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Nutritive Value and Quality of Sweet Basil Leaves in an Animal Model of Nonalcoholic Fatty Liver Disease

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THIS study was carried out to identify the nutritive value of sweet basil leaves and investigate their effects as a functional food on CCl_4-intoxicated rats previously fed on high fat diet as a novel nonalcoholic fatty liver disease (NAFLD) model. Fresh sweet basil leaves were chemically analyzed in order to determine their nutritive value. The biological experiment was conducted using thirty two male albino rats (Sprague Dawley strain) weighing 80 ± 5 g, which were divided into four groups including normal control group, untreated liver – injured group, while the other two groups were treated with 2 and 4% sweet basil leaves powder (SBLP), respectively. The curative trial lasted for 4 weeks. Results showed that protein, total fat, carbohydrates, dietary fiber, calcium, iron and tocopherols contents in 100 g of the used sample of fresh sweet basil leaves represent 6.4, 0.77, 0.89, 6.07, 14.23, 17.78 and 5% of the new DVs for adults, while vitamin C content was found to represent 17.8% and 21.36% of the new DVs for males and females (19-30 years), respectively. Thus, they are considered good source of calcium and iron and good/ excellent source of vitamin C. Results of the biological experiment showed that the developed NAFLD model characterized by overweight, liver enlargement and dysfunctionalong with oxidative stress, which was further confirmed by histological staining using H&E. Due to its known high antioxidant capacity, supplementation of basal diet with SBLP, especially at the high concentration, reduced the abnormalities noticed in liver tissue and alleviated the disorders associated with its dysfunction. Accordingly, the present study shows that sweet basil leaves are a good source of some health promotive nutrients, and recommends that they should be consumed regularly(about 2 tablespoons/day as shade dried leaves) and implicated in the dietary interventions directed to patients with NAFLD.

Keywords: Sweet basil leaves, Nutritive value, Nonalcoholic fatty liver disease.

Introduction

Worldwide, non-alcoholic fatty liver disease (NAFLD) is a major cause of morbidity and mortality. It is a major health problem as it can rapidly develop and lead to end-stage liver disease and liver transplant (Carr et al., 2016). NAFLD can be defined as a condition characterized by accumulation ofneutral lipids to more than 5% of total liver weight in hepatocytes of patients with no history of alcohol abuse (Rossoet al., 2014; Kang et al., 2018 and Panera et al., 2018) and its clinical manifestation spans from bland steatosis to steatohepatitis, which usually progress to fibrosis and cirrhosis. The pathogenesis includes the effects of hormones, insulin resistance, nutritional and intestinal dysbiosis, lipotoxicity, hepatic inflammation and genes (Carr et al., 2016). NAFLD is considered the hepatic manifestation of metabolic syndrome, as it is associated with obesity, type 2 diabetes, hypertension and dyslipidemia (Lopez-Velazquez et al., 2014).

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The estimated worldwide prevalence of NAFLD is 25% and of NASH is 3%-5% (Kim et al., 2017). Both adults and children can present with NAFLD; and sex, ethnicity and genetic polymorphisms contribute to the onset and progression of it (Scorletti and Byrne, 2018 and Jump et al., 2018). It is no doubt that NAFLD will be more prevalent in the next few years, indicating the presence of several risk factors. For example, an unhealthy lifestyle involving poor dietary habits and low physical activity is a major risk factor (Rector et al., 2008).

High fat diet (HFD) -fed mice (Deng et al., 2005 and Zou et al., 2006) 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 (Fujii et al., 2010). Chheda et al., (2014) 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.

Worldwide, basil (*Ocimum basilicum* L.) is a typical ingredient of the healthy Mediterranean diet (Sestili et al., 2018). It is also known as sweet basil (S.B) and is a universally cultivated herbaceous, perennial plant (Bantis et al., 2016). The extracts of its essential oils are used as flavors in food products. It is used as a kitchen, culinary and ornamental herb (Gulcin et al., 2007). It has also been used as commercial fragrances, flavors and to improve the shelf life of food products (Makinen et al., 1999; Suppakul et al., 2003 and Nguyen and Niemeyer, 2008). It has high antioxidant power (Pandey et al., 2016). The antioxidative effect of basil is mainly due to its content of phenolic components, such as flavonoids, phenolic acids, rosmarinic acid and aromatic compounds. Additionally, basil had been found to contain linalool, eugenol,methyl chavicol, methyl cinnamate, ferulate, methyl eugenol,triterpenoids and steroidal glycoside (Gulcin et al., 2007) which are responsible for its abilities asanti-hyperlipidemic (Amrani et al., 2009), anticonvulsant (Nyugen et al., 2010 and Freire et al., 2006), anti-inflammatory (Raina et al., 2016), anti-thrombotic (Tohti et al., 2006), antiplatelet property (Amrani et al., 2009), anti-microbial (Makinen et al., 1999; Suppakul et al., 2003 and Nguyen and Niemeyer, 2008), insecticidal (Freire et al., 2006) and immuno modulatory (Okazaki et al., 2011). It also acts against digestive and neurodegenerated disorders and used as cardiotonic and reliever of abdominal pain (Bais et al., 2002).

Owing to the current importance of dietary sources as nutritive, cheap and safe natural agents, the scarce of studies investigated the hepatocurative effects of sweet basil leaves (SBLs) and the difficulty of controlling all the factors affecting patients with NAFLD, especially non volunteer patients, this study was carried out to identify the nutritive value of SBLs and investigate their effects as a functional food on CCl_4-intoxicated rats previously fed on HFD as a novel NAFLD model.

