Ameliorative Effect of *Melissa officinalis L.* Fortified Cupcake on Hepatotoxicity in Rats

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Abstract

This study was conducted to investigate the effect of cupcakes with different levels of *Melissa officinalis L*. **(**Melissa Powder MP) on impaired liver in rats injected with Carbon Tetrachloride (CCl_4) in paraffin oil (1:1) (2 ml/ kg b.w twice a week for 2 weeks. Thirty male mature albino rats, weighing 180 gm were divided into 2 main groups, the first group (6 rats) still fed on basal diet and the second main group (24 rats) had given CCl_4 twice a week for 15 days to induce liver impaired in rats, then classified into 4 groups (6 rats in each group) one of them left as positive control, and the other three groups fed on melissa powder (MP) at levels of 2.5, 5 and 10% for 28 days. Serum liver functions (AST, ALT and ALP), lipid profile (TG, TC, LDL-c, HDL-c and VLDL-c), kidney functions (uric acid, urea and creatinine) and histopathological changes of liver and spleen have been evaluated. The obtained results of hepatic rats revealed that 10% from MP cupcake showed a significant increase in HDL, but with significant decreases in the rest of the analyses referred to, previously, as compared with positive control group.

In conclusion, it was recommended that using Melissa powder (MP) cupcake in daily diets to improve the liver functions**.**

Keywords: *Melissa officinalis L.,* cupcake, Liver functions, lipid profiles, Histopathological structure.

Introduction

Liver is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells, and the production of hormones. Liver diseases are common and represent the major cause of human mortality in the world. They are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma ***(*Lok*., 2004 and Fu-Sheng *et al.*, 2014*).***

Carbon tetrachloride (CCl_4) is a chemical agent widely used for experimental induction of fatty liver and liver fibrosis in animals **(Grover *et al.*, 2002).**

Plants used in traditional medicine require detailed investigation from an ethno-pharmacological approach for the treatment of liver disorders because hepatic ailments remain a serious health problem caused by drugs, chemicals and alcohol **(Anju *et al.*, 2012).**

Melissa officinalis known as lemon balm, bee balm, honey balm, ***(*Rasmussen*., 2011)*** is a perennial herb. It is a member of the Lamiaceae (mint) family, and lemon balm (*Melissa officinalis L.,*) belongs to a genus that includes 5 species of perennial herbs native to Europe, Central Asia,***(Jastrzebska et al., 2013)*** **and Iran*.***

Although *Melissa officinalis* originated primarily in Southern Europe, it is now naturalized around the world, from North America to New Zealand**.**

Melissa officinalis **has a large use in traditional medicine, food** industry, and aromatherapy, due to its fresh smell and its medicinal properties including hypoglycemic, hepatoprotective, antimicrobial, antidepressant, hypnotic, and sedative. In fortified, there are studies that pointed out the cytotoxic effect of lemon balm extract on breast cancer and colon carcinoma ***(*Weidner *et al.*, 2015 and Sepide *et al.*, 2017*).***

Melissa officinalis) lemon balm) contains 15.3%, 0.94% and7.37% as moisture, lipid and protein content. They are moderate source of minerals such as Na, Mn, Fe, Zn, Ni and Cu (83.34, 16.41,119,4, 29, 163, 1.067, 6.589 ppm respectively**) *(*Negrea *et al.*, 2017*).*** *Melissa officinalis* had very high levels of phenolics in 32 plant spices. It had the highest levels of phenolics and flavonoids **(Dias *et al., 2012).***The leaf of *Melissa officinalis* contains flavonoids (quercitrin, rhamnocitrin, and luteolin), polyphenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (ursolic and oleanolic acids), sesquiterpenes, tannins, and essential oils (citral) ***(*** **Sofowora *et al.*, 2013).** Lemon balm infusion improves plasma levels of catalase, superoxide dismutase, and glutathione peroxidase and a marked reduction in plasma DNA damage, myeloperoxidase, and lipid

peroxidation. Due to its iron chelating activity of the extract, its antioxidant potential was increased *(*Dastmalchi *et al.,* 2008*)*.

Its activity is comparable with synthetic antioxidants (BHA and BHT), and antioxidant activity is related to phenolic compounds like citronellal and neral. It was found that flavonoids aglycones were responsible for the free radical scavenging activity and contains compounds that have anti-oxidant activity with the ability to reverse liver fibrosis. Frequent consumption of MP may lower liver damage and prevent development of some liver diseases *(*Meftahizade *et al.,* 2010 *and* Pereira *et al.,* 2014).Therefore, this study aimed to investigate the effect of *Melissa officinalis* powder levels on impaired liver in rats injected with Carbon Tetrachloride (CCl_4).

