Egypt. J. of Nutrition and Health Vol. 17 No. 1 Jan (2022)

Bay and camphor leaf powders as inhibitors for hyperlipidemia and hepatotoxicity associated with industrial trans-fats consumption in male rats

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Abstract

Trans-fats are found in large numbers of food products as well as naturally in some animal sources in small amounts.Their consumption was associated with a lot of health problems. The main objective of this study was to investigate the hypolipidemic and hepatoprotective effects of bay and camphor leaf powders (BLP and CLP, respectively) in trans-fat enriched diet -fed rats. Thirty six adult male albino rats were used and divided into six equal groups, including the control group while groups 2 to 6 were fed for six weeks on trans-fats enriched diet(TFED, 12% hydrogenated vegetable fat) only (group 2), TFED containing 0.5 and 1 % of BLP (groups 3 and 4, respectively), or TFED containing 0.5 and 1 % of CLP (groups 5 and 6, respectively). At the end, body weight gain was calculated. Lipid profile, liver functions, pancreatic lipase and insulin levels were determined in sera, while cholesterol and phospholipids levels and oxidative stress markers were determined in liver tissue. Moreover, the histopathological examination of liver was performed and phenolic compounds in both powders were determined. Feeding TFED resulted in overweight, hyperlipidemia and liver dysfunction along with oxidative stress, which was confirmed histopathologically. Due to their content of phenolic compounds rather than other antioxidant agents, BLP and CLP exerted anti-obesity and hypolipidemic protective properties and alleviated the risky effects of TFED on hepatocytes. Thus, bay and camphor leaf powders can be considered efficient inhibitors for hyperlipidemia and hepatotoxicity associated with trans-fats consumption.

Keywords: Hyperlipidemia,hepatotoxicity,hydrogenated fats,bay leaves,camphor leaves, rats

Introduction

Trans-fats are composed of *trans*-fatty acids (TFAs) which are the stereoisomers of the naturally occurring *cis*-fatty acids (CFAs). They are produced industrially during hydrogenation of unsaturated oils via a process called “hardening” ***(Emken, 1984)***. This process reduces most of the double bonds in unsaturated fatty acid moieties of the oils but isomerizes some of the *cis* double bonds to a *trans* configuration, resulting in improved stability, a longer shelf life and the acquisition of desirable tactile, functional and sensory properties. Trans-fats are found in foods originating from ruminant animals, such as cows and sheep, and are found in foods containing partially hydrogenated vegetable oils (PHVO). Industrial trans-fats are widely used to produce a variety of foods, including margarines, cookies, pastries, salad dressings and cooking oils ***(Korver and Katan, 2006)***.

Approximately 540,000 deaths each year can be attributed to intake of industrially produced trans-fatty acids ***(Wang et al., 2016)***. High trans-fat intake increases the risk of death from any cause by 34%, coronary heart disease deaths by 28% ***(De Souza et al., 2015)***.Trans-fat increases low density lipoprotein cholesterol (LDL-c) levels while lowering high density lipoprotein cholesterol (HDL-c) levels ***(De Souza et al., 2015; Mozaffarian et al., 2006)***.

Animal trans-fat levels can comprise up to 6% of a product's fat content in ruminant foods, and industrial trans-fat levels can comprise up to 60% of a product's fat content in foods containing partially hydrogenated vegetable oil (PHVO). The discovery of adverse effects on the blood cholesterol profile and the increased risk of coronary heart disease of industrial trans-fat ***(Mozaffarian et al., 2006; De Souza et al., 2015)*** have led to public health recommendations to lower total trans-fat intake to below 1% of total energy intake, primarily by the removal of industrial trans-fat **(FAO/WHO, 2010)**.
On the other hand, high intake of dietary trans-fats has been reported to enhance the steatogenic and pro-fibrotic properties of the western diet in mice***(Hansen et al., 2017)***. Trans-fats likely sensitize mice to the effects of high fat diet by increasing insulin resistance, hepatic lipogenesis and oxidative stress ***(Machado et al., 2010)***.

Plants are natural source of treatment and are used from ages for food and medicine ***(Shah etal., 2021)***.Herbal plants have also been found to nourish the body and provide vitamins, minerals, and many trace elements that are easy to absorb ***(Melkegna and Jonah, 2021)***.*Laurus nobilis* L.(*L. nobilis*, bay) leaves are used mainly as a seasoning in cooking. They are rich in active compounds such as phenols, flavonols and flavones, and have antioxidant and antimicrobial effects ***(Palazzo et al., 2020)***.

L. nobilis has been positively evaluated by various researchers and explored wide range of pharmacological activities. Antioxidant and wound-healing effects of the ethanolic extract of *L. nobilis* have been well reported ***(Vardapetyan et al., 2013)***.Bay leaves have many other biologic activities such as antibacterial, antiviral, immunostimulant,anticholinergic, antifungal, insect repellant, anticonvulsant, anti-mutagenic,analgesic and anti-inflammatory activities***(Batool et al., 2020)***. *L. nobilis* was also used for preventing and treating type II diabetes because it reduces the level of serum glucose ***(Khan et al., 2009)***.