Materials and Methods

Materials

Plant material

Fresh sweet basil (*Ocimum basilicum* L.) leaves were sampled from several parts of Nawag village, Tanta City, Al-Gharbia Governorate, Egypt. The herb was identified by the Department of Flora, Agricultural Museum, Ministry of Agriculture and the Herbarium of the Department of Botany, Faculty of Science, Cairo University.

Animals

A total of 32 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, kits and other required materials:

Casein, vitamins, minerals, cellulose, choline chloride, DL-methionine, CCl_4 (assay purity > 98%)and other required chemicals were obtained from El-Gomhoreya Company for trading drugs, chemicals and medical appliances, Cairo, Egypt. Kits used for biochemical determinations were obtained from Gama Trade Company for chemicals, Cairo, Egypt. Sheep tallow, sucrose, soybean oil and corn starch were obtained from the local market, Tanta City, Al-Gharbia Governorate, Egypt.

Methods

Quality characteristics of chemical analysis of fresh sweet basil leaves

Fresh sweet basil leaves were chemically analyzed in order to determine its macronutrients

including crude protein, total fat, dietary fiber according to A.O.A.C. (2000). Total carbohydrates were calculated by difference. The energy value was calculated using the Atwater factors of 4, 9 and 4 for protein, fats and carbohydrates, respectively, according to Chaney, (2006). Fresh sweet basil leaves were also wet acid-digested, using a nitric acid and perchloric acid mixture (HNO :HClO , 5:1 w/v), then the total amounts of calcium (Ca) and iron (Fe) were determined by atomic absorption spectrophotometry (Thermo–Elmental, Model 300VA, UK) according to Lindsey and Norwell, (1969). Vitamin C (vit. C) concentration was spectrophotometrically (Model No 6300, Designed and manufactured in UK by I en way LTD) determined by the method in which 2, 6 -dichlorophenolendophenol dye is reduced by ascorbic acid according to Anonymous, (1966). Tocopherols (vit. E) were determined in lipid extracts from fresh sweet basil leaves. Extractions were performed in the dark according to the method of Quartacciet al., (2001) and under continuous flux of nitrogen. The tocopherol forms (α-, β-, γ- and δ-Toc) were determined by isocratic RP-HPLC using a Shimadzu apparatus (model LC-20AD, Suisse) with an electrochemical detector (Metrohm model 791, Shimadzu, Suisse) equipped with a glassy carbon electrode and LC Solution software (Shimadzu) for the integration of peaks. Detection was performed according to Galatro et al. (2001) at +0.6 V at 25°C with a Nova Pak C-18 4 µm column (3.9 × 150 mm; Shimadzu, Suisse). The extracts were eluted with 95% methanol containing 20 mM $LiClO_4$ at a flow rate of 1 ml min^{-1}. For identification and quantification of peaks, a calibration curve was prepared using standard mixtures of α-, β-, γ- and δ-tocopherol (Sigma, Steinheim, Germany) in the range of 50–150 pmol.

Drying of fresh sweet basil leaves

Fresh sweet basil leaves were washed thoroughly, allowed to drain and then spread thinly on clean aluminum trays in a wellventilated room at 25°C away from sunlight forseven days. Natural current of air was used for shadow drying and the leaves were constantly turned to avert fungal growth according to Vanderhulst et al. (1990) with some modification.

Milling and storage of driedsweet basil leaves

After drying, leaves of sweet basil were milled to a fine powder using a hammer mill (Thomas Willey mills, model Ed-5, Germany). After that, they were sieved with a screen of 2 mm pore size and stored at room temperature in airtight glass containers in the dark until used.

Diets

Basal diet used in the experiment was formulated according to Reeves et al., (1993) with the modifications of El-Hashash (2014), while HFD was formulated according to Woods et al. (2003) and Liu et al. (2004) with the modifications of El-Hashash (2014) (Table 1).

Animals & study design

Male albino rats (n = 32) of Sprague Dawley strain weighing (80± 5g) were housed in well-aerated cages under hygienic conditions "22-25°C and a 12 h light-dark cycle" and fed on basal diet for one week for adaptation. After that, rats were weighed and divided into four groups. The first group (n = 5) was fed on basal diet as a normal control group for ten weeks, while groups from 2 to 4 (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. (1997). At the end of the induction period (phase 1 = 6 weeks), all animals were weighed and 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 a representative sample from liver -injured groups versus 155 and 87 U/L, respectively in normal control group. However, by the end of the 4th week, 3 HFD –fed rats died, while another 6 rats died by the end of the 6th week after CCl_4 injection. The total mortality was nearly equal in the three liver -injured groups. Afterwards, the second group was kept untreated and fed on basal diet only, while the third and the fourth groups were fed on basal diet supplemented with 2 and 4% of sweet basil leaves powder (SBLP), respectively. The curative period lasted for four weeks (phase 2). 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 tubesand kept frozen at - 20C 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

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

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

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.

Preparation of liver homogenate

In order to prepare liver homogenate, one gram of liver tissue was homogenized in ice-cold 1.15% solution of potassium chloride in 50 mmol L-1 potassium phosphate buffer solution (pH 7.4) to yield a liver homogenate 10% (W/V). Homogenization was performed using Sonicator, 4710 Ultrasonics Homogenizer (Cole- Parmer Instrument Co., USA). The homogenate was centrifuged at 4,000×g for 5 min at 4°C. The supernatant was collected and stored at -80ºC until used.