Materials and methods

Material

Fresh plants of *Melissa officinalis* were obtained from National Research Center (NRC), Dokki, Giza, Egypt. Kits were used for the quantitative determination of the different parameters were purchased from Bio diagnostic Co., Dokki, Giza, Egypt. Casein, carbon tetrachloride, vitamin and salt mixtures were purchased from El-Gomhoria Company for Trading Drugs, Chemicals and Medicals Instruments, Cairo, Egypt. Thirty male albino rats of Sprague Dawley strain were obtained from Research Institute of Ophthalmology, Medical Analysis Department, and Giza, Egypt.

Wheat flour (72 % extraction) sucrose, skimmed milk, baking powder, butter, eggs and salt obtained from local market.

Methods

Preparation of *Melissa officinalis L.* powder

Melissa officinalisL. plant washed thoroughly under running tap water, dried in shade and then dried in an air oven at 50°C, and ground to obtain fine powder using a mill. The resulting product was kept under refrigeration at -20 ℃ until ready for use **(Ibrahim, 1999) .**

Preparation of cupcakes

The cupcakes are prepared according the processing method proposed by **A.A.C.C. (2000)** which was used in the experiment and involves the following steps: The butter (125g) was thoroughly melted, and then salt (2g) and sucrose sugar (175g) were added to the mixture and vigorously stirred. Whole eggs (120g) were mixed with vanilla (3g) and whipped for several minutes to make a cream with a smooth texture. Moreover, white flour (72%+ extraction) (320g) for control sample while in tested samples MP were fortified with white flour (72% extraction)at different levels, 2.5, 5 and 10%, which were individually mixed with approximately 10g baking powder and 125g skimmed milk powder . The resulting mixture was then gradually added to the whipped egg and gently mixed to form a homogenous dough with the help of a handheld mixer (MK-4H-W, Panasonic Co, Malaysia). The resulting homogeneous mixture was then poured into different cups of similar sizes and baked at a temperature of 180 ℃ (+/- 5) for 30 to 35 minutes. The product was cooled and then stored for use in the experiment.

Chemical composition of *Melissa* Powder and its products.

Moisture, total nitrogen, total lipids, ash, crude fibers of plant and its product were determined according to the methods described **by AOAC (2010).** Total carbohydrates were calculated by difference**.**

Determination of total flavonoids, total phenolics and antioxidant activity of *Melissa officinalis L.* powder.

Total flavonoids were estimated using the method of **Ordonez *et al.* (2006).** Total phenolic contents were determined by Folin Ciocalteu method as adopted by **Wolfe *et al.* (2003)** and the results were

expressed as mg gallic acid equivalent (GAE).Antioxidant activity were determined by the method of **Roberta *et al.* (1999).**

Sensory evaluation

The sensory properties of control and different fortified cupcakes were carried out according to ***Khalifa et al. (2015)***. The average of total score was converted to a descriptive category as follows(Judging scale) very good (9-8), Good (6-7), Fair (4-5), Poor (2-3) and Very Poor (1)

Experimental animals

Preparation of basal diet

The basal diet (Casein-basal diet) was composed of 12.3g casein (10% protein), 10g corn oil (10% fat), 4g minerals (4% minerals), 1g vitamin mixture (1% vitamin), 4g cellulose (4% fiber), choline chloride (0.2%), methionine (0.3%), and the remained was corn starch according to **Campbell (1963).**

The salt mixture which used in the experiment was recommended by **Hegsted *et al.* (1941)** and the vitamin mixture were used in the experiment was that of **Muller (1964)**.

Induction of experimental liver damage

Chronic liver damage was induced in normal healthy mal albino rats by intra-peritoneal injection of CCl_4 in paraffin oil (50% v/v 2 ml/kg body weight) twice a week (for two weeks), according to the method described by **Jayasekhar *et al.* (1997).**

Experimental design and animal groups

A total of thirty white male albino Sprague Dawley rats weighing approximately 180 gm were used in this experiment. The experimental pant was done in Research Institute of Ophthalmology Giza. Each rat was housed individually in a bottomed, stainless steel cage under standard conditions. It was well-aerated cages in an animal room and maintained in a temperature- controlled room (23 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. The animals were processed according to the suggested international ethical guidelines for the care of laboratory animals. These rats were randomly sampled into five groups comprising 6 rats each.

The rats were weighed each week. The basal diet proposed by the **AIN-93** diet guidelines was prepared to feed these animals **(AIN, 1993**) for one week to help them adapt to the environment. Thirty adult albino rats, weighing 180 g were divided into 2 main groups, the first group (G1) (6 rats) still fed on basal diet and the second main group (24 rats) had given CCl_4 (2 ml/kg bw) twice a week for 15 days to induce liver impaired in rats, then classified into 4 groups (6 rats in each group) one of them (G2) left as positive control, and the other three fed on basal diet with10% cupcakes which contained2.5, 5 and 10%of MP for 28 days.