On the other hand, *Cinnamomum camphora*L. (*C. camphora*) is an ever green tree native in China and distributed to some Asian countries. *C. camphora* is famous for its ornamental, economic, and medicinal value ***(Chen and Dai, 2015)***. The leaves are considered one of the most important parts of this plant.They are rich in bioactive compounds ***(Zhang et al., 2020)*** which have strong fumigant and anthelmintic activities***(Chen et al., 2014)***. Also, the oil of the seed kernel has antioxidant, anti-inflammatory ***(Fu et al., 2016)***, anti-aging and anti-bacterial effects ***(Chen et al., 2018; He et al., 2018)***. However, little information is available regarding the preventive influences of *Laurus nobilis* L. and *Cinnamomum camphora*L. leaf powders on hyperlipidemia and hepatotoxicity associated with industrial trans-fats consumption *in vivo*;hence this study was carried out.

Materials and Methods

Materials:

Plant leaves:

Dry leaves of bay (*Laurus nobilis* L.) were purchased from Arab company for Pharmaceutical and Medicinal plants, MEPACO, Egypt. Fresh champhor (*Cinnamomum camphora.*) leaves were sampled from several parts of Nawag village, Tanta City, Gharbiya Governorate, Egypt.

Animals:

A total of 36 adult male albino rats (*Sprague_ Dawley strain*) weighing 150±10 g were obtained from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt.

Chemicals and other ingredients:

Casein (≥ 80% protein), cellulose, vitamins, minerals, corn starch, DL–methionine, choline chloride, formalin, diethyl ether and other required chemicals were obtained from Elgomhouria Company for Trading Drugs, Chemicals and Medical Appliances, Cairo, Egypt.Soybean oil and the vegetable ghee were purchased from the local market, Tanta City, Gharbiya Governorate, Egypt.

Methods:

Drying of fresh camphor leaves:

Fresh camphor leaves were washed thoroughly, allowed to drain and then subjected to solar energy for drying.

Preparation of leaf powder:

Dry leaves of the studied plants were milled to fine powders, sieved and stored at room temperature in closed glass bottles in dark place until used.

Phenolic profile identification of plant samples*:*

Phenolic compounds of leaf powders were identified and determined by high-performance liquid chromatography (HPLC) according to ***Goupy et al. (1999)***.

Experimental diets:

Basal diet was prepared from fine ingredients per 100g. It had the following composition: Casein (≥ 80% protein) 14%, soybean oil 4%, cellulose 5%, mineral mixture 3.5%, vitamin mixture 1%, choline chloride 0.25%, DL-methionine 0.3% and corn starch up to 100g ***(Reeves et al., 1993)***.
Trans-fats enriched diet (TFED) had the same composition of basal diet mentioned formerly with substituting soybean oil with a source of industrial trans-fats(vegetable ghee) and increasing the percentage from 4% to 12% according to ***Angelis-Pereira et al. (2017)***. For treated groups, TFED was supplemented with the powder of either bay or camphor leaves(BLP or CLP, respectively) at two percentages (0.5 and 1%)

Experimental design:

Animals were kept in clean wire cages under hygienic conditions in a room maintained at a relative humidity 40-60%, 20–25°C and 12/12 h day light/darkness cycle. Feed was introduced (*ad libitum*) to the rats for adaptation for one week in special food containers to avoid scattering. Similarly,

fresh water was provided *ad-libitum* and checked daily. After that, rats were randomly assigned to 6 equal groups. The 1st group was fed on basal diet only as a negative control group(-veg), while the 2nd group was fed on trans-fats enriched diet (TFED) only as a positive control group(+veg). The 3rd and 4th groups were fed on TFED containing BLP (0.5 and 1%, respectively), while the 5rd and 6th groups were fed on TFED containing CLP (0.5 and 1%, respectively).

The experiment lasted for six weeks. Meanwhile, rats were weighed weekly. At the end, animals were weighed, fasted overnight, and then sacrificed under very light ether anesthesia. Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 round per minute for 15 minutes at room temperature, transferred into dry clean Eppendorf tubes, then kept frozen at - 20˚C for biochemical determinations. Livers were removed from rats by careful dissection, washed in saline solution (0.9%) and dried using filter paper. A specimen of each liver was kept in formalin solution (10%) for later histopathological examination, while other specimen was kept at (-80 ˚C) for later homogenization and analysis.

Calculation of body weight gain:

Body weight gain (BWG) in grams was calculated by subtracting the initial weight of each rat from its final weight.

