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Effects of Frankincense on Rats Suffering from Nonalcoholic Fatty Liver Disease

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Abstract: The aim of this study was to investigate the effects of frankincense on rats suffering from nonalcoholic fatty liver disease. Twenty three male albino rats (Sprague Dawley strain) weighing 80 ± 5 g were used and divided into three groups one of them kept normal and served as a control group (5 rats), while the others (18 rats) were fed on HFD for 4 weeks, then injected subcutaneously with CCl_4 (2 ml/kg body weight) twice a week for two weeks. The second group was then kept untreated and fed on basal diet only, while the third group was fed on basal diet supplemented with 4% of frankincense powder. The curative trial continued for 4 weeks. Body weight gain and liver weight/ body weight (%) were finally calculated. In addition, lipid profile, liver enzymes, total protein and albumin were determined in serum. Moreover, a specimen from each liver was homogenized and used to determine lipid profile, antioxidant enzymes as well as MDA and NO while other specimen was histopathologically examined. Results indicated that feeding rats on high fat diet followed by CCl_4 injection resulted in an increase of body and relative liver weights. Liver dysfunctions as well as significant increase in liver TG and total cholesterol and oxidative stress were also found. Supplementation of basal diet with 4% of frankincense powder reduced the marked lesions noticed in liver tissue, hence, led to a significant decrease in body weight gain, liver TG and total cholesterol besides the activities of ALT and GGT enzymes in serum and MDA in liver tissue homogenate. Conversely, phospholipids, nitric oxide and antioxidant enzymes in liver tissue homogenate as well as total protein in serum were significantly increased. Accordingly, the present study recommends patients with nonalcoholic fatty liver disease to consume frankincense regularly.

Key words: Frankincense · High Fat Diet · Carbon Tetrachloride · Nonalcoholic Fatty Liver Disease · Rats

INTRODUCTION

Nonalcoholic-fatty liver disease (NAFLD) is a burgeoning public health concern worldwide because of its high morbidity and its association with cardiovascular diseases and type 2 diabetes [1, 2]. In recent years, pathogenesis and treatment of NAFLD are attracting greater attention. The prevalence of NAFLD is 20-30% in adults and is higher in industrialized countries [3].

NAFLD represents a spectrum of disorders ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis and liver cancer [4]. NAFLD is a condition characterized by deposition of fat in hepatocytes of patients with no history of excessive alcohol consumption [5]. The pathogenesis includes the roles of hormones, nutritional and intestinal dysbiosis, insulin resistance, lipotoxicity, hepatic inflammation and genes [6].

High fat diets (HFD) - fed mice [7, 8] were used as an example of diet modulations leading to NAFLD. Similarly, carbon tetrachloride (CCl_4)-treated mice are a well-known chemical-induced model of NAFLD [9]. Chheda *et al*. [10] investigated the development of steatosis, steatohepatitis and fibrosis in the fast food diet-CCl_4 model when compared to the individual effects of a fast food diet (FFD) or a micro dose of CCl_4 in rats. The serum biochemical profile of the FFD-CCl_4 model showed an increase in liver injury and extensive fibrosis. This was also accompanied by a significant increase in liver triglycerides, inflammation and oxidative stress.

Frankincense, resin of *Boswellia* tree, had been an important trade material for the civilizations located in the Arabian Peninsula and North Africa for at least 3000 years. It is a natural oleo-gum resin obtained through incisions made in the trunks of trees of the genus *Boswellia* (Family *Burseraceae*) [11-13]. It is known as Olibanum, Kendar, Luban Dakar or Bakhor (In Arabic) and

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Salai Guggal (In Ayurvedic medicine) [14, 15]. In general, the genus *Boswellia* comprises 25 species. The species are widely distributed on the Arabian Peninsula (*Boswellia sacra*), in India (*Boswellia serrata*), in North Africa, Somalia (*Boswellia carterii* and *Boswellia frereana*), Ethiopia (*Boswellia papyrifera* and *Boswellia rivae*) and Eritrea (*Boswellia neglecta*) [11-13]. Traditionally, the oleo-gum resin of some *Boswellia* species such as *Boswellia serrata* and *Boswellia carterii* has been used in many countries for the treatment of rheumatic and other inflammatory diseases, including Crohn's disease and ulcerative colitis [16, 17]. Furthermore, the extracts and essential oils of frankincense have been used as antiseptic agents in a mouth-wash as well as in the treatment of coughs and asthma [18]. The anticancer, anti-inflammatory, immunomodulatory, antimicrobial and even antidiabetic activities of several *Boswellia* species have been reported [16, 18-22].