Body weight gain and relative liver weight calculation

Body weight gain 1 (BWG 1) was calculated by subtracting the initial weight of each rat from its first final weight (final weight 1), while BWG 2 was calculated by subtracting the first final weight of each rat from its second final weight (final weight 2). As for relative liver weight (RLW), it was calculated according to Angervall and Carlström, (1963).

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, (1960) andRichmond, (1973), respectively. Phospholipids (PhLs) concentration also was determined in liver tissue homogenate according to the method of Ray et al., (1969).In addition, high density lipoprotein cholesterol (HDL-c) was determined according to the method proposed by Friedwald et al., (1972), while low and very low-density lipoprotein-cholesterols, (LDL-c and VLDL-c) were calculated according to the equations of Friedwald et al. (1972).

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, (1984); Beauchamp and Fridovich (1971) and Ellman (1959), respectively. On the other hand, lipid peroxidation expressed as malondialdehyde (MDA) was determined following the method suggested by Ohkawa et al. (1979). Nitric oxide (NO) was similarly measured by the Griess reaction (Miranda et al., 2001).

Determination of liver enzymes and serum proteins

In serum, the activities of liver enzymes including aminotransferases´ (AST and ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954) and gamma – glutamyltransferase (GGT) (Kytzia, 2005) were determined. Moreover, total protein (T.P) and albumin were determined according to the methods described by Gornall et al. (1949) and Doumas et al. (1971), 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, (1980).

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 $p<0.05$ (Sendcor and Cochran, 1979).

Results

Macronutrients, some minerals and antioxidant vitamins in fresh sweet basil leaves

In Table 2, the concentrations of macronutrients, some minerals and antioxidant vitamins in fresh sweet basil leaves were presented.

It was found that 100 g of the used sample of fresh sweet basil leaves contain 3.2, 0.6 and 4.15 g of crude protein, total fat and carbohydrates, respectively. Thus, the total energy provided is 28 kcal. Dietary fiber content also was 1.7 g/100 g. As for mineral content, calcium and iron contents were found to be 185 and 3.20 mg, respectively, while total ash was 0.55 g/100 g. Regarding the antioxidant vitamins, 100 g of the sample were found to contain 16.02 and 0.75 mg of vit. C and vit.E, respectively.

Body weight gain & relative liver weight

Effects of different concentrations of sweet basil leaves on body weight gain and relative liver weight in CCl_4-intoxicated rats previously fed on high fat diet versus normal rats were illustrated in Table 3. It could be noticed that there were no significant differences in body weight among all groups at the beginning of the experiment. At the end of the phase 1 of the experiment "induction period" (6 weeks), liver -injured groups (CCl_4-intoxicated groups previously fed on high fat diet) were found to gain more body weight than normal control group with a significance at $P<0.05$, while the body weight gain of untreated liver -injured group only was significantly higher than that of normal control group by the end of the phase 2 of the experiment "curative period" (4 weeks), as herb –fed groups recorded no significant differences compared with normal control group, with no significant differences between them. On the other hand, liver weight as well as relative liver weight of untreated liver -injured group was significantly higher than those of normal control group. 4% was more efficient than 2% of SBLP in reducing both liver weight and relative liver weight significantly compared with untreated liver –injured group and returning it toward its normal value recorded by normal control group.

Lipid profiles

Effects of different concentrations of sweet basilleaves on lipid profiles in liver and serum of CCl_4-intoxicated rats previously fed on high fat dietversus normal rats were illustrated in Table 4. 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. Supplementation with SBLP led to significant reductions in both parameters, however it could not return them toward their normal values recorded by normal control group. The nearest TG level from that of normal control group was recorded by the group fed on basal diet supplemented with 4% SBLP, while there was no significant difference between the two concentrations regarding liver T.C. In contrast, liver phospholipids were significantly lower in untreated liver -injured group compared with normal control group. Both concentrations induced a significant increase in liver phospholipids compared with untreated liver –injured group, with significant decreases as compared to normal control group, and no significant difference between them. In serum, there were no significant differences in the mean values of triglycerides, total cholesterol, HDL-c and VLDL-c among all studied groups. Only serum LDL-c was significantly elevated in untreated liver –injured group compared with normal control group. Both concentrations of SBLP reduced the mean value of serum LDL-c as compared to untreated liver –injured group, however, the differences were not significant.

Antioxidant enzymes & oxidative markers

Effects of different concentrations of sweet basilleaves on antioxidant enzymes and oxidative markers in liver tissue homogenate of CCl_4-intoxicated rats previously fed on high fat dietversus normal rats were illustrated in Table 5. 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. Herb -fed groups recorded significant increases in the activities of the three enzymes compared with untreated liver –injured group, with no significant differences between them regarding both CAT and SOD. Regarding GSH activity, 4% SBLP –

TABLE 2. Chemical analysis of fresh *Ocimum basilicum* L. leaves per 100 g

Nutrient	Concentration
Macronutrient:	
Moisture	91.50 g
Crude protein	3.20 g
Total fat	0.60 g
Ash	0.55 g
Dietary fiber	1.70 g
Carbohydrates	4.15 g
Energy	28.00 kcal
Minerals:	
Calcium	185 mg
Iron	3.20 mg
Antioxidant vitamins:	
Vit. C	16.02 mg
Vit. E	0.75 mg