Determination of lipid profile:

Triglycerides (TG), total cholesterol (T.C) and high density lipoprotein cholesterol (HDL-c) were determined in serum according to the method described by ***Trinder and Ann (1969)***, ***Richmond (1973)*** and***Lopes- Virella et al. (1977)***,respectively.On the other hand, very low and low density lipoprotein cholesterols(VLDL-c and LDL-c, respectively) were calculated using the equations of ***Friedwald et al. (1972)***.Atherogenic coefficient(AC) was calculated according to ***Brehm et al. (2004)*** and ***Nimmanapalli et al. (2016)*** using the following equation: AC = (T.C - HDL-c)/HDL-c.

Determination of pancreatic lipase:

Pancreatic lipase (PL) was determined in serum according to the method described by ***Lykidis et al. (1994)***.

Determination of liver cholesterol and phospholipids:

Cholesterol and phospholipidswere determined in liver tissue homogenate (Liv. Cho and Liv. PhLs)according to the methods of ***Richmond (1973)*** and ***Ray et al. (1969)***, respectively.

Determination of total antioxidant capacity and malondialdehyde level in liver tissue homogenate:

In liver tissue homogenate, total antioxidant capacity (TAC) was determined according to ***Koracevic et al. (2001)***. Also, lipid peroxidation, expressed as malondialdehyde (MDA), was determined in liver tissue homogenate according to the method described by ***Ohkawa et al. (1979)***.

Determination of liver functions:

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in serum according to the method described by ***Reitman and Frankel (1957)***, while total protein (T. P) and albumin (ALB) were estimated according to ***Sonnenwirth and Jaret (1980)***.

Histopathological examination:

After sacrificing rats, livers were removed and immersed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. After that, they were cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with haematoxylin and eosin***(Drury and Wallington, 1980)***.

Statistical analysis:

Statistical analysis was carried out using one way analysis of variance(ANOVA) test followed by Duncan test through the program of statistical packages for the social science (SPSS). Results were expressed as mean± standard deviation **(**mean± SD). The differences among means at $p < 0.05$ were considered significant ***(Snedecor and Cochran, 1989)***.

Results and Discussion

Phenolic profile in both bay and camphor leaves:

Bay and camphor leaf powders were analyzed by HPLC for their phenolic compounds. The obtained results were shown in table 1. Except for chlorogenic acid, bay leaf powder recorded lower content of all analyzed phenolic compounds than camphor leaf powder. Chlorogenic acid was not found in camphor leaf powder, while it was found in bay leaf powder (133.49 ppm). Phenolic compounds that were found in high concentrations in bay leaf powder (> 500 ppm) were catechin and ellagic acid (1536.12 and 586.95 ppm, respectively). This is in accordance with ***El-Gawishet al. (2021)*** who showed that *Laurus nobilis* recorded higher content of catechin and ellagic acid. On the other hand, the presence of some phenolic compounds such as pyrogallol as well as vanillic and ferulic acids is in agreement with ***Muñiz-Márquez et al. (2013)*** and ***Muchuweti et al. (2007)***. In camphor leaf powder, phenolic compounds that were found in high concentrations (> 1000 ppm) were ellagic acid, catechin, catechol and P-OH-benzoicacid (3474.51, 2918.73, 2307.79 and 1340.86 ppm, respectively). Thus, it can be concluded that the two compounds found in both powders in great amounts are ellagic acid and catechins, but in different arrangement. Regarding animal studies, ellagic acid was proved to have a significant hypolipidemic effect in mice liver ***(Xu et al., 2021)***. Catechins in green tea were found to induce a specific hypolipidemic effect in rats ***(Kim et al., 2011)***.

Table (1)
Analysis of phenolic compounds in both bay and camphor leaf powder samples

	Phenolic compounds (ppm)	
	Bay leaf powder	Camphor leaf powder
Catechin	1536.12	2918.73
Ellagic acid	586.95	3474.51
Ferulic acid	403.05	513.65
Caffeine	271.67	321.14
Catechol	271.09	2307.79
Chlorogenic acid	133.49	--
Pyrogallol	105.17	715.81
Coumarin	84.82	438.25
Vanillic acid	65.84	227.38
Caffeic acid	55.78	519.23
P-OH-benzoic acid	30.63	1340.86
4-Aminobenzoic acid	15.77	69.33
Gallic acid	3.14	77.69

Body weight gain &serum lipid profile:

Data presented in table 2 showed the effect of feeding ratsTFED containing bay and camphor leaf powders on body weight gain,serum lipid profile as well as atherogenic coefficient (AC). The mean values of BWG, TG, T.C,LDL-c,VLDL-c and AC were significantly increased in TFED -fed group compared with the control group. Feeding TFED supplemented with bay or camphor leaf powder (0.5, 1%) caused significant decrease compared with feeding on TFED only. In contrast, the mean value of HDL-c was significantly lower in TFED -fed group compared with the control. TFED containing bay or camphor leaf powders (0.5, 1%) led to significant increase compared with TFED alone. In general, the best results were obtained in the groups fed on TFED+1% of BLP, followed by TFED+ 1% CLP, with no significant difference between them.