Blood sampling collections

At the end of experiment period blood samples were collected after 12 hours fasting from the portal vein; the rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r. p. m to separate the serum. Serum was carefully aspirated and transferred into clean covet tubes and stored frozen at -20°C for analysis **(Malhotra, 2003).**

Biochemical parameters

The serum levels of alanine amino transferase **(ALT**), aspartate amino transferase **(AST)** and alkaline phosphatase **(ALP**) were calculated as mg/dl according to **Tietz (1976), Henry (1974) and Moss (1982).** Serum **(TG), (TC), (HDL-c), (LDL-c) and (VLDL-c)** were determined according to the methods described by **Fossati and Principe (1982), Richmond (1973), Allain etal., (1974**), **Castelli *et al.,* (1977**), **Lee and Nieman (1996)** respectively**.** Serum urea, creatinine and uric acid were calculated as mg/dl according to **Patton and Crouch (1977), Schirmeister (1964) and While *et al.* (1970) respectively.**

Histopathological examination

Specimens from liver and spleen were collected directly after scarification of animals at the end of experimental period, fixed in 10% neutral buffered formalin, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin, 4-6 thick sections were prepared and stained with

Hematoxylen and Eosin (**Bancroft and Cook, 1998).**

Statistical analysis

Data are presented as means ± SD and the analysis was conducted using **SPSS** program**, Version 16.0 (2007).**

Results and discussion

Chemical composition of *Melissa officinalis L.*

Table (1) indicated that the proximate composition analysis of the dried sample. The results clearly shown that moisture content was 19.73%in the dried sample. The percentage of crude fat content was 5.07%.the amount of crude fat present sample seems to be moderate and may be adequate for consumption without any health threat. This observation agreed with the report of **Pereira *et al*. (2014)**, who noted that excess fat consumption was implicated in certain cardiovascular disorder such as atherosclerosis, cancer and ageing. The crude protein in the dried sample was 6.69% which is a close agreement with previous results. However, the percentage of ash content 6.7%. The percentage of fiber 9.34% while the sample have 52.47% of carbohydrates when compared to some vegetable the carbohydrates content are higher in dried leaves than dried leaves of *Telfaria occidentalis (***Dorman *et al.,* 2018)*.***

Total phenolic, total flavonoid and antioxidant activity of *Melissa officinalis* determined were 13.57 mg GAE/g dried weight (DW) and 24.04 mg QE/g DW, respectively (Table 2). Total equivalent antioxidant activities, was86.96%. The antioxidants are known to play an important role in protection against disorders caused by oxidant damage. Reactive oxygen species (ROS) production can overcome cellular antioxidant defenses and can lead to a condition termed oxidative stress. Oxidative stress has been implicated in the installation and progression of several degenerative diseases, via DNA mutation, protein oxidation, and/or lipid peroxidation. Literature data have given special attention to the role of ROS and oxidative stress in diabetes, cardiovascular diseases, chronic neurodegenerative disorders such as Parkinson's and Alzheimer's diseases .It was revealed that *Melissa officinalis* L have good potential for antioxidant activity and can be used in lipid-containing foods. It is a rich

source of antioxidants, in particular from the group of phenolic compounds. Its activity is comparable with synthetic antioxidants (BHA and BHT), and antioxidant activity is related to phenolic compounds like citronellal and neral ***Koksal et al., 2011 and Kamdem et al., 2013).***

Chemical composition of control and fortified cupcakes with different levels of Melissa powder.

The chemical analysis of control and fortified cupcake with 2.5, 5% and 10% Melissa powder is demonstrated in table (3).The moisture content of fortified cupcake was higher with different levels of Melissa powder than control cupcake. On the other hand, the fortified cupcake with tested levels of Melissa powder showed increasing in fat, ash and crude fibers than that of the control one. While carbohydrate values in fortified cupcake with levels of Melissa powder decreased by increasing the level of Melissa powder. The results of carbohydrates and moisture showed nonsignificat changes as compared with control sample. Adding 10% of Melissa powder led to significant changes with the control and other Melissa samples. The obtained result agree with **Ivelina *et al.*(2018*)*** who reported that fortified of melissa powder increased dietary fiber content and antioxidant activity of bread.

Sensory evaluation of control and fortified cupcake with different levels of melissa powder.

Table (4) illustrates sensory evaluation scores of fortified cupcake with Melissa powder. Cupcake fortified with 2.5% Melissa powder had a non-significant in all sensory properties when compared with the control sample. While cupcakes fortified with 5 and 10% Melissa powder showed significant differences with the control and 2.5% Melissa samples. Color and texture were the most sensory properties affected by increasing the level of Melissa. Therefore fortified cupcake with 2.5% recorded the best results in sensory evaluation followed by 5%. The differences between the tested sample led to increase the fiber content which effect on water absorption between melissa fiber and other ingredients when mix with flour and sugars resulting in a significant factor in the contribution of these

ingredients to overall cupcake color and texture in some formulations *(*Jasberg *et al.,* 1989*).*

Effect of different levels of Melissa cupcake on feed intake (FI) , body weight gain (BWG), and some relative organs weight of hepatic rats.