Drugs used to control and/or treat NAFLD are available. However, owing to the current importance of dietary sources as cheap and safe natural agents and the scarce of studies investigated the hepatocurative effects of frankincense, in particular, on NAFLD, this study was carried out.

MATERIALS AND METHODS

Plant Materials: Frankincense (Oleogum resin of *Boswellia* species) was purchased from the local market for medicinal plants and herbs, Tanta City, Al-Gharbia Governorate, Egypt.

Animals: A total of 23 normal male albino rats (Sprague_ Dawley strain) weighing 80 ± 5g were obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt.

Chemicals and Other Required Materials: Casein, vitamins, minerals, cellulose, choline chloride, DL-methionine, CCl_4 and other required chemicals were obtained from El-Goumhouria Company for trading drugs, chemicals and medical appliances, Cairo, Egypt. Sheep tallow, sucrose, soybean oil and corn starch were obtained from the local market, Tanta City, Al-Gharbia Governorate, Egypt.

Preparation of Plant Materials: Frankincense was milled to a fine powder using pestle and motor (Stainless steel) and stored at room temperature in closed glass bottles in the dark until used.

Table 1: Composition of basal and high fat diets used in the experiment (g/kg diet)

HFD	Basal diet	Ingredient
140	140	Casein (80% protein)
10	40	Soybean oil
190	-	Sheep tallow
50	50	Cellulose
10	10	Vitamin mixture
35	35	Mineral mixture
2.5	2.5	Choline chloride
3	3	DL-Methionine
100	100	Sucrose
459.5	619.5	Corn starch

Diets: Basal diet used in the experiment was formulated according to Reeves *et al*. [23] with some modifications, while HFD was formulated according to Woods *et al*. [24] and Liu *et al*. [25] with some modifications, too (Table 1).

Animals & Study Design: Male albino rats (n = 23) of Sprague Dawley strain weighing (80± 5g) were housed in well-aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. After that, rats were weighed and divided into three groups. The first group (n = 5) was fed on basal diet as a normal control group for ten weeks, while the second and third groups (Each consisted of 9 rats) were fed on high fat diet (HFD) for four weeks, then injected subcutaneously with CCl_4 in paraffin oil (50% v/v, 2 ml/kg body weight) twice a week for two weeks according to Jayasekhar *et al*. [26]. At the end of the induction period (6 weeks), liver injury was diagnosed through the determination of aminotransferases activities, as the mean values of AST and ALT activities were 250 and 140 U/L, respectively in the serum of liver -injured groups versus 155 and 87 U/L, respectively in normal control group. However, 6 rats died by the end of the 6^{th} week after CCl_4 injection. Afterwards, the second group was kept untreated and fed on basal diet only, while the third group was fed on basal diet supplemented with 4% of frankincense (Frankin.) powder. The curative period continued for four weeks. Meanwhile, diet and water were provided *ad-libitum* and body weight was recorded once a week.

Blood and Tissue Sampling: At the end of the curative period, animals were weighed and fasted overnight before sacrificing. Blood samples were collected from the aorta of each rat into dry clean centrifuge tubes. Sera were carefully separated by centrifugation of blood samples at 3000 rpm (Round per minute) for 10 minutes at room temperature, then transferred into dry clean ebendorf tubes and kept frozen at - 20°C till analyzed. Moreover,

livers were removed by careful dissection, washed in ice-cold NaCl (0.9%), dried using filter paper and weighed. After that, a specimen from each liver was stored at -80ºC until homogenate preparation, while other specimen was immersed in 10% buffered neutral formalin solution for latter histopathological examination.

Body Weight Gain and Relative Liver Weight Calculation: Body weight gain (BWG) was calculated by subtracting the initial weight of each rat from its final weight. As for relative liver weight (RLW), it was calculated according to Angervall and Carlström [27].