TABLE 3. Effects of sweet basil leaves on body weight gain and relative liver weight in CCl_4-intoxicated rats previously fed on high fat diet versus normal rats

Groups / Parameters	Normal control	Liver –injured	Liver –injured + 2% SBLP	Liver –injured + 4% SBLP
Initial weight (g)	86.40±8.95	93.40±14.59	93.00±12.00	92.00±11.42
Final weight 1 (g)	93.80±7.85^{b}	116.20±13.76^{a}	116.00±13.00^{a}	113.20±11.82^{a}
Final weight 2 (g)	104.40±14.50^{b}	130.20±16.50^{a}	125.75±12.87^{ab}	123.20±16.48^{ab}
BWG 1 (g)	7.40±1.20^{b}	22.80±2.39^{a}	23.00±2.50^{a}	21.20±2.45^{a}
BWG 2 (g)	10.60 ±1.52^{b}	14.00±2.24^{a}	9.75±1.35^{b}	10.00±1.46^{b}
Liver weight (g)	2.18±0.21^{b}	4.00±0.41^{a}	3.80±0.68^{a}	2.25±0.33^{b}
RLW (%)	2.09±0.29^{b}	3.07±0.35^{a}	3.02±0.32^{a}	1.83±0.23^{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 4. Effects of sweet basil leaves on lipid profiles in liver and serum of CCl_4-intoxicated rats previously fed on high fat diet versus normal rats

Groups / Parameters	Normal control	Liver –injured	Liver –injured + 2% SBLP	Liver –injured + 4% SBLP
Liver TGs (mg/g)	35.42±6.30^{d}	79.14±10.42^{a}	60.37±8.43^{b}	48.31±8.18^{c}
Liver T.C (mg/g)	40.19±5.65^{c}	65.05±9.38^{a}	56.00±9.00^{b}	53.31±8.22^{b}
Liver PhLs (mg/g)	50.07±8.41^{a}	27.47±4.31^{c}	35.05±7.00^{b}	38.54±6.88^{b}
Serum TGs (mg/dl)	58.00±7.38	70.25±9.06	66.18±10.00	63.20±9.86
Serum T.C (mg/dl)	98.60 ±12.82	117.40±17.59	111.45±15.80	110.80±14.10
Serum HDL-c (mg/dl)	46.40±8.56	36.80±6.22	35.12±5.41	40.80±7.40
Serum LDL-c (mg/dl)	40.60±6.75^{b}	66.55±9.03^{a}	63.09±8.00^{a}	57.36±7.68^{a}
Serum VLDL-c (mg/dl)	11.60±1.48	14.05±1.81	13.24±1.65	12.64±1.97

- 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 5. Effects of sweet basil leaves on antioxidant enzymes and oxidative markers in liver tissue homogenate of CCl_4-intoxicated rats previously fed on high fat diet versus normal rats

Groups / Parameters	Normal control	Liver –injured	Liver –injured + 2% SBLP	Liver –injured + 4% SBLP
CAT (Mmol/g)	0.39±0.05[a]	0.15±0.03[c]	0.25±0.04[b]	0.28±0.04[b]
SOD (U/g)	0.36±0.05[a]	0.11±0.03[c]	0.22±0.03[b]	0.24±0.03[b]
GSH (ng/g)	0.35±0.04[a]	0.13±0.03[c]	0.23±0.03[b]	0.31±0.03[a]
MDA (Mmol/g)	0.10±0.02[c]	0.33±0.06[a]	0.24±0.03[b]	0.19±0.03[b]
NO (Mmol/g)	0.32±0.03[a]	0.09±0.02[d]	0.19±0.02[c]	0.24±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.

fed group was better than 2% SBLP –fed one as it recorded no significant difference as compared to normal control 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. Both herb -fed groups recorded significant increases in liver NO compared with untreated liver –injured group, and the nearest level from that of normal control group was noticed in 4% SBLP –fed group, with a significant increase compared with 2% SBLP –fed group. Conversely, liver MDA increased significantly in untreated liver –injured group as compared to normal control group. Both concentrations of SBLP resulted in significant decreases in this parametercompared withuntreated liver –injured group, with no significant differences between them.

Liver enzymes

Effects of different concentrations of sweet basil leaves on the activities of liver enzymes in serum of CCl_4-intoxicated rats previously fed on high fat dietversus normal rats were illustrated in Table 6. 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. Both herb –fed groups recorded significant decreases in the activities of these enzymes compared with untreated liver –injured group, except for AST and ALP activities which decreased in 2% SBLP –fed group, but the reductions were not significant. Generally, the high concentration of SBLP was more efficient than the low concentration in lowering the activities of these enzymes in serum.

Serum proteins

Effects of different concentrations of sweet basil leaves on total protein and albumin in serum of CCl_4-intoxicated rats previously fed on high fat dietversus normal rats were illustrated in Table 7. 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. Although only the group fed on basal diet supplemented with the high concentration of SBLP recorded significant increase in serum total protein compared withuntreated liver –injured group, both concentrations led to significant increases in serum albumincompared withuntreated liver –injured group, with no significant differences between them.