The present results were in agreement with ***Kavanagh et al. (2007)*** who revealed that monkeys fed on a trans-fat diet gained 7.2% of their body weight, as compared to 1.8% for monkeys on a monounsaturated fat diet.Trans-fats enriched diet has an indirect dyslipidemic action. It increases the fat mass in the body***(Kavanagh et al., 2007)***, which in turn is associated with insulin resistance***(Arita et al., 1999; Ishibashi et al., 2018)***. It is thought that in the insulin-resistant state, dyslipidemia occurs ***(DeFronzo and Ferrannini, 1991)***.

The hypolipidemic effect of bay leaf powder, in the present study,was in agreement with ***Al–Samarrai et al. (2017)***. ***Gasparyan et al.(2015)***also mentioned that extract of bay leaves decreased serum T.C and TG in carbon tetrachloride–intoxicated male Wistar rats. Similarly, ***Casamassima et al. (2017)*** showed that bay leaves improved lipid profile in hyperlipidemic rabbits. In fact, nearly no studies were found on the hypolipidemic action of camphor leaves. However, other parts of the plant were analyzed and studied. ***Fu et al. (2016)***found that levels of blood total cholesterol, triglycerides and free fatty acid in the *Cinnamomum camphora* seed kernel oil group were decreased significantly compared to lard and soy groups.

This hypolipidemic action of the both powders can be attributed to its phenolic content. As mentioned above, the two compounds found in both powders in great amounts were ellagic acid and catechins. Both compounds were reported to have a significant hypolipidemic action in animal studies***(Xu et al., 2021; Kim et al., 2011)***. Of phenolics, flavonoids and their derivatives play an important role in enhancing lipid profile.For example, quercetin was found to decrease serum LDL-c and TG levels in rats ***(Rafieian-Kopaei et al., 2013)***.***AIchalabi et al., (2020)***supported the previous suggestion, as they attributed the hypolipidemic action of *Laurus nobilis* alcoholic extract in diabetic rats to flavonoids found in the leaves, since they have the ability to manage lipid profile.

Table (2)

Effect of bay and camphor leaf powders on body weight gain,serum lipid profile and atherogenic coefficient in TFED -fed rats

Parameters / Groups	BWG (%)	TG (mg/dl)	T.C (mg/dl)	HDL-c (mg/dl)	VLDL- c (mg/dl)	LDL- c (mg/dl)	AC
Control(-ve g)	6.78± 0.57^{d}	63.80± 7.33^{d}	72.50± 4.70^{d}	44.05± 3.40^{a}	12.76± 1.47^{d}	15.56± 1.75^{d}	13.12± 1.46^{d}
TFED(+ve g)	17.1± 1.25^{a}	176.98± 16.64^{a}	129.90± 4.09^{a}	28.38± 2.03^{c}	35.40± 3.33^{a}	66.13± 1.79^{a}	37.73± 3.25^{a}
TFED + BLP (0.5%)	15.03± 0.68^{b}	108.30± 8.91^{bc}	87.93± 8.61^{bc}	34.43± 4.59^{b}	21.66± 1.78^{bc}	31.84± 2.40^{b}	22.61± 1.98^{bc}
TFED + BLP (1%)	11.12± 1.30^{c}	96.95± 2.47^{c}	76.13± 6.53^{d}	36.30± 2.87^{b}	19.39± 0.49^{c}	20.44± 2.64^{c}	20.04± 0.54^{c}
TFED + CLP (0.5%)	14.78± 0.93^{b}	121.73± 7.66^{b}	90.48± 8.69^{b}	35.05± 3.57^{b}	24.35± 1.53^{b}	32.08± 3.46^{b}	25.25± 1.70^{b}
TFED + CLP (1%)	11.82± 1.31^{c}	108.20± 4.78^{bc}	79.85± 3.83^{cd}	35.50± 3.05^{b}	21.64± 0.96^{bc}	22.66± 2.05^{c}	22.29± 1.01^{c}

-Values that have different letters in each column differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Pancreatic lipase in serum:

The effect of bay and camphor leaf powders on pancreatic lipase activity in serum of TFED -fed rats was presented in table 3.The activity of pancreatic lipase (PL) was significantly increased in serum of TFED -fed group compared with the control group. Marked improvement was noticed as a result of supplementing TFED with bay or camphor leaf powder (0.5, 1%). In general, the best results were obtained in the groups fed on TFED supplemented with the higher percentages of bay or camphor leaf powder.