Determination of Lipid Profile in Serum and Liver Tissue Homogenate: Triglycerides (TG) and total cholesterol (T.C) were determined in serum as well as liver tissue homogenate according to the methods described by Jacobs and VanDenmark [28] and Richmond [29] respectively. Phospholipids (PhLs) concentration also was determined in liver tissue homogenate according to the method of Ray *et al*. [30]. In addition, high density lipoprotein cholesterol (HDL-c) was determined according to the method proposed by Friedwald *et al*. [31] while low and very low-density lipoprotein-cholesterols, (LDL-c and VLDL-c) were calculated according to the equations of Friedwald *et al*. [31] too.

Assessment of Antioxidant/oxidant Biomarkers in Liver Tissue Homogenate: In liver tissue homogenate, catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities were measured according to the methods described by Aebi [32], Beauchamp and Fridovich [33] and Ellman [34] respectively. On the other hand, lipid peroxidation expressed as malondialdehyde (MDA) was determined following the method suggested by Ohkawa *et al*. [35] Nitric oxide (NO) was similarly measured by the Griess reaction [36].

Determination of Liver Enzymes and Serum Proteins: In serum, the activities of liver enzymes including aminotransferases´ (AST and ALT) [37] alkaline phosphatase (ALP) [38] and gamma -glutamyltransferase (GGT) [39] were determined. Moreover, total protein (T.P) and albumin were determined according to the methods described by Gornall *et al*. [40] and Doumas *et al*. [41] respectively.

Histopathological Examination: After sacrificing, specimen from each liver was taken and immersed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with haematoxylin and cosin according to Drury and Wallington [42].

Statistical Analysis: Statistical analysis was carried out using the programme of statistical package for the social sciences (SPSS), PC statistical software (Version 20; Untitled - SPSS Data Editor). The results were expressed as mean ± standard deviation (mean ± SD). Data were analyzed using one-way classification, analysis of variance (ANOVA) test. The differences between means were tested for significance using Duncan test at $p<0.05$ [43].

RESULTS

Body Weight Gain & Relative Liver Weight: Effects of frankincense on body weight gain and relative liver weight in CCl_4-intoxicated rats previously fed on high fat diet were illustrated in Table (2). It could be noticed that body weight gain of untreated liver -injured group was significantly higher than that of normal control group by the end of the experiment, as frankincense (Frankin.) -fed group recorded no significant difference compared with normal control group. On the other hand, the relative liver weight of untreated liver -injured group was significantly higher than that of normal control group. Frankincense reduced relative liver weight compared with untreated liver -injured group, however, the decrease was not significant.

Lipid Profiles: Effects of frankincense on lipid profiles in liver and serum of CCl_4-intoxicated rats previously fed on high fat diet were illustrated in Table (3). It was found that untreated liver - injured group recorded significant increases in both triglycerides and total cholesterol in liver tissue homogenate as compared to normal control group. Frankincense feeding led to significant reductions in both parameters, however it could not return them toward their normal values recorded by normal control group. In contrast, liver phospholipids were significantly lower in untreated liver -injured group compared with normal control group. Frankincense induced a significant increase in liver phospholipids compared with untreated liver -injured group, with significant decreases as compared to normal control group. In serum, there were no significant differences in the mean values of triglycerides, total cholesterol, HDL-c and VLDL-c among

Table 2: Effects of frankincense on body weight gain and relative liver weight in CCl_4-intoxicated rats previously fed on high fat diet.

	Groups		
Parameters	Normal control	Liver -injured	Liver -injured + Frankin.
BWG (g)	10.60 ±1.52[b]	14.00±2.24[a]	10.00±1.58[b]
RLW (%)	0.021±0.00[b]	0.032±0.01[a]	0.024±0.01[ab]

- Values that have different letters in each row differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table 3: Effect of frankincense on lipid profiles in liver and serum of CCl_4-intoxicated rats previously fed on high fat diet.