Histopathological findings

Results of the histopathological examination of rat livers from different experimental groups were illustrated in Fig. 1-5: Fig. 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 number of binucleated hepatocytes, cytoplasmic degeneration (Fig. 2), fatty change of hepatocytes (Fig. 2 and 3) and deteriorated blood sinusoids (Fig. 3). Supplementation of basal diet with SBLP somewhat decreased these lesions. For example, Fig. 4 represents liver section of rat from 2% SBLP –fed group, in which haemorrhage, Kupffer cell activation, pronounced nuclear changes: pyknotic nuclei and karyolitic onescan be noticed. In Fig. 5, liver section of rat from 4% SBLP –fed group was represented, in which Kupffer cell activation and irregular blood sinusoids can be observed.

TABLE 6. Effects of sweet basil leaves on the activities of liver enzymes in serum of CCl_4-intoxicated rats previously fed on high fat diet versus normal rats

Groups / Parameters	Normal control	Liver –injured	Liver –injured + 2% SBLP	Liver –injured + 4% SBLP
AST (U/L)	148.00±18.91^{c}	235.33±30.30^{a}	222.90±28.74^{ab}	190.00±29.15^{b}
ALT (U/L)	83.20±11.88^{c}	159.25±20.47^{a}	116.41±18.32^{b}	106.20±12.76^{b}
ALP (U/L)	197.00±21.68^{c}	304.20±40.55^{a}	285.85±40.03^{ab}	244.60±30.70^{b}
GGT (U/L)	26.33±3.63^{d}	65.25±6.40^{a}	49.48±6.19^{b}	37.80±5.07^{c}

- 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 7. Effects of sweet basil leaves on total protein and albumin in serum of CCl4-intoxicated rats previously fed on high fat diet versus normal rats

Groups / Parameters	Normal control	Liver –injured	Liver –injured + 2% SBLP	Liver –injured + 4% SBLP
T.P (g/dl)	6.74±0.74^{a}	4.60±0.60^{c}	5.00±0.63^{bc}	5.80±0.72^{b}
Albumin (g/dl)	4.68±0.68^{a}	3.02±0.36^{c}	3.70±0.46^{b}	3.83±0.50^{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.

Discussion

Results of the chemical analysis indicated that 100 g of the used sample of fresh sweet basil leaves provided 28 kcal/100g, i.e. 1.4% of the caloric intake for the human adult (2000 kcal/ day). According to Food and Drug Administration (FDA), 2016, protein, total fat, carbohydrates and dietary fiber contents in 100 g of the used sample of fresh sweet basil leaves represent 6.4, 0.77, 1.51 and 6.07% of the new daily values (DVs) based on a caloric intake of 2000 kcal for adults.

Also, calcium, iron and tocopherols contents in 100 g of the used sample represent 14.23, 17.78 and 5% of the new DVs for adults reported by FDA, (2016). Thus, fresh sweet basil leaves are considered good source of calcium and iron. Thenew DVs of vit. C are not equal in males and females. It was reported to be 90 mg for males and 75 mg for females. Accordingly, vit. C content in 100 g of the used sample represent 17.8% and 21.36% of the new DVs for males and females (19-30 years), respectively. So, fresh sweet basil leaves can be considered good source of vit. C foradult males and excellent source for adult females.

According to The United States Department of Agriculture (USDA) Food Composition Databases, 100 g of fresh basil contain 92.06 g moisture. Protein, total fat, dietary fiber and carbohydrate contents are 3.15, 0.64, 1.6 and 2.65 g, respectively. Also, calcium, iron, vit. C and □-tocopherol contents are 177, 3.17, 18 and 0.80 mg. Thus, the concentrations of protein, dietary fiber, total carbohydrates, Ca and Fe in the used sample were higher compared to USDA determinations. In contrast, the concentrations of total fat, vit.C and vit.Ewere lower. As vit. C is a water soluble vitamin and vit. E is a fat soluble one, the decreased moisture and total fat contents in the used sample can account for their decreased concentrations, respectively. In general, all present determinations are near from USDA determinations to a large extent.

Except fot vitamin C and carotenoids, shadow drying was found to help concentrate the nutrients of leafy vegetables per unit. This is an indication that use of a relatively small amount of the shade dried leaves could significantly raise the content of minerals and phenolics components in the diet and enables the individuals to meet the RDAs for these micronutrients (Acho et al., 2016).

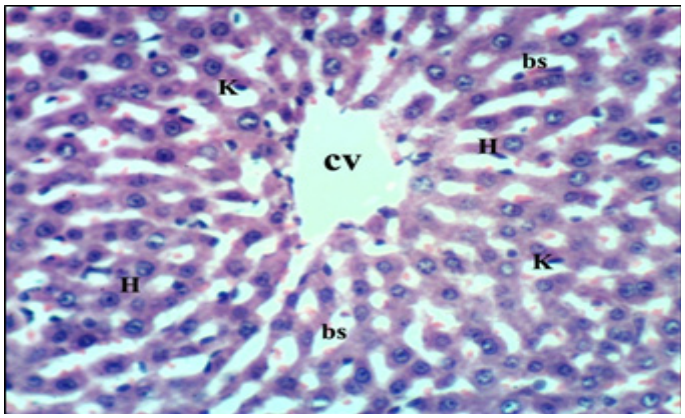

Fig. 1. Liver section of rat from normal control group showing the normal histological structure of hepatic lobule, central vein (cv) and radiating polygonal hepatocytes (H). The hepatocytes had homogenous granular cytoplasm with centrally located nucleus with prominent envelops and normal distributed 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)