The effect of different levels of Melissa cupcake on feed intake (g/day/each rat), body weight gain (BWG), and relative some organs weight including "liver, kidney, heart and spleen" of hepatic rats are presented in table (5). It could be noticed that the positive control group was significantly decreased in the feed intake and BWG when compared to normal rats. Treated rats with diet containing fortified cupcakes with 2.5%, 5% and 10% Melissa powder showed significant decreasing in FI and BWG when compared to positive control group and increasing as compared to negative control rats. Our results agree with **(Sief *et al.*, 2015**) who cleared that treated animals with *Melissa officinalis* (MO) shows a marked significant improvement in body weight, feed intake. Data in the same table, the liver, kidneys, heart and spleen of positive control group were significantly increased than the negative control group. While treated rats with different cupcake samples significant decreased their relative weight as compared to the positive control group and the weight improved by increasing the level of MP in cupcakes. The body weight is a sensitive indicator that reflects the state of health of experimental animals and the decrease in body weight correlates with defects in body metabolism **(Bhatiaand Khera, 2013).** Significant improvement may be due to active compounds such as Eugenol, Terpenoids and Flavonoids, works as antioxidants and substances that improve digestion by stimulating the release of digestive enzymes, which increases the utilization of nutrients, leads to weight gain and weight gain **(Kennedy *et al.,* 2016).** The proportion of flavonoids, that affected the appetite, consequently, which led to an increase in feed consumption, reflected the weight gain.

Effect of different levels of Melissa cupcakes on serum lipids fractions of hepatic rats

From these results which presented in table (6) it could be concluded that, injected rats with CCl4 induced elevation of serum cholesterol**,**

triglycerides, LDL-c and VLDL-c, while HDL-c decreased than that of non-injected rats. Feeding rats on fortified cupcake with tested different levels of Melissa powder led to significant decreasing in serum levels of TC, TG, LDL and VLDL and significant increasing in serum HDL which nearly returned toward the normal levels when treated with 10% cupcake fortified with MP. This in fact indicates that *Melissa officinalis* extract is more effective in reducing TG levels. The toxic liver in such a way that it reduces the number of LDL receptors in its cells due to increased levels of blood LDL-c. The obtained results agreed with **Bolkent *et al.* (2005)** who reported that *Melissa officinalis L* (MO) decreased levels of serum total cholesterol, total lipid. Fortified ally**, Ashtiyani *et al.* (2011)** found that reducing cholesterol involves reducing its excretion, and inhibiting its synthesis and absorption. The Melissa *officinalis* also suggested that it includes phenolic alkaloids among the materials that can impede the synthesis of cholesterol. The lipid lowering effect of the extract might be due to the action of flavonoids and other phenolic compounds, triterpenoids, alkaloids, steroids and glycosides. Normalized rate of lipogenesis is due to the insulin-like activity of triterpenoids or activating normoglycemia by the insulin tropic effect of flavonoids**.**MO is a perennial herb of the *Lamiaceae* family that has been shown to modulate the serum lipid profile **(Sakurai *et al.,* 2002).**In another study, showed that toxicity rats caused by KbrO3 have substantial rises in TG, TC, and LDL-c serum levels, and decreased HDL-c serum levels. Fortified with MO proved to be as a rich source of antioxidants and active compounds, and can be beneficial in the reversal of LDL-c serum levels with borderline hyperlipidemia **(Parisa *et al.*, 2016).**

Effect of different levels of Melissa cupcakes on serum uric acid, nitrogen urea and creatinine levels of hepatic rats (mg/dl).

Results in table (7) exhibited that levels of creatinine, urea and uric acid of nephritic positive control group were markedly increased significantly (P≤0.05) as compared to negative control group. All hepatic rats fed on diet containing cupcakes with 2.5,5 and 10% Melissa powder

showed dramatically decreases significant in levels of kidney functions comparison to hepatic positive control group. The best treatment was recorded for group 5 (hepatic rats fed on 10% MP). The oral administration of Melissa powder to rats injured with renal toxicity due oxidative stress by cisplatin led to improving on renal function where markedly reduced level of urea, uric acid and creatinine and increase glomerular filtration rate, this due to it contained flavonoid and polyphenolic compounds which is responsible for its strong antioxidant capacity and decline the risks complications of acute kidney failure (**Ashtiyani *et al.*, 2011**). **Also, Sepide *et al., (*2017*)*** reported that MP had effect in preventing and treating oxidative stress-related diseases, radical scavenging effects and anti-inflammatory activities**.**

Effect of different levels of Melissa cupcakes on liver functions (U/L) of hepatic rats.