	Groups		
Parameters	Normal control	Liver -injured	Liver -injured + Frankin.
Liver TGs (mg/g)	35.42±6.30[c]	79.14±10.42[a]	64.20±9.41[b]
Liver T.C (mg/g)	40.19±5.65[c]	65.05±9.38[a]	54.00±8.19[b]
Liver PhLs (mg/g)	50.07±8.41[a]	27.47±4.31[c]	37.00±6.69[b]
Serum TGs (mg/dl)	58.00±7.38	70.25±9.06	63.60±9.56
Serum T.C (mg/dl)	98.60 ±12.82	117.40±17.59	113.60±14.48
Serum HDL-c (mg/dl)	46.40±8.56	36.80±6.22	39.00±6.12
Serum LDL-c (mg/dl)	40.60±6.75[b]	66.55±9.03[a]	61.88±8.67[a]
Serum VLDL-c (mg/dl)	11.60±1.48	14.05±1.81	12.72±1.91

- Values that have different letters in each row differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table 4: Effect of frankincense on antioxidant enzymes and oxidative markers in liver tissue homogenate of Ccl_4-intoxicated rats previously fed on high fat diet

	Groups		
Parameters	Normal control	Liver -injured	Liver -injured + Frankin.
CAT (Mmol/g)	0.39±0.05[a]	0.15±0.03[c]	0.28±0.03[b]
SOD (U/g)	0.36±0.05[a]	0.11±0.03[c]	0.25±0.02[b]
GSH (ng/g)	0.35±0.04[a]	0.13±0.03[c]	0.21±0.03[b]
MDA (Mmol/g)	0.10±0.02[c]	0.33±0.06[a]	0.22±0.04[b]
NO (Mmol/g)	0.32±0.03[a]	0.09±0.02[c]	0.18±0.02[b]

- Values that have different letters in each row differ significantly, while the difference among those with similar letters completely or partially is not significant.

all studied groups. Only serum LDL-c was significantly elevated in untreated liver -injured group compared with normal control group. Frankincense reduced the mean value of serum LDL-c as compared to untreated liver -injured group, however, the difference was not significant.

Antioxidant Enzymes & Oxidative Markers: Effects of frankincense on antioxidant enzymes and oxidative markers in liver tissue homogenate of CCl_4-intoxicated rats previously fed on high fat diet were illustrated in Table (4). It was found that the activities of all studied antioxidant enzymes including CAT, SOD and GSH were significantly lowered in liver tissue homogenate of untreated liver -injured group as compared to normal control group. Frankincense -fed group recorded significant increases in the activities of the three enzymes compared with untreated liver -injured group. Similarly, the mean value of NO in liver tissue homogenate of untreated liver -injured group was significantly lower as compared to normal control group. Frankincense -fed group recorded significant increase in liver NO compared with untreated liver -injured group, but could not normalize it. Conversely, liver MDA increased significantly in untreated liver -injured group as compared to normal control group. Frankincense -fed group recorded significant decrease in this parameter compared with untreated liver -injured group, with significant increase compared with normal group.

Liver Enzymes: Effects of frankincense on the activities of liver enzymes in serum of CCl_4-intoxicated rats previously fed on high fat diet were illustrated in Table (5). It could be noticed that the activities of all studied liver enzymes, namely AST, ALT, ALP and GGT in serum of untreated liver -injured group were significantly higher compared with normal control group. Frankincense -fed group recorded significant decreases in the activities of ALT and GGT compared with untreated liver -injured group, while AST and ALP activities were decreased in frankincense -fed group, but the decreases were not significant.

Table 5: Effect of frankincense on the activities of liver enzymes in serum of CCl_4-intoxicated rats previously fed on high fat diet

	Groups		
Parameters	Normal control	Liver -injured	Liver -injured + Frankin.
AST (U/L)	148.00±18.91[b]	235.33±30.30[a]	220.60±28.14[a]
ALT (U/L)	83.20±11.88[c]	159.25±20.47[a]	110.40±16.44[b]
ALP (U/L)	197.00±21.68[b]	304.20±40.55[a]	259.40±34.69[a]
GGT (U/L)	26.33±3.63[c]	65.25±6.40[a]	47.20±7.79[b]

- Values that have different letters in each row differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table 6: Effect of frankincense on total protein and albumin in serum of CCl_4-intoxicated rats previously fed on high fat diet.

	Groups		
Parameters	Normal control	Liver -injured	Liver -injured + Frankin.
T.P (g/dl)	6.74±0.74[a]	4.60±0.60[c]	5.68±0.65[b]
Albumin (g/dl)	4.68±0.68[a]	3.02±0.36[b]	3.78±0.58[b]

- Values that have different letters in each row differ significantly, while the difference among those with similar letters completely or partially is not significant.

