

Effect of Fortified Pan Bread with *Melissa Officinalis* L. on Induced Oxidative Stress in Rats.

Asmaa M.I.El Gamel

¹Fellow (Lecturer) of Nutrition and Food Science, Ahmed Maher Teaching Hospital, Egypt.

Abstract

Melissa officinalis L(MO) is also known as bee balm, lemon balm, honey balm, and is a perennial herb. It is a member of the Lamiaceae family Which is often referred to as (the mint family). The influence of diet containing fortified bread with(5, 7.5 , 10%)*Meliss* powder(MP) on rats suffering from oxidative stress has been studied. Fortified bread with (5 , 7.5 , 10%) MP were prepared and were subjected to sensory evaluation.The best level of fortified bread after sensory evaluation was 5% but the best level of fortified bread after the biological study was 10%.The chemical composition of bread was estimated at 5% due to its acceptance in sensory evaluation. Twenty five male albino rats(170-190g) were randomly distributed into 5 equal groups,of 5 rats each. Group 1 was fed on the basal diet containing unfortified pan bread and kept as a negative control group. The other 4 groups (n=20 rats), were injected with a single intraperitoneal dose of 125mg/kgbw of potassium bromate (KBrO₃) for oxidative stress induction group 2 was fed on the basal diet, which includes unfortified pan bread as positive control rats, groups 3 , 4 and 5 were fed on diet containing fortified pan bread with (5 , 7.5 & 10%)MP respectively. The obtained results revealed that the injected groups with levels 10% of diet containing fortified bread with MP had significant decrease in serum levels of lipid profile (total cholesterol, triglycerides , LDL , VLDL), kidney,liver function and serum malondialdehyde (MDA) and significant increased in serum levels of HDL, total antioxidant capacity (TAC), glutathione (GSH), levels of glutathione peroxidase (GPX),superoxide dismutase (SOD) and catalase (CAT) enzymes. Moreover, there was a significant reduced in MDA and increase in GSH levels and the activity of antioxidant enzymes(GPX, SOD and CAT)in liver tissues as well as partially improvements in liver structures compared to positive intoxicated “control group”, but non significant increase in(BWG%) , FI and FER which nearly returned toward the negative control group. The best improvements among all the biochemical parameters and histological structures of liver which were tended toward normal results were observed in group treated with bread fortified 10%MP.In conclusion, the present findings suggested that regular intake from 10% fortified bread with MP may be useful

in improving liver and kidney functions and may protect against potassium bromate (KBrO_3) which induced oxidative stress in rats.

Keywords: Antioxidant enzymes, liver functions, kidney functions, Lipid profile, *Melissa officinalis* L, oxidative stress, rats.

Introduction

Oxidative stress is defined as increased production of reactive oxygen species (ROS) that exceeds existing antioxidant defence mechanisms. (Hyunjin Noh and Hunjoo Ha, 2011). It plays a major role in the aetiology and pathogenesis of many chronic diseases, (Krajcovicova –Kudlackova *et al.*, 2012). The hallmarks of many chronic diseases and cell death are oxidative stress associated with oxygen-free radicals and the subsequent tissue destruction (Mates *et al.*, 2002). The healing ability of medicinal plants as natural antioxidants to mitigate tissue damage inflicted by free radicals and to preserve health and defence against such age-related degenerative diseases such as cancer and coronary heart disease (Pourmorad *et al.*, 2006).

Melissa officinalis L(MO) is also known as bee balm, lemon balm, honey balm, and is a perennial herb. It is a member of the *Lamiaceae* family Jastrzebska-Stojko *et al.*, (2013). *Melissa officinalis* (*M. officinalis*) is a medicinal herb native to the Eastern Mediterranean and Western Asia (Emamghorishi and Talebianpour, 2009). (MO) can be considered an effective agent rats for the prevention of different neurological disorders associated with oxidative stress. (Sepide Miraj *et al.*, 2017). (MO) contains a rich amount of natural antioxidants and is beneficial in many diseases of oxidation. (Hasani-Ranjbar *et al.*, 2009). A recent research found that Lemon balm protects against oxidative stress in subjects exposed to long-term low-dose ionizing radiation (Zeraatpishe *et al.*, 2011). The antioxidants are known to play an important role in protecting against oxidant damage-induced disorders. Production of reactive oxygen species (ROS) can surpass Cellular antioxidant defenses called oxidative stress (Capecka *et al.*, 2005).