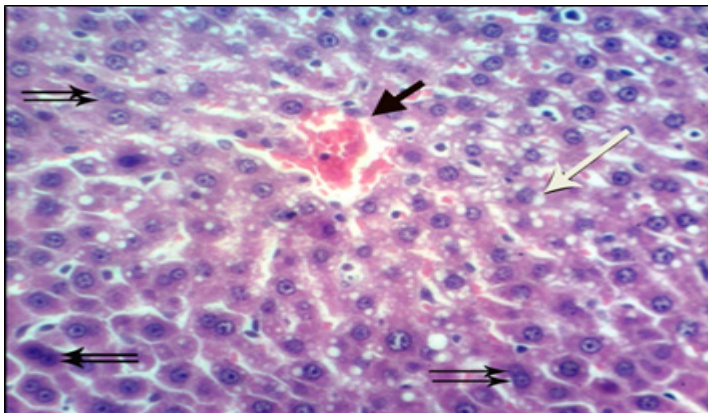

Fig. 2. Liver section of rat from untreated liver –injured group showing irregular and congested central vein (arrow), increase number of binucleated hepatocytes (double arrows), cytoplasmic degeneration and fatty change of hepatocytes (white arrow) (H & E X 400)

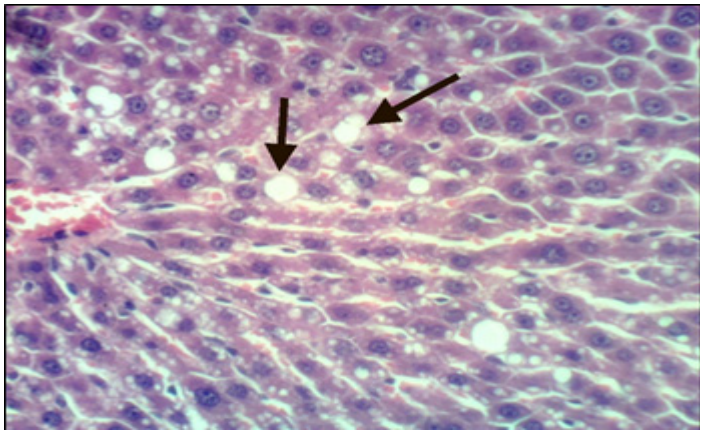

Fig. 3. Liver section of another rat from untreated liver –injured group showing fatty change of hepatocytes and deteriorated blood sinusoids (H & E X 400)

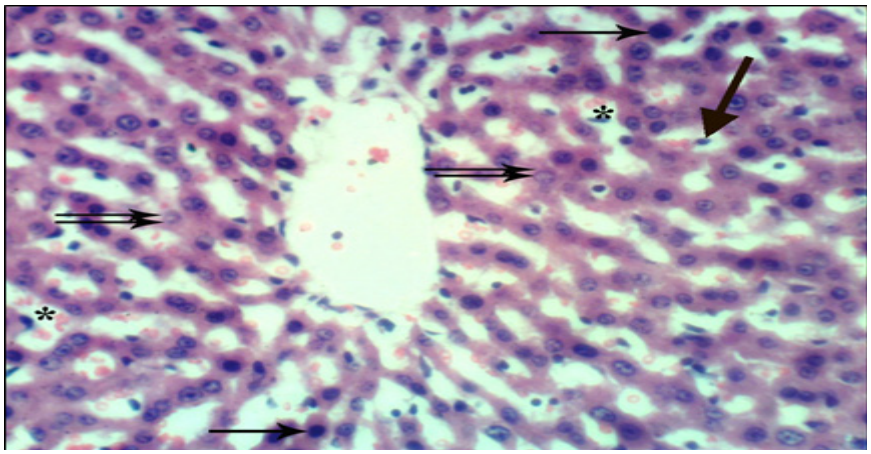

Fig.4. Liver section of rat from 2% SBLP showing haemorrhage (*), Kupffer cell activation (thick arrow), pronounced nuclear changes: pyknotic nuclei (thin arrows) and karyolitic ones (double arrows) (H & E X 400)

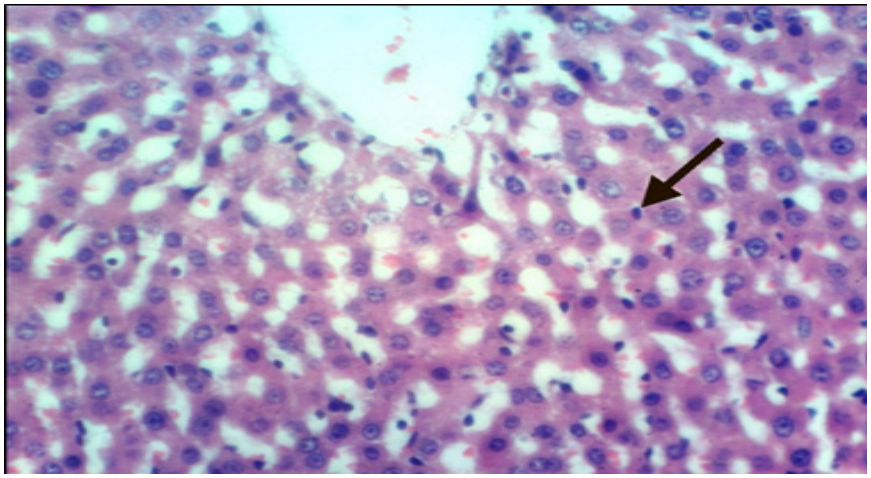

Fig. 5. Liver section of rat from 4% SBLP –fed group showing Kupffer cell activation (arrow) and irregular blood sinusoids (H & E X 400)

Accordingly, in the present study, shadow drying is expected to elevate the concentrations of all analyzed nutrients except for vitamin C.