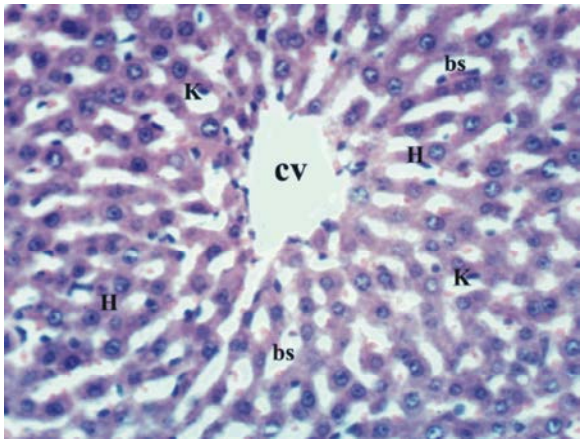

Fig. 1: Liver section of rat from normal control group showing the normal histological structure of hepatic lobule, central vein (cv) and radiating polygonal hepatocytes (H). The hepatocytes had homogenous granular cytoplasm with centrally located nucleus with prominent envelops and normal distributed chromatin. The liver strands were alternating with narrow blood sinusoids (bs) lined by endothelial cells and distinct phagocytic Kuffer cells (K) (H & E X 400)

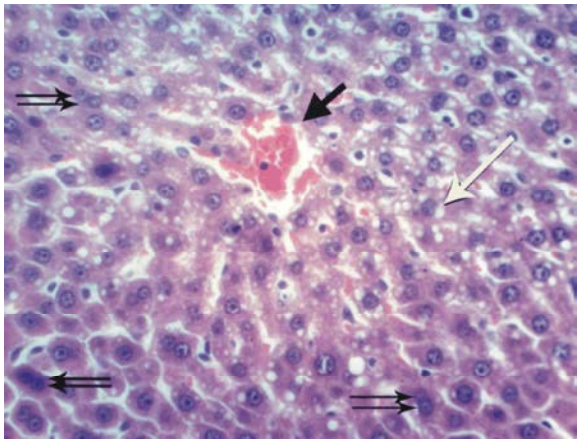

Fig. 2: Liver section of rat from untreated liver -injured group showing irregular and congested central vein (Arrow), increase number of binucleated hepatocytes (Double arrows), cytoplasmic degeneration and fatty change of hepatocytes (White arrow) (H & E X 400).

Serum Proteins: Effects of frankincense on total protein and albumin in serum of CCl_4-intoxicated rats previously fed on high fat diet were illustrated in Table (6). It was found that the mean values of total protein and albumin in serum of untreated liver -injured group were significantly lower than those of normal control group. Frankincense -fed group recorded significant increase in serum total protein compared with untreated liver -injured group, while it exhibited an elevation in serum albumin, but the increase was not significant.

Histopathological Findings: Results of the histopathological examination of rat livers from different experimental groups were illustrated in Figures (1-4). Figure (1) represents liver section of rat from normal control group, in which the normal histological structure of hepatic lobule, central vein and radiating polygonal hepatocytes can be observed. The hepatocytes had homogenous granular cytoplasm with centrally located nucleus with prominent envelops and normal distributed chromatin. The liver strands were alternating with narrow blood sinusoids lined by endothelial cells and distinct phagocytic Kuffer cells. HFD feeding followed by CCl_4 exposure led to marked lesions in liver tissue including irregular and congested central vein, increase

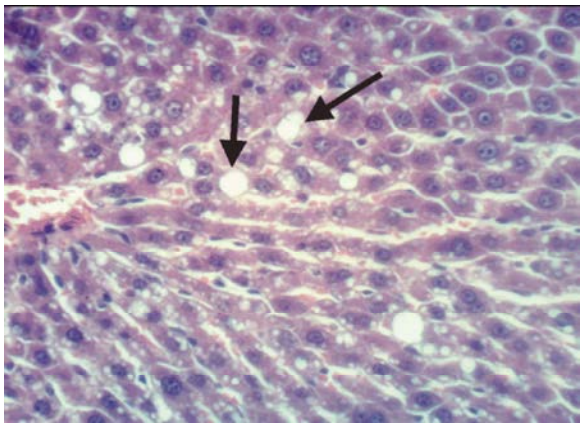

Fig. 3: Liver section of another rat from untreated liver -injured group showing fatty change of hepatocytes and deteriorated blood sinusoids (H & E X 400)