The antioxidants in plants are widely known for shielding humans from free radicals and avoiding other diseases. The leaves of *Melissa officinalis* contains polyphenolic compounds, such as rosmarinic acid, trimeric compounds and other flavonoids, which can scavenge free radicals and have antioxidant effects. (Zeraatpishe *et al.*, 2011). Lemon balm (*Melissa officinalis*) is considered herbal medicine to treat headaches, gastrointestinal disorders, nervousness and rheumatisms and the essential oil of (MO) which is rich in aldehydes and terpenic alcohols (Robeiro *et al.*, 2004) is reported to have anti-

microbial properties as well as a strong protective ability against lipid peroxidation (**Mimica-Dukic et al., 2004**).

Melissa officinalis that plant rich in flavonoids and phenols in general, which are the highly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used to prevent and cure of various diseases (**Deepa et al., 2009**). Flavonoids, a group of polyphenolic compounds with known properties, such as free radical *Melissa* activity, inhibition of hydrolytic and oxidative enzyme and anti inflammatory action (**Omale and Okafor , 2008**). Dry herbs were abundant in tannins and rutin. Tannins exhibit many biologically important functions (**Atanassova and Christova-Bagdassarian, 2009**). In human diets tannins are the most available antioxidants (**Has et al., 2007**). *Lemon balm* infusions boost oxidative stress in workers as used as a dietary supplement (**Davood Fazli et al., 2012**). A collection of flavonoids from medicinal plants were investigated for their function as microsomal lipid peroxidation inhibitors and in vitro oxygen-free radicals scavengers as well as a xenobiotic toxicity model in the mouse (**Sanz et al., 1994**). *Melissa officinalis* is regarded as a useful herbal plant for the prevention of various neurological diseases such as Alzheimer's (**Mohammad et al., 2012**).

Melissa powder contain moisture, protein, fat , ash, fiber and carbohydrates as follow 17.73 , 7.60, 1.30, 0.71, 1.41 and 71.35% respectively(**Hayam, 2020**).

Therefore the present study was designed to study of the effect of fortified bread with *Melissa officinalis L.* against (KBrO₃)induced oxidative stress in" rats"as animal model.

Materials and Methods

Materials

- Fresh plant of *Melissa officinalis L* were obtained from National Research Center (NRC) in Dokki, Cairo, Egypt.
- Casein, vitamins, minerals, cellulose, choline chloride and potassium bromate(KBrO₃) were purchased from El-Gomhoreya Company, Cairo, Egypt.
- Oil and starch, sugar, salt and wheat flour(72% extraction) were purchased from local market, Cairo, Egypt.
- Twenty-five male albino rats (Sprague Dawley Strain) were obtained from Food Technology Res. Institute, Giza.

Methods

Preparation of *Melissa officinalis L* powder:

Melissa officinalis L, was washed with flowing tap water and dried with solar energy,(10.5 in hours) temperature(50°C) relative humidity 21% **Ibrahim, (1999)**. The whole quantity was transferred to the solar cabinet dryer

chamber of the solar energy laboratory at the National Research Center for dehydration after which the dried plant was grounded in fine powder.

Preparation of *Melissa officinalis* L Fortified Pan Bread :

The standard formula was according to Abdelghafor *et al.*,(2011) The ingredients Included 1000g of wheat flour(wheat flour extracting rate 72%), 15g instant yeast, 10g salt(NaCl) , 10g Improver(goldenpack), 40g sugar (sucrose). Three formulas were prepared with different levels of *Melissa officinalis* L powder 5%, 7.5% and 10% on wheat flour replacement basis and pure water added to make the dough. All dry ingredients were weighed and placed in a mixer for 5 sec, and then a suspension of the yeast in water was added. The mixture was further run at high speed for 92sec and water was added to the mixture for creating the dough.The dough was scaled into three portions, rounded into balls by hand in a fermentation bowls and placed in fermentation cabinet at 30°C and 85 percent relative humidity for 20 min. The fermented doughs were placed in pans and finally returned into the fermentation cabinet for 50 min. The pans were put in a convection oven at 212 ° C for 18 min. sensory assessment of loaves was registered.