Results of alanine amino transferase, aspartate amine transaminase and alkaline phosphatase enzymes of rats are presented in table (8). It has been found that ALT, AST and ALP of positive control rats were significant increased in comparison to negative control group. On the other hand, treated groups fed on cupcakes fortified with 2.5, 5 and 10 %MP had significantly decreased in ALT,AST and ALP comparison to CCl4 intoxicated group and the best group was fed on 10% MP. The results agree with ***Bolkent et al.,* (2005*).*** Who reported that administration of *Melissa officinalis* extract increased the content of glutathione (GSH), in liver and blood of hyperlipidemic rats**. Dastmalchi *et al.* (2008)**. Who cleared that antioxidant components and scavenging effects, Melissa officinalis L may improve antioxidant defensive activity and reduce oxidant stress and AST in Al workers**. Sofowora *et al.* (2013*)*** reported that a radical scavenging and antioxidant potential of polar extracts from Melissa for its content of flavonoids, rosmarinic acid, and the benzodioxole. The antioxidant effects of these compounds are up to 10 times stronger than the effects of those of vitamins B and C.

Also Ali*et al.* (2014) cleared that the liver enzyme production in the treatment groups receiving Melissa officinalis (MO) had decreased liver

enzyme levels. ***Sepide et al. (2017)*** reported that MO had a diminishing effect on hepatic enzyme activity in treatment groups. Because of its antioxidant properties, polyphenolic compounds can neutralize free radicals and inhibit their destructive effects. Another study by **Bolkent *et al.* (2005)** found that previous experiments and research conducted suggested that *M. officinalis* (MO) may lower liver function, and was effective in improving lever function and treating liver disease. Also, **Xufeng *et al.* (2016)** results showed that total flavonoids (TFs) reduced serum AST, ALT and improved hepatic histopathology.

The applied products

The control cupcake and cupcakes with different levels of MP (2.5,5 and 10%) were presented in photos (1,2,3 and 4). it could be noticed that the differences between the fortified cupcakes and control sample in their volume and color , the increasing of MP level led to decreasing their volume and increasing the dark color. These changes in volume due to the high level of fiber which absorbed water in the dough and its dark color led to the effect of heating on the content of fiber ***(*Jasberg *et al.* 1989).** The formation treatment made with 2.5%MP as partial replacement of wheat flour had very good acceptance followed by 5% and the last degree was 10%.

Histopathological examination

Liver of rat fed on basal diet (control-) showing the normal histological structure of hepatic lobule (**photo 5).**Meanwhile, liver of injected rat with CCL4 and fed on basal diet (control+) showing portal infiltration with leucocytes and vacuolations of hepatocytes and focal hepatic necrosis associated with leukocyte cells infiltration (**Photos 6 and 7)**.Moreover, liver of rat suffering from chronic liver diseases and treated daily with Melissa powder 2.5% (MP 2.5%) showing vacuolations of centrilobular hepatocytes (**photo 8**). Liver of rat suffering from chronic liver diseases and treated daily with MP 5% showing slight congestion of central vein (**photo 9).** Liver of rat suffering from chronic liver diseases and treated daily with MP 10 % showing no histopathological changes (**photo 10).** In case the effect of

different tested samples of cupcake on the histological results of spleen, it could be noticed that spleen of rat from control negative group showing normal lymphoid follicle (**photo 11**) while spleen of rat from positive control group showing slight lymphocytic necrosis (**photo 12**). Moreover, spleen of hepatic rat fed on basal diet and 2.5 % MP showing slight lymphocytic necrosis (**photo 13)** and spleen of hepatic rat fed on basal diet and 5 % MP showing slight lymphocytic necrosis. However, spleen of hepatic rat fed on basal diet and 10%MPshowing no histopathological changes **(photo 14)**. Histopathological changes of liver and spleen tissue in groups receiving 2.5, 5and 10% of PM led to perooxidant or antioxidant activity of plant and chemical compounds which has near relationship with consumed concentration that corresponds with liver pathologic lesions at doses of 10%. Studies on the cellular models have shown that some antioxidant polyphenols like quercetine, catechins (Epigallocatechin-3-gallate and epicatechin) and gallic acid have perooxidative activity (**Valko *et al*. 2007 and Ozden *et al*. 2015).**

Conclusion

Previous studies and studies done together with this one show that *Melissa officinalis* and its products by decreasing AST, ALP, and ALT as well as cholesterol is effective in improving the function of liver and treating liver diseases. Its metabolic intervention occur when the plant content acts to protect the liver and reduce the amount of lipid profiles and influence the kidney function. The presence of active and effective antioxidants, especial ability to inhibit the production of free radicals, as well as cytotoxic and anti-mutagenic effects, have given a unique feature to the plant**.**