High fat diet –fed animals are one of diet modulations leading to NAFLD (Zou et al., 2006) wherein, NAFLD severity depends on diet content, feeding duration, strain, species and gender of animals. On the other hand, $CCl_4$is one of the toxins known to induce NAFLD (Fujii et al., 2010). Despite of many advantages, considerable disadvantages have been revealed in all induction methods (Starkel and Leclercq, 2011). Chheda et al., (2014) 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 sweet basilleaves onthis model.

The current results indicated that liver –injured groups, by the end of the induction period, gained more body weight than normal control group with significance at $P<0.05$, with no significant differences among them. This effect is attributed to the 4 weeks HFD feeding and is in agreement withWoods et al. (2003) who demonstrated that high fat diet-fed rats weighed more than low fat controls. El-Hashash, (2014) found that BWG of HFD –fed group was 66.92 ±0.76 g versus 13.111.11± g in normal control group. In the present study, the mean values of

BWG in liver –injured groups were 22.80±2.39, 23.00± 2.50 and 21.20±2.45 g, respectively versus 7.401.20± g in normal control group. Despite of significance, the lower gain rate noticed in the present study can be attributed to CCl_4 injection, as CCl_4 exposure led to weight loss as a result of direct toxicity and/or indirect toxicity related to the liver injury (Mohamed et al., 2009).

By the end of the curative period, the BWG of untreated liver –injured group only was significantly higher than normal control group, while herb -fed groups recorded no significant differences. In agreement with the current data, Irondi et al., (2016) concluded that *O. basilicum* could be used as a functional food for obesity management as a result of inhibition of pancreatic lipase activity which in turn is due to the combined effects of flavonoids and phenolic acids present in the leaves.

The liver weight significantly increased in untreated liver –injured group compared with normal 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. (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%). Zivkovic et al. (2007) explained that excessive dietary fat intake combined with peripheral insulin resistance, continued insulin-promoted triglyceride hydrolysis via lipoprotein lipase which led to higher blood free fatty acid levels. This caused increased fat accumulation in skeletal muscles as well as increased liver TG and cholesterol esters. On the other hand, Shiratori et al., (1986) 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 shown by hematoxylin and eosin staining, in the present study, as well as the significant increase in triglycerides concentration in liver tissue homogenate support the significant increase of both absolute and relative liver weights.

Like HFD, CCl_4 increases liver cholesterol. This may be due to increased cholesterol synthesis (Boll et al., 2001). Compared to other lipid classes, phospholipids, the vital biomembrane components, are the most sensitive to lipid peroxidation induced by CCl_4 (Morrow et al., 1992). Lamb et al. (1988) also explained that the decreased levels of phospholipids in liver tissue can be assigned to the increased phospholipase activity. Similarly, high fat diet feeding was found to increase phospholipid peroxidation in rat liver (Burdeos et al., 2012) which in turn is involved in the pathophysiology of many abnormalities.

Although both used concentrations of sweet basil leaves powder (2 and 4%) lowered TG concentration significantly in liver tissue homogenate and the fatty changes in hepatocytes disappeared, absolute and relative liver weight decreased significantly only in the group fed on the high concentration of SBLP. In harmony with the present results concerning the effect of SBLP on liver levels of TG and total cholesterol, Harnafi et al. (2009) declared that the polar products present in sweet basil leaves could eliminate dyslipidemia and correct the lipid profile in liver of hypercholesterolemic rats.

Except for LDL-c, lipid profiles in serum, unlike liver lipids, did not significantly respond to the co-effects of HFD and CCl_4 or herb feeding. While serum LDL-c increased significantly in untreated liver –injured group compared with control group, its decrease noticed in herb -fed groups was not significant. CCl_4 has a hypotriglyceridemic effect because it rapidly rises the triglyceride accumulation in the liver due to a failure in their secretory mechanisms (Shi et al., 1998 and Hamdy and El-Demerdash, 2012) and also increased triglycerides uptake into the liver. In contrast, HFD exerts a hyperlipidemic effect through increasing both pancreatic lipase activity and insulin resistance, as revealed by El-Hashash, (2014). In the current study, it could be suggested that the hypertriglyceridemic effect of HFD was somewhat reversed by the hypotriglyceridemic effect of CCl_4. Consequently, serum TG was not significantly increased. The insignificant changes noticed in total cholesterol and HDL-c in all groups may be due to low experimental 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 an end product of lipid peroxidation was significantly increased. Both HFD and CCl_4 are responsible for these effects. In accordance with these results, Deng et al., (2019) 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., (2008) 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. CCl_4 induces liver injury through its conversion into a trichloromethyl free radical ($\bullet CCl_3$) by cytochrome P450 in the liver. The $\bullet CCl_3$ free radical further causes polysome disaggregation, a distorted structure and dysfunction of endoplasmic reticula and plasma membranes (Smuckler, 1976) and stimulation of lipid peroxidation (Tomasi et al., 1987).

The beneficial effects of SBLP on antioxidant defense system in liver tissue, as noticed in the present study, were in line with Marinava and Ynishlieva, (1997) who reported that *Ocimum basilicum* contains several active antioxidant compounds. The antioxidant property of *O. basilicum* is due to the polyphenoidrosmarinic acid which is a derivative of cinnamic acid (Phippen and Simon, 1998). In goats, the ethanolic extract of *O. basilicum* leaves showed significant hepatoprotective effects against H2O2 and CCl_4 -induced liver injury. Moreover, significant anti-lipid peroxidation effect was noticed (Meera et al., 2009).

