Meanwhile, the treatment with guggulsterone resulted in a not significant minor decrease in serum TG levels compared with HFD group, such outcome might be attributed to differences in animal model and the time period in which this study was carried out, since some studies showed that the treatment with guggulsterone in Fischer rats lowered serum triglycerides and concomitantly increased serum HDL levels[13][14]. Liver enzymes mainly the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) considered as liver toxicity markers, the increment in their level that is due to increased ROS generation[15][16]. This study data showed a reduction in the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in guggulsterone treated groups remarkably with the higher concentration, compared to the HFD-treated group, thus consequently alleviated the damage in the liver caused by HFD. This is due to the antioxidant activity of guggulsterone, which reported to be hepatoprotective agent[17]. Guggulsterone enhance the transcription of bile salt export pump that lead to reduced hepatic lipid content, also due to guggulsterone own activity as antioxidant, guggulsterone reduce the need for the bilirubin that act as free radical scavenger this results in reduced oxidative stress [18][19] eventually reduced production of bilirubin, as noted in the presented results that serum TB is significantly lower in guggulsterone treated groups compared with the HFD group that showed a markedly elevated TB level. The results showed that the level of CK elevated remarkably in the group that maintained on HFD only. Studies showed that the higher serum level of CK is associated with higher body fat mass, and consider as biochemical evidence of metabolic abnormality and the presence of NAFLD[20][21], Guggulsterone treatment resulted in lowering the level of CK, the higher the concentration result in the lower level of CK, this can be attributed to the hypolipidemic ability of guggulsterone that resulted in lower body visceral fat[19][18].TNF-α is used as a predictor of fibrosis in patients with Steatohepatitis[22].The elevation in TNF-α is related to aberrant production of cytokines by adipose macrophages and Kupffer cells[23]through the activation of TLR4 in macrophages that initiates downstream signaling pathways including nuclear factor-kappa B (NF-κB) complex that play an important role in acute and chronic inflammatory conditions in response to liver damage caused by HFD[24][25]. The treatment with guggulsterone as shown in the results had significantly decreased the elevated serum level of both TNF-α compared to the HFD group. This reduction in cytokines level is related to the anti-inflammatory effect of guggulsterone, as it has the ability to Inhibit the NF-κB signaling pathways as it had been shown in previous studies[26][27]. The histological assessment of liver tissue in this study showed that the HFD group clearly developed an early sign of fibrotic NASH with marked hepatosteatosis and ballooning degeneration. While the guggulsterone treated groups had showed a healthy like liver tissue especially with higher concentration. The result supports the role of guggulsterone as hepatoprotective and antifibrotic agent[6].

Conclusion

The results obtained from this study demonstrate that guggulsterone protected the treated mice against high-fat diet induced NAFLD changes, and the (1000 and 2000) ppm concentration of guggulsterone effectively provided the highest level of protection to the liver against high-fat diet induced NAFLD. Therefore, guggulsterone may be useful in preventing the development of steatohepatitis.

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