Table (1): The proximate composition analysis of the dried Melissa powder

Parameters	Chemical composition (%) Mean ±SD
Moisture	19.73±1.07
Protein	6.69±0.08
Fat	5.07±9.32
Ash	6.7±0.94
Fiber	9.34±0.76
Carbohydrates	52.47±4.97

The results presented as means±SD

Table (2): Total phenolic compounds, total flavonoid, and Antioxidant Activity of Melissa powder

Components	Values Mean±SD
Total phenolic (mg/g)	13.57±0.24
Total flavonoid (mg/g)	24.06±0.96
Antioxidant Activity (AOA %)	86.96±0.28

The results presented as means±SD

Table (3): Chemical composition of control and fortified cupcakes with different levels of Melissa powder

Parameters / Samples	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate
Control cupcake	17.73^{a}±1.92	7.60^{a}±1.03	1.40^{c}±0.22	0.71^{d}±0.05	1.31^{d}±0.76	71.25^{a} ±3.45
Cupcake with MP 2.5%	17.78^{a}±0.99	7.59^{a}±0.87	1.50^{b}±0.34	0.89^{c}±0.02	1.51^{c}±0.34	70.73^{a} ±4.12
Cupcake with MP 5%	17.83^{a}±1.43	7.56^{a}±1.54	1.58^{b}±0.04	1.1^{b}±0.23	1.71^{b}±0.07	70.22^{a} ±3.09
Cupcake with MP 10%	17.92^{a}±1.64	7.51^{b}±1.75	1.77^{a}±0.92	1.31^{a}±0.11	2.1^{a} ±0.65	69.39^{a} ±2.75

All results are expressed as mean± SD. means followed by different superscripts within columns are significantly different (P≤0.05).

Table (4): Sensory evaluation of control and fortified cupcakes with different levels of Melissa powder (MP)

Premaster / Samples	Color	Taste	Texture	Odor	Appearance	Overall acceptability
Control cupcake	9.64^{a}±0.25	9.7^{a}±0.15	9.6^{a}±0.12	9.82^{a}±0.09	9.71^{a}±5.86	9.63^{a}±0.29
Cupcake with MP 2.5%	8.81^{a}±9.69	8.9^{a}±0.17	8.73^{a}±0.35	8.82^{a}±0.36	8.89^{a}±0.15	8.82^{a}±0.18
Cupcake with MP 5%	7.8^{b}±1.34	7.8^{b}±1.13	7.67^{b}±1.54	7.76^{b}±1.48	7.73^{b}±1.17	7.73^{b}±0.12
Cupcake with MP 10%	6.53^{c}±0.65	6.6^{c}±0.46	6.60^{c}±0.10	6.30^{c}±0.97	6.53^{c}±0.33	6.53^{c}±0.76

Control: Wheat flour (72% extraction). All results are expressed as mean± SD.means followed by different superscripts within columns are significantly different (P≤0.05).

Table (٥): Effect of different levels of Melissa cupcake on feed intake (FI), relative body weight gain (BWG %), and some relative organs weight of hepatic rats

Parameters / Groups	FI (gm/day)	BWG%	Organ weight/body weight (%)			
			Liver	Kidney	Heart	Spleen
Negative Control (-)	16.92 [a] ± 1.89	33.88 [a] ± 1.12	2.89 [e] ± 0.28	0.42 [d] ± 0.01	0.32[c] ±0.01	0.22[e] ±0.02
Positive Control (+)	6.58 [d] ± 1.12	8.05 [e] ± 1.03	5.32 [a] ± 0.77	0.63 [a] ± 0.02	0.38[a] ±0.01	0.45[a] ±0.0.01
Cupcake with MP 2.5%	7.12 [c] ± 1.12	10.85 [d] ± 1.43	4.96 [b] ± 0.86	0.59 [b] ± 0.01	0.36[a] ±0.02	0.41 [b] ±0.00
Cupcake with MP 5%	7.91 [c] ± 1.12	13.84 [c] ± 1.97	4.57 [c] ± 0.98	0.48 [c] ± 0.04	0.35[b] ±0.01	0.33 [c]±0.00
Cupcake with MP 10%	9.75 [b] ± 1.12	18.22 [b] ± 0.82	4.22 [d] ± 0.85	0.47 [c] ± 0.03	0.31[c] ±0.015	0.28 [d] ±0.01
LSD	1.09	1.925	0.051	0.027	0.028	0.018

All results are expressed as mean± SD. means followed by different superscripts within columns are significantly different (P≤0.05).