It is well known that those components able to reduce nitric oxide production in the liver tissue possess hepatoprotective effects (Majano et al., 2004). According to Meera et al. (2009), the ethanolic extract of O. basilicum leavesshowed significant activity in superoxide radical and nitric oxide radical scavenging.

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. (2015) 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. (2008) demonstrated that in CCl_4 -intoxicated rats, hepatic lipids levels and plasma aminotransferases activities were increased, while antioxidant defense system was impaired. The same effects were reported by Ma et al. (2014) who attributed them to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred (Liu et al., 2013 and Ozturket al., 2009). Chheda et al., (2014) 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., (2016) found that serum albumin was decreased after high fat feeding in both Wistar and Sprague-Dawley Rats. Similarly,Shittu et al., (2015)observed a marked decrease in the total proteins in liver and serum of CCl_4-administered rats when compared with the control rats. This decrease in serum proteins induced by HFD and/or CCl_4 suggests a reduction of the synthetic ability of the liver. Such decrease could, however, lead to hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals (Adeyemi et al., 2012).

Feeding on basal diet supplemented with the high concentration of SBLP (4%), as noticed in the present study, reduced the activities of all studied liver enzymes in serum, while induced a significant increase in serum levels of total protein and albumin, while 2% SBLP could only decrease ALT and GGT activities and increase albumin level significantly. These effects suggest that SBLP could preserve liver integrity and increased its synthetic ability. These beneficial effects happened markedly in the group received the high concentration of SBLP, while were less obvious in the group received the low concentration.

Conclusion

According to the present results, sweet basil leaves are a good source of some health promotive nutrients. In addition, the present study recommends that approximately 22-33 g “about 2 tablespoons” of shade dried sweet basil leaves/day should be consumed regularly and implicated in the dietary interventions directed to adult patients with nonalcoholic fatty liver disease.

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القيمة الغذائية وجودة أوراق الريحان في نموذج حيواني تجريبي لمرض الكبد الدهنى غير الكحولى

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أجريت هذه الدراسة بهدف تحديد القيمة الغذائية لأوراق الريحان وبحث تأثيراتها كغذاء وظيفى على الفئران المسممة برابع كلوريد الكربون والتى سبق وأن تغذت على الغذاء عالى الدهن كنموذج حيوانى تجريبى مستحدث لمرض الكبد الدهنى غير الكحولى. تم تحليل أوراق الريحان كيميائيا بهدف التعرف على قيمتها الغذائية . فى حين أجريت التجربة البيولوجية باستخدام عدد ٣٢ فأر ذكر ألبينو (سلالة سبراجيو داولى) يبلغ متوسط أوزانهم ٨٠± ٥ جم . حيث تم تقسيمهم إلى أربع مجموعات تشمل المجموعة الضابطة السليمة . المجموعة المصابة بتلف الكبد والتى تركت دون معالجة بالإضافة إلى مجموعتين تم علاجهم بالتغذية على الغذاء القياسى مدعما بـ ٢ . ٤٪ منمسحوق أوراق الريحان على التوالى. هذا وقد استمرت التجربة العلاجية لمدة أربعة أسابيع. أظهرت النتائج أن محتوى العينة المستخدمة من أوراق الريحان الطازجة (١٠٠ جم) من كل من البروتين . الدهون الكلية . الكربوهيدرات . الألياف الغذائية . الكالسيوم . الحديد . فيتامين هـ يمثل ٦,٤ . ٠,٧٧ . ٠,٨٩ . ٦,٠٧ . ١٤,٢٣ . ١٧,٧٨ . ٥٪ من القيم اليومية الحديثة للبالغين . فى حين أن محتواها من فيتامين ج يمثل ١٧,٨ . ٢١,٣٦٪ من القيم اليومية الحديثة للبالغين والبالغات (سن ١٩-٣٠ عام) على التوالى. وهكذا. فهى تعد مصدرا جيدا للكالسيوم والحديد ومصدر جيد/ممتاز لفيتامين ج. أما نتائج التجربة البيولوجية فأظهرت أن هذا النموذج التجريبى المستحدث لمرض الكبد الدهنى غير الكحولى يتسم بزيادة فى وزن الجسم وتضخم الكبد واختلال وظائفهمصحوبا بالإجهادالتأكسدى للكبد.وهوماأكدهالفحص الهستوباثولوجى للكبدفيمابعد.أدى تدعيم الغذاءالقياسى بمسحوق أوراق الريحان نظرا لكفائتها العالية والمعروفة فى اعاقة تفاعلات الأكسدة «وخاصة بالتركيز العالى» إلى تقليل المظاهر غير الطبيعية التى لوحظت بنسيج الكبد وكذلك تخفيف حدة الاضطرابات المرتبطة بخلل وظائفه. وبناءا عليه . فإن الدراسة الحالية تبين أن أوراق الريحانتعتبر مصدر جيد لعدد من المغذيات الداعمة للصحة . كما توصى باستهلاكها بصورة منتظمة «بمعدل ٢ ملعقة طعام يوميا تقريبا كأوراق مجففة فى الظل» واستخدامها ضمن وسائل التدخل الغذائى الموجهة لمرضى الكبد الدهنى غير الكحولى.