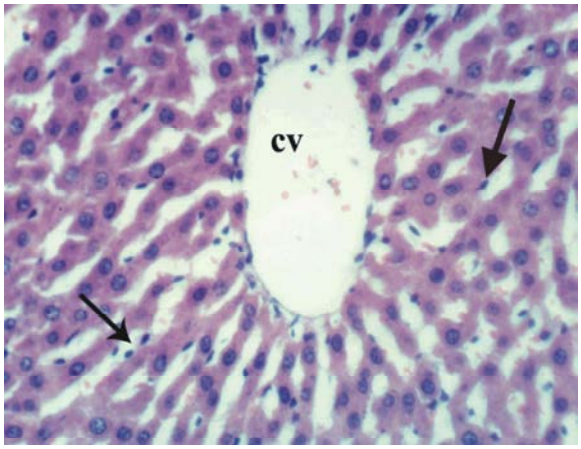

Fig. 4: Liver section of rat from frankincense -fed group showing widening central vein (cv) engorged with RBCs, irregular cords of hepatocytes and Kupffer cell activation (Arrows) (H & E X 400)

number of binucleated hepatocytes, cytoplasmic degeneration (Figure 2), fatty change of hepatocytes (Figures 2 and 3) and deteriorated blood sinusoids (Figure 3). Supplementation of basal diet with frankincense decreased these lesions. Figure (4) represents liver section of rat from frankincense -fed group, in which widening central vein engorged with RBCs, irregular cords of hepatocytes and Kupffer cell activation can be noticed.

DISCUSSION

High fat diet -fed animals are one of diet modulations leading to NAFLD [8]. On the other hand, CCl_4 is one of the toxins known to induce NAFLD [9]. Chheda *et al*. [10] presented NAFLD rat model developed over 8 weeks using a modified fast food diet with a CCL_4 dose (0.5 ml CCl_4/kg body weight). The present study not only presented a new rat model of NAFLD, but also it investigated the curative effects of frankincense on this model.

The current results indicated that liver -injured group, by the end of the experiment, gained more body weight than normal control group with significance at P□0.05. This effect is attributed to HFD feeding and is in agreement with Woods *et al*. [24] who demonstrated that high fat diet-fed rats weighed more than low fat controls.

By the end of the curative period, the BWG of untreated liver -injured group was significantly higher than normal control group, while frankincense -fed group recorded no significant difference compared with normal control group. In agreement with the current data, Zaitone *et al*. [44] found that boswellic acids (Components of frankincense) induced anti-obesity properties in HFD -fed rats and this effect can be attributed to the reduction of feed intake. Singh *et al*. [45] noticed a significant decrease in body weight in Wistar rats received *Boswellia serrata* (1000 mg/kg body weight). This effect was suggested to be due to the high concentration of guggalsterones in *Boswellia serrate*. Guggalsterones stimulates the thyroid and increases its efficiency, leading to metabolic up-regulation and increased caloric burn and therefore possible weight loss [46].

The relative liver weight significantly increased in untreated liver -injured group compared with control group as a result of the synergistic effect of both high fat diet and CCl_4. In line with these results, Bravo *et al*. [47] demonstrated that the high fat diet used to induce nonalcoholic fatty liver disease in rats caused an increase in liver TG (× 2.6) and cholesterol (+ 30%). On the other hand, Shiratori *et al*. [48] found that fat-storing cells from CCl_4-treated rats divided rapidly, in the presence of Kupffer cells, as compared with untreated rats. Fatty change noticed in hepatocytes of untreated liver -injured rats, in the present study, as well as the significant increase in triglycerides concentration in liver tissue homogenate support the significant increase of relative liver weight.

Like HFD, CCl_4 increases liver cholesterol. This may be due to increased cholesterol synthesis [49]. Compared to other lipid classes, phospholipids, the vital biomembrane components, are the most sensitive to lipid peroxidation induced by CCl_4 [50]. Similarly, high fat diet feeding was found to increase phospholipid peroxidation in rat liver [51] which in turn is involved in the pathophysiology of many abnormalities.