TFED, used through the present study, is a type of high fat diet (HFD) as it consisted of 12% fats versus 4% only in basal diet. So, the present results were in agreement with***El-Hashash (2014)***. Previously, ***Gidez (1973)*** demonstrated that levels of pancreatic lipase were increased when the fat content of the diet was raised from about 5% to 15–22%.

Inhibitory effect of some plant extracts on pancreatic lipase was presented by ***Gholam hoseinian and co-worker,(2010)***,where they have shown the percent (%) inhibition of pancreatic lipase. According to the data given in a research paper percent inhibition of *Laurus nobilis* leaves against pancreatic lipase was 20.5% ***(Gholamhoseinian et al., 2010)***. Dietary fibers exert inhibitory actions on pancreatic lipase ***(Dukehart et al., 1989)***. Thus, the high content of crude fibers in bay leaf powder (26.3± 0.33/100 g) according to***Tawfek and Ali (2022)*** can account the reduced effect of BLP

–containing TFED compared with TFED alone on serum pancreatic lipase.
On the other hand, the decreasing effect of both powders on pancreatic lipase activity is a cause for their hypolipidemic effect.

Table (3)
Effect of bay and camphor leaf powders on pancreatic lipase (PL) activity in serum of TFED -fed rats

Parameters / Groups	PL (U/L)
Control(-veg)	10.07 ± 1.34^{c}
TFED(+veg)	16.70 ± 1.40^{a}
TFED + BLP (0.5%)	12.63 ± 0.91^{b}
TFED + BLP (1%)	10.37 ± 0.40^{c}
TFED + CLP (0.5%)	13.40 ± 1.41^{b}
TFED + CLP (1%)	10.53 ± 0.85^{c}

-Values that have different letters in each column differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Lipid profile and oxidation indices in liver tissue homogenate:

The mean values of cholesterol and malondialdehyde in liver tissue homogenate (Liv. Cho and MDA, respectively) were significantly increased in TFED -fed group compared with the control group. In contrast, the mean values of phospholipids and total antioxidant capacity in liver tissue homogenate (Liv. PhLs and TAC, respectively) were significantly decreased. Feeding TFED supplemented with bay or camphor leaf powder (0.5, 1%) improved all these indices significantly as compared to feeding on TFED only, except for TAC level as TFED+0.5% CLP could not affect it significantly. In general, the best results were obtained in the groups fed on TFED+1% of BLP, followed by TFED+ 1% CLP (Table 4).

In line with the present results, ***Bravo et al. (2011)*** 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%). Regarding the oxidative effect ofHFD, Also,***Machadoet al. (2010***) reported that trans-fats may sensitize mice to the effects of high fat diet by increasing insulin resistance, hepatic lipogenesis and oxidative stress. Moreover, ***Dhibi et al. (2011)*** revealed that the rates of hepatic lipid peroxidation were markedly higher in margarine -fed groups than in the control group.It was indicated that trans-fatty acids impair fat cell membrane fluidity. When they are incorporated into cell membranes, the membrane fluidity is reduced and the cells do not function as well. The resulting effect is then to promote further production of reactive oxygen species causing lipid peroxidation.

On the other hand, the antioxidant effect of bay leaves were in agreement with ***Fang et al. (2005)*** who reported that sesquiterpene lactones identified in bay leaf were found to have different pharmacological properties including enhancement of liver glutathione S-transferase (GST) activity. ***Elmastaş et al. (2006)***explained that the antioxidant activity of ethanol extract of *L. nobilis* leaves may be due to phenolic compounds present in the extract. Thus, it can be concluded that managing of lipid profile in liver tissue and the antioxidant effect of both studied leaf powders are due to their content of phenolic compounds such as ellagic acid ***(Xu et al., 2021)***.

Table (4)

Effect of bay and camphor leaf powders on lipid profile and oxidative indices in liver tissue homogenate of TFED -fed rats

Parameters / Groups	Liv. Cho (mg/dl)	Liv. PhLs (mg/dl)	TAC (mm/L)	MDA (nmol/g tissue)
Control(-veg)	77.67± 6.43^{e}	305.67± 19.56^{a}	0.46± 0.06^{a}	39.33± 5.51^{d}
TFED(+veg)	149.33± 3.06^{a}	115.33± 8.33^{e}	0.18± 0.08^{c}	99.00 ± 6.56^{a}
TFED + BLP (0.5%)	111.00 ±14.18^{c}	190.67± 9.45^{c}	0.30± 0.05^{b}	75.00± 8.19^{b}
TFED + BLP (1%)	86.67± 2.89d^{e}	249.00± 16.70^{b}	0.37 ± 0.05^{ab}	57.00 ± 9.00^{c}
TFED + CLP (0.5%)	123.33± 2.08^{b}	157.67± 8.14^{d}	0.20± 0.04^{c}	81.67± 9.45^{b}
TFED + CLP (1%)	98.00± 3.61^{d}	238.00± 9.54^{b}	0.33± 0.05^{b}	65.33 ± 14.57^{bc}

-Values that have different letters in each column differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Liver functions:

The activities of transaminases (ALT and AST) were increased significantly in serum of TFED group compared with the control group. In contrast, the mean values of total protein and albumin (T.P and ALB) were significantly decreased. Feeding TFED supplemented with bay or camphor leaf powder (0.5, 1%) improved all these indices significantly as compared to feeding on TFED only. The protective effects of bay and camphor leaf powders, as noticed here, increased by increasing their concentrations (Table 5).