Chemical analysis of un-fortified and fortified bread with (5%) *Melissa officinalis* L powder:

Chemical analysis of un-fortified and fortified bread with(5%) *Melissa officinalis* L powder including protein, carbohydrate, fat, moisture and ash were determined in Food Technology Res. Institute according to the method described by the(A.O.A.C.,2003).

Determination of total flavonoids for un-fortified and fortified bread with (5 ,7.5 ,10%) *Melissa officinalis* L powder:

Total flavonoids were estimated using the method of Ordonez *et al.*,(2006).

Determination of total phenolics for un-fortified and fortified bread with (5 ,7.5 ,10%) *Melissa officinalis* L powder:

Total phenol contents were determined by the modified Folin- Ciocalteu method of Wolfe *et al.*,(2003).

Sensory evaluation of un-fortified and fortified bread with (5% ,7.5 and 10%) *Melissa officinalis* L powder:

Sensory evaluation was performed by invited ten panelists of the staff members from Food Technology Res. Institute , Ministry of Agriculture, Giza. Each panelist was asked to evaluate unfortified and fortified bread samples with 5, 7.5 and 10 % (Melissa powder), according to color, odor, taste, volume, texture tenderness and general acceptability (Abd El-Latif 1990). According to general acceptability the best level of fortified bread was 5%.

The biological assay:

Male albino rats Spargue Dawley Strain (25 rats) weighing (170 -190 g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet according to **Reeves *et al.*, (1993)** for adaptation at ad libitum in the animal house of Agricultural Res. Center in Ministry of Agriculture , Giza. After this period, the rats were divided into two main groups , the first main group (n=5 rats) fed on basal diet containing(300g/kg diet) un-fortified bread and kept as a control negative group. The second main groups (20 rats) were injected by a single intraperitoneal dose of potassium bromate at dose of 125 mg/kg body weight for induction of oxidative stress according to the described methods by **Khan and Sultana(2004)** the injected groups were divided into 4 subgroups as the following , subgroup 2 fed on basal diet containing (300g/kg diet) un-fortified bread as positive control group, subgroups 3 ,4 and 5 were fed on diet containing (300g/kg diet) fortified bread with (5% , 7.5% and 10%), respectively.

During the experimental period (five weeks), each rat was weighed every week and food consumption was recorded. The body weight gain%(BWG) and feed efficiency ratio (FER) were determined according to **Chapman *et al.*, (1959)** using the following formula:

$$(BWG\%) = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} * 100$$

$$(FER) = \frac{\text{Daily body Weight gain(g)}}{\text{Feed intake (g/d)}}$$

At the end of the experimental period rats were fasted over night before sacrificing blood was collected then centrifuged. Serum was separated and stored at -20°C for biochemical analysis i.e. serum total cholesterol(TC) and triglycerides(TG)were according to **Schettler and Nussel, (1975)**, high density lipoprotein cholesterol (HDL-c) **Lope Virella *et al.*, (1977)**, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c)were according to **Fried wald *et al.*, (1972)**, aspartate amino transferase (AST) and alanine amino transferase (ALT) **Reitman and Frankel, (1957)**, serum alkaline phosphates (ALP) **Belfield and Goldberg, (1971)**, serum uric acid **Fossati *et al.*, (1980)**, urea (**Marsch *et al.*, 1965**), Creatinine **Bartels and Bohmer, (1971)**,serum malondialdehyde (MDA) **Draper and**

Hadley (1990),glutathione (GSH) **Beutler *et al.*,(1963)**.Serum activity of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) enzymes were assayed according to the method of **Hissin and Hiff (1976)**, **Kakkor *et al.* (1984)** and **Sinha(1972)**, respectively. Serum total antioxidant capacity(TAC) **Woodford and Whitehead (1998)**.