Because frankincense lowered TG concentration in liver tissue homogenate, the fatty changes in hepatocytes somewhat disappeared and relative liver weight decreased. However, the decrease of relative liver weight in frankincense - fed group was not significant. This may be due to low experimental period. In harmony with the present results concerning the effect of the frankincense on liver levels of TG and total cholesterol, Zutshi *et al*. [52] found that fat deposits in various organs including iris were significantly less marked in the kendar -treated group. The effect was probably at the biosynthesis level.

Except for LDL-c, lipid profiles in serum did not significantly respond to the co-effects of HFD and CCl_4 or frankincense feeding. While serum LDL-c increased significantly in untreated liver -injured group compared with control group, its decrease noticed in frankincense - fed group was not significant. The insignificant changes noticed in serum triglycerides, total cholesterol and HDL-c in all groups may be due to low curative duration.

In the current study, significant reductions were noticed in nitric oxide and the activities of CAT, SOD and GSH in liver tissue homogenate of untreated liver -injured group, while MDA level as lipid peroxide was significantly increased. Both HFD and CCl_4 are responsible for these effects. In agreement with these results, Deng *et al*. [53] observed that rats fed a HFD exhibited a higher MDA level along with lower SOD and GSH levels. On the other hand, Wu *et al*. [54] revealed that exposure to CCl_4 caused decreases in hepatic SOD activity and the total antioxidant status, as well as an increase in the hepatic malondialdehyde level.

The beneficial effects of frankincense on antioxidant defense system in liver tissue, as noticed in the present study, were in line with Zaitone *et al*. [44] who revealed that boswellic acids at the used doses (125 and 250mg/kg) decreased MDA level and increased GSH activity in liver tissue compared to HFD group. In RBC, Masoud *et al*. [55] demonstrated that the administration of frankincense extract enhanced the antioxidant defense system in alloxan-induced diabetic rats. This recovery may be due to the activation of enzymes, resulting in higher CAT activity, an antioxidant enzyme important at higher H2O2 concentration [56] and higher thiol levels which lead to a reduce ROS level and hence reduce oxidative stress by preventing the generation of free radicals and, thus inhibiting the development of diabetes mellitus. In general, pharmacokinetic tests of gum resin of different *Boswellia* species have shown that they are moderate to potent inhibitors of CYP enzymes, a large and diverse group of enzymes that catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones [57].

It is well known that those components able to reduce nitric oxide production in the liver tissue possess hepatoprotective effects [58]. According to Pandey *et al*. [59] various terpenoid compounds in frankincense were found to inhibit nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages.

As expected, untreated liver -injured group, in the present study, recorded a significant increase in the activities of transaminases (AST and ALT), alkaline phosphatase as well as gamma -glutamyltransferase in serum as compared to control group, while serum total protein and albumin were significantly decreased. Both HFD and CCl_4 are responsible for these effects, as indicated by many previous studies. As for liver enzymes, Zaitone *et al*. [44] revealed that high fat feeding resulted in elevations in the serum activities of ALT and AST. Similar effects were reported in CCl_4 -treated animals. Wu *et al*. [54] demonstrated that in CCl_4 -intoxicated rats, hepatic lipids levels and plasma aminotransferases activities were increased, while antioxidant defense system was impaired. Chheda *et al*. [10] insured these results, as they reported that fast food diet-CCl_4 animals showed an increase in liver injury confirmed by marked elevation in serum AST, ALT, GGT and ALP.

Regarding serum proteins, Marques *et al*. [60] found that serum albumin was decreased after high fat feeding in both Wistar and Sprague-Dawley Rats. Similarly, Shittu *et al*. [61] observed a marked decrease in the total proteins in liver and serum of CCl_4-administered rats when compared with the control rats. Frankincense feeding, as noticed in the present study, reduced the activities of all studied liver enzymes in serum, while induced an increase in serum levels of total protein and albumin. These effects suggest that the liver health was improved. However, the insignificant decrease in AST and ALP activities and the insignificant increase in serum albumin may be due to low curative period.

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