These results were in agreement with ***Dhibi et al. (2011)*** who found that the activities of transaminases, alkaline phosphatase and lactate dehydrogenase increased significantly in margarine-consumed group compared to the control group.***Lin et al. (2010)*** concluded that higher saturated fat intake is significantly associated with the presence of high albuminuria.As a result, serum albumin decreased significantly. Regarding the effective role of *Laurus nobilis*, the present results were in line with ***Alchalabi et al. (2020)*** who mentioned that statistical reduction of the ALT, AST and ALP activities in diabetic group given *Laurus nobilis* alcoholic extract compared to normal control group and this was attributed to the antioxidant compounds in the extract. Also,***Casamassima et al. (2017)***reported a decrease in the activities of ALT and AST in New Zealand white growing rabbits as a result of feeding on dried leaves of *Laurus nobilis*-contained meals. In rats,***Mohammed et al. (2021)*** mentioned that albumin and total protein levels in *L. nobilis* extract group were insignificantly increased compared to untreated diabetic group.In general, the hepatoprotective effects of either bay or camphor leaf powder can be attributed to its antioxidant and hypolipidemic effects.

Table(5)

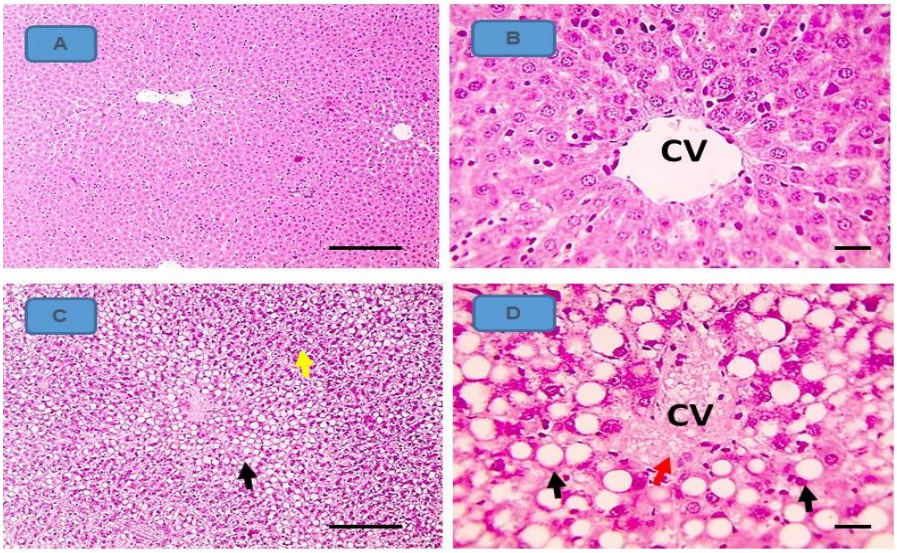
Effect of bay and camphor leaf powderson liver function indices in serum ofTFED -fed rats

Parameters / Groups	ALT (U/L)	AST (U/L)	T.P (g/dl)	ALB (g/dl)
Control(-veg)	11.35±2.85^{c}	86.75±8.66^{b}	7.30±0.23^{a}	4.27± 0.15^{a}
TFED(+veg)	39.58 ±4.55^{a}	197.58±17.26^{a}	5.18± 0.66^{c}	2.95± 0.41^{c}
TFED + BLP (0.5%)	11.75±1.74^{c}	85.73±7.72^{b}	6.73± 0.20^{a}	4.01± 0.10^{ab}
TFED + BLP (1%)	12.00± 1.84^{c}	82.40±6.90^{b}	6.92± 0.53^{a}	4.08± 0.21^{ab}
TFED + CLP (0.5%)	16.43± 2.54^{b}	90.28±7.41^{b}	6.11± 0.24^{b}	3.72± 0.22^{b}
TFED + CLP (1%)	11.43± 2.77^{c}	89.65±8.13^{b}	6.88± 0.32^{a}	4.06± 0.19^{ab}

-Values that have different letters in each column differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