Table (6): Effect of different levels of Melissa cupcake on serum lipids fractions (mg/dl) of hepatic rats

Parameter / Groups	Lipid profile fraction(mg/dl)				
	TC	TG	HDL	LDL	VLDL
Control Negative (-)	97.89 [d] ± 3.82	96.54 [e] ± 5.53	55.50 [a] ± 1.36	23.08 [e] ± 0.79	19.31 [c] ± 0.61
Control Positive(+)	152.92 [a] ±1.35	173.13 [a] ±1.90	30.56 [e] ±1.21	87.73 [a] ± 2.21	34.63 [a] ± 0.49
Cupcake with MP 2.5%	135.13 [b] ±1.10	165.04 [b] ±1.29	35.79 [d] ± 0.83	66.33 [b] ± 0.79	33.01 [b] ± 0.49
Cupcake with MP 5%	117.27 [c] ±0.52	147.40 [c]±2.92	40.35 [c] ±0.14	47.44 [c] ± 2.50	29.48 [b] ± 0.03
Cupcake with MP 10%	100.48 [d] ±0.61	110.11 [d] ±1.19	46.75 [b] ± 1.33	31.71 [d] ± 0.75	22.02 [b] ± 0.59
L.S.D	5.12	3.21	3.21	1.97	2.93

HDL-c:High-density lipoprotein cholesterol, TC: Total Cholester , TG: Triglycerides, lLDL-c: Low-density lipoprotein cholesterol, VLDL-c: Very low-density lipoproteincholestero. All results are expressed as mean± SD. Means followed by differentsuperscripts within columns are significantly different (P≤0.05).

Table (7): Effect of different levels of Melissa cupcake on serum uric acid creatinine and urea nitrogen levels (mg/dl) of hepatic rats.

Parameters / Groups	Uric acid mg/dl	Urea nitrogen mg/dl	Creatinine mg/dl
Control Negative (-)	3.46 ^{e} ± 0.05	21.28 ^{e} ± 0.99	0.57 ^{e} ± 0.02
Control Positive (+)	7.99 ^{a} ± 0.01	44.40 ^{a} ± 0.93	1.95 ^{a} ± 0.06
Cupcake with MP 2.5%	6.63 ^{b} ± 0.002	40.58 ^{b} ± 0.67	1.85 ^{b} ± 0.02
Cupcake with MP 5%	5.09 ^{c} ± 0.14	36.94 ^{c} ± 0.23	1.69 ^{c} ± 0.02
Cupcake with MP 10%	4.40 ^{d} ± 0.01	30.12 ^{d} ± 1.89	1.46 ^{d} ± 0.05
LSD	0.128	1.991	0.077

All results are expressed as mean± SD. means followed by different superscripts within columns are significantly different (P≤0.05).

Table (8): Effect of different levels of Melissa cupcake on liver functions (U/L) of hepatic rats

Parameters / Groups	AST	ALT	ALP
Control Negative (-)	39.07 ^{e} ± 0.11	27.38 ^{e} ± 0.33	87.29 ^{e} ± 1.00
Control Positive (+)	128.75 ^{a} ± 1.50	147.67 ^{a} ± 0.51	308.62 ^{a} ± 2.19
Cupcake with MP 2.5%	116.66 ^{b} ± 3.71	135.73 ^{b} ± 1.83	290.09 ^{b} ± 3.51
Cupcake with MP 5%	105.2 ^{c} ± 2.45	128.51 ^{c} ± 0.64	274.87 ^{c} ± 4.33
Cupcake with MP 10%	90.31 ^{d} ± 0.65	97.41 ^{d} ± 0.96	254.69 ^{d} ± 5.62
L.S.D	3.85	9.02	6.73

All results are expressed as mean± SD. means followed by different superscripts within columns are significantly different (P≤0.05).

The applied product

Photo (1): The cupcakes without MP

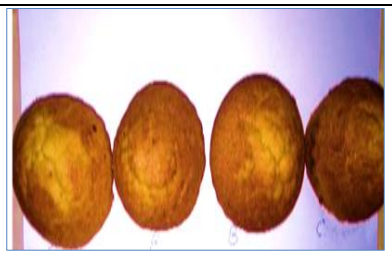

Photo (2): The cupcakes fortified with 2.5% MP

as a control sample

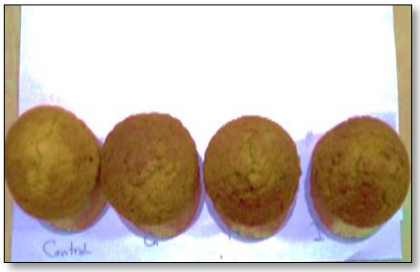

Photo (3): The cupcakes fortified with 5% MP

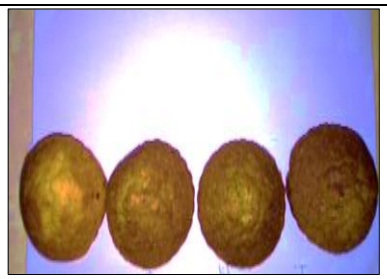

Photo (4): The cupcakes fortified with 10% MP

Histopathological changes of liver and spleen

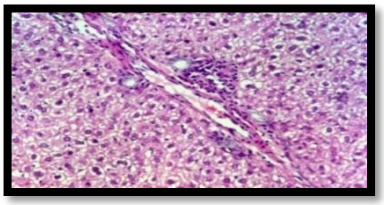

Photo (5): Liver of rat fed on basal diet as negative control showing no histopathological changes (H and E x 200).