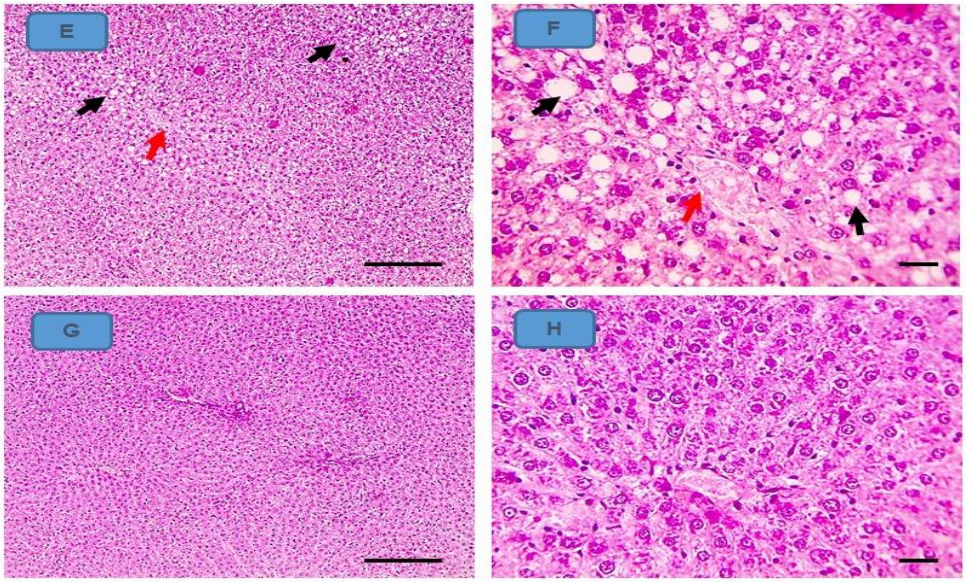
Histological examination results:

Histological examination results were illustrated in the following figures**:**



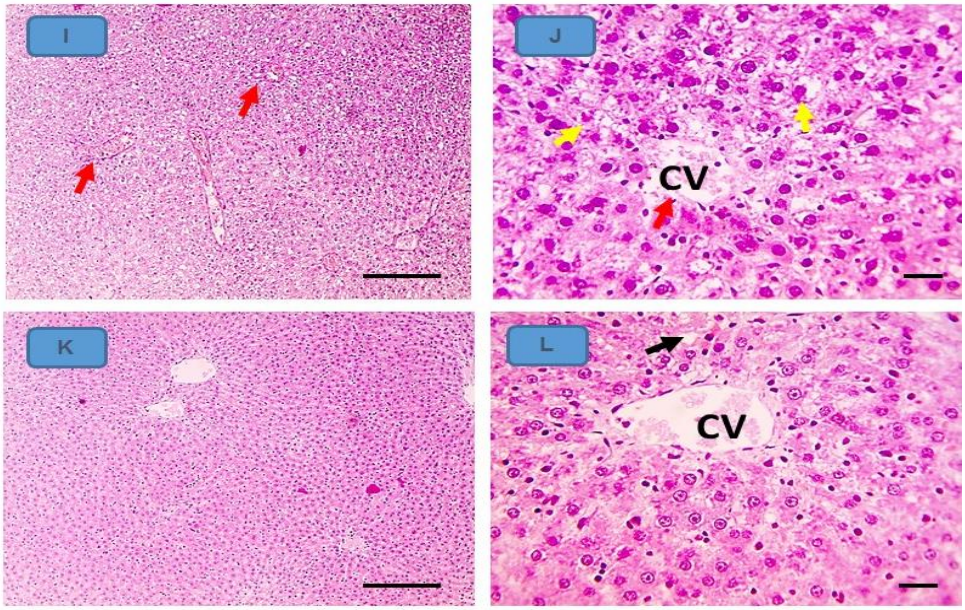
Figs. A&B:

Microscopic pictures of H&E stained liver sections show normal hepatocytes arranged in radiating plates around a central vein (CV) with normal sinusoids (s) in the control group. **Figs.C&D:** Liver sections from TFED –fed group show prominent centrilobular macrovesicular steatosis (black arrow) in hepatocytes around congested central vein (red arrow) with occluded sinusoids and the other hepatocytes show hydropic degeneration (yellow arrow). Low magnification X: 100 bar 100, high magnification X: 400 bar 50



Figs. E&F:

Microscopic pictures of H&E stained liver sections of rats fed on TFED containing 0.5% BLP show milder centrilobular macrovesicular steatosis (black arrow) in hepatocytes around mildly congested central vein (red arrow) compared to TFED-fed group. **Figs. G&H:** Liver sections from rats fed on TFED containing 1% BLP show retained normal histological picture of hepatocytes, central vein and sinusoids (s). Low magnification X: 100 bar 100, high magnification X: 400 bar 50



Figs. I&J:

Microscopic pictures of H&E stained liver sections of rats fed on TFED containing 0.5% CLP show mild hydropic degeneration (yellow arrow) in hepatocytes with occluded sinusoids and mild congestion (red arrow). **Figs. K&L:** Liver sections from rats fed on TFED containing 1% CLP show greatly improved histological picture with very few large cytoplasmic vacuoles (black arrow) and normal sinusoids (s). Low magnification X: 100 bar 100, high magnification X: 400 bar 50.

The abnormal changes noticed in hepatocytes of TFED group were in accordance with many animal studies.***Popescu et al. (2013)***found that HFD consumption for one month by male mice was associated with some abnormalities in hepatocytes includinghepatocyte swelling, sometimes associated with karyomegaly, granular or finely vacuolated cytoplasm,numerous activated Kupffer cells, indistinct sinusoids(no visible lumen), because of the volume increase of the hepatocytes, unaltered portal fields. These lesions were consistent with a medium hepatopathy (granular and vacuolar degeneration of the hepatocytes). Similarly,***El-Hashash (2014)*** reported that liver sections from rats fed on high saturated fat diet showed vaculation of cytoplasm, kupffer cells activation, fatty degeneration of hepatocytes, congestion of portal vein, proliferation of bile ductules and fibroplasia in portal triad that was associated with a portal infiltration with inflammatory cells (H and E ×400).

Insulin resistance and oxidative stress are the most important mechanisms through which high fat diet can negatively affect hepatocytes ***(Mehta et al., 2002)***. So, the protective effects noticed in the groups fed on TFED containing either BLP or CLP can be attributed to their content of polyphenolic compounds, rather than other antioxidants, which were proved to decrease insulin resistance and alleviate oxidative stress (***Fang et al. 2005***;***Elmastaş et al. 2006)***.

Conclusion

Bay and camphor leaf powders can be considered efficient semi protective agents against hyperlipidemia and hepatotoxicity associated with TFED consumption

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Egypt. J. of Nutrition and Health Vol. 17 No. 1 Jan (2022)

مسحوقى أوراق الغار والكافور كموانع لإرتفاع دهون الدم والتسمم الكبدي المرتبطين باستهلاك الدهون المتحولة الصناعية في ذكور الجرذان

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الملخص العربي

توجد الدهون المتحولة فى عدد كبير من المنتجات الغذائية كما تتواجد طبيعيا بكميات بسيطة فى بعض المصادر الحيوانية، وقد ارتبط استهلاكها بكثير من المشاكل الصحية.الهدف الرئيس من هذه الدراسة هو بحث التأثيرات الخافضة للدهون والواقية للكبد لمسحوقي أوراق الغار والكافور فى الجرذان التي تم تغذيتها على غذاء غنى بالدهون المتحولة. تم استخدام عدد 36 من ذكور جرذان الألبينو حيث تم تقسيمها إلى ست مجموعات متساوية العدد تشمل المجموعة الضابطة بينما تم تغذية المجموعات من 2 إلى 6 لمدة ستة أسابيع على غذاء غنى بالدهون المتحولة(والذى تألف من 12% من الزيوت النباتية المهدرجة)فقط بالنسبة للمجموعة الثانية أو محتويًا على مسحوق أوراق الغار بنسب 0.5، 1% بالنسبة للمجموعتين الثالثة والرابعة على الترتيب أو محتويًاعلى مسحوق أوراق الكافور بنسب 0.5، 1% بالنسبة للمجموعتين الخامسة والسادسة على الترتيب. فى النهاية، تم حساب الزيادة المكتسبة في وزن الجسم، كما تم تقدير الدهون ووظائف الكبد وانزيم ليبيز البنكرياس والإنسولين فى السيرم ومستويات الكوليسترول والفوسفوليبيدات ودلائل الإجهاد التأكسدي في نسيج الكبد. علاوة على ذلك تم إجراء الفحص الهستوباثولوجي للكبد، وتقدير المركبات الفينولية فى كلا المسحوقين.أسفرت التغذية على الغذاء الغني بالدهون المتحولة عن حدوث زيادة في وزن الجسم وارتفاع في دهون الدم وخلل في وظائف الكبد مصحوبًا بالإجهاد التأكسدي وهو ما أكده الفحص الهستوباثولوجي. أظهر مسحوقي أوراق الغار والكافور خصائص وقائية مضادة للسمنة وخافضة لدهون الدم، كما ساعدا في تقليل التأثير الضار للغذاء الغني بالدهون المتحولة على الخلايا الكبدية ويرجع هذا إلى محتواهما من المركبات الفينولية فضلًا عن العوامل الأخرى المضادة للأكسدة. وهكذا فإنه يمكن اعتبار مسحوقي أوراق الغار والكافور موانع فعالة لارتفاع دهون الدم وتسمم الكبد المصاحبين لاستهلاك الدهون المتحولة.

الكلمات المفتاحية: ارتفاع دهون الدم، التسمم الكبدي، الدهون المتحولة، أوراق الغار، أوراق الكافور، الجرذان.