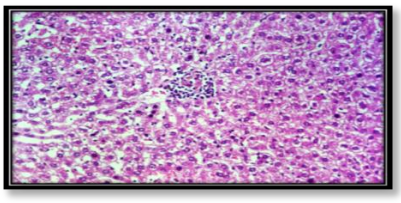

Photo (6): Liver of hepatic rat fed on basal diet as positive control group showing portal infiltration with leucocytes (H and E x 200).

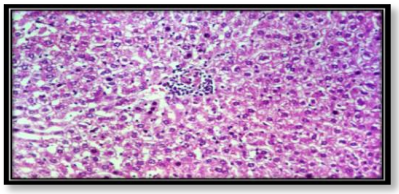

Photo (7): Liver of hepatic rat and fed on basal diet as positive control group showing vacuolations of hepatocytes and focal hepatic necrosis associated with leucocytic cells infiltration.

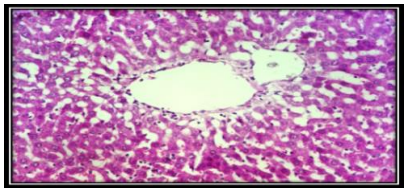

Photo (8): Liver of hepatic rat fed on basal diet with 2.5% MP showing vacuolationof Centro lobular hepatocytes (H and E x 200).

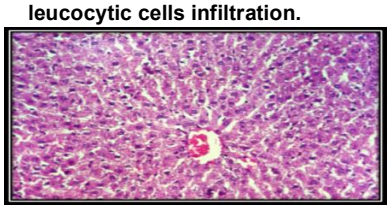

Photo (9): Liver of hepatic rat fed on basal diet with 5% MP showing slight congestion of central vein (H and E x 200).

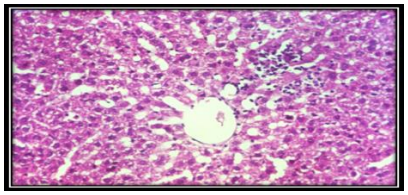

Photo (10): Liver of hepatic rat fed on basal diet with 10 % MP showing no histopathological changes (H and E x 200).

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التأثير المحسن للكب كيك المدعم بعشبة المليسيا على التسمم الكبدي في الجرذان

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الملخص العربي

أجريت هذه الدراسة لمعرفة هدف تأثير عشبة الميلسيا على الفئران المصابة بسمية الكبد والاستفادة منها فى اعداد منتج من الكب كيك كوقاية لسمية الكبد من اجل ذلك تم استخدام ثلاثين ذكرًا من الجرذان البيضاء البالغة وزنها ١٨٠ جرام ثم قسمت إلى مجموعتين رئيسيتين ، المجموعة الأولى (٦ فئران) لا تزال تتغذى على النظام الغذائي الأساسي (المجموعة الضابطة السالبة)، والمجموعات الثانية والثالثة والرابعة والخامسة وعددهم (٢٤ فأر) فقدُحقنت تحت الجلد لمدة أسبوعين برابع كلوريد الكربون بزيت البرافين الذائب (١:١ حجم/حجم) ٢ ملجم/ كجم) مرتان فى الاسبوع ،لمدة اسبوعين متتاليين لاحداث خلل كبدي واعتبرت المجموعة الثانية حيث تناولت الغذاء الاساسى فقط (المجموعة الضابطة الموجبه) ، أما المجموعة الثالثة والرابعة والخامسة فقد تناولت الفئران الغذاء الاساسى في التجربة مضاف إلية١٠ % كب كيك مدعم بمسحوق عشبة المليسيا ٢,٥ ، 5 ،10% على التوالي ولقد أسفرت النتائج عن حدوث نقص معنوي في صورة الكوليسترول الكلى والجليسريدات الثلاثية وكذلك إنزيمات الكبد و نيتروجين اليوريا وحامض اليورك والكرياتنين التي تغذت على الغذاء الاساسى والمضاف إلية نسب عالية من مسحوق عشبة المليسيا. وكانت أفضل النتائج للمجموعة التى تغذت على كب كيك المدعم بعشبة المليسيا ١٠ .% وتوصى الدراسة بتدعيم الوجبات والمنتجات الغذائية بمسحوق عشبة المليسيا وذلك لما أظهره من نتائج ايجابية في تحسين وظائف الكبد والكلى وكذلك صورة الدهون، وكونه كمصدرا جيدا لعمل منتج غذائي وظيفي جديد كوقاية لسمية الكبد .

الكلمات الافتتاحية: مسحوق ميليسا, وظائف الكبد والكلى, كب كيك, هستوباثولوجى.