Assay levels of (GSH) and activity of antioxidant enzyme in liver tissues:

Oxidant and antioxidant status in liver tissues includes(MDA) **Albro *et al.* (1986)**. Non-enzyme antioxidant as (GSH) in liver tissues **Beutler *et al.* (1963)**. Activities of antioxidant enzyme of GPx, SOD and CAT in liver tissues **Paglia and Valentine (1979)**, **Nishikimi *et al.* (1972)** and **Aebi (1984)**,respectively.

Preparation of liver tissue homogenate:

Part of liver of all animals were cut into small pieces and were determined as described by(**Montgomery and Dymock, 1961**).

Histopathological Examination

liver tissue was separated from each rat and examined histopathologically **Bancroft *et al.*, (2012)**.

Statistical analysis

The results were expressed as means \pm SD statistically analyzed using one-way (ANOVA) test, $p < 0.05$ was used to signal significance **Steel and Torri, (1980)**.

Results and Discussion

Table (1): Chemical analysis of un-fortified and fortified bread with 5% *Melissa* powder.

The chemical analysis of un-fortified and fortified pan bread with 5% *Melissa* powder(moisture,protein, fat, ash , crude fibers and carbohydrates) is demonstrated in **Table (1)**.The moisture content of un-fortified bread was higher than fortified bread with 5% *Melissa* powder. On the other hand,the fortified bread with 5% *Melissa* powder showed increase in ash and crude fibers than that of the un-fortified bread.While carbohydrate , protein and fat values in fortified bread with 5% *Melissa* powder nearly returned toward the un-fortified bread values.

Table (2):Active Component of un-fortified and fortified bread with (5,7.5 and 10%) *Melissa* powder.

The three types of fortified bread and *Melissa* powder showed increase in total (phenolic and flavonoid) than the un-fortified bread.While the three types of fortified bread recorded decrease in active component than that of *Melissa*

powder. On the other hand, active component recorded the highest value in fortified bread with 10% *Melissa* powder than that of fortified bread with 5% while fortified bread with 10% nearly returned toward the fortified bread with 7.5%. The findings are in accordance with **Pereira *et al.*, (2009)** who showed that *M. officinalis* is highly antioxidant and has phenolic compounds. Additionally, **(Sepide Miraj *et al.*, 2017)** revealed that The dried *Melissa officinalis* leaves 11.8 percent of total polyphenol compounds and 0.5 percent of total flavonoid compounds. **(Maria Atanassova and Silviya Georgieva, 2010)** who found that The overall *Melissa officinalis* phenolic and flavonoid content ranged from (48.86 mg GAE/100 g to 45.06 mg GE/100 g).

Table (3): Sensory evaluation of pan bread un-fortified and fortified with different levels of *Melissa* powder.

The results in **Table(3)** indicated that bread fortified with 5% *Melissa* powder had a non-significant in (taste, odor, volume, texture, tenderness and general acceptability except in external color and internal color and showed bread fortified with 7.5% MP had a non-significant in taste in comparing with control un-fortified bread, except external color, internal color, odor, volume, texture, tenderness and general acceptability compared with control un-fortified bread. While, bread fortified with 10% MP recorded the lowest significant in external color, internal color, odor, volume, texture, tenderness and general acceptability in compared to the control un-fortified bread. Therefore fortified bread with 5% recorded the best results in sensory evaluation (general acceptability).

Table (4): Feed intake (FI), feed efficiency ratio (FER) and body weight gain (BWG%) in normal, intoxicated and treated rats treated with different levels of *Melissa* powder.

Results in **Table (4)** indicated that the positive control group was non-significantly in the feed efficiency ratio, BWG% and FI when compared to normal rats. Comparing treated rats with diet containing fortified bread with 5% *Melissa* powder, 7.5% and 10% revealed non-significant in FI, BWG and FER compared to negative and positive control group 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.

Table (5): Serum levels of Triglycerides, Total cholesterol, High density lipoprotein Cholesterol, Low density lipoprotein cholesterol, Very low density lipoprotein Cholesterol in normal, intoxicated and treated rats with different levels of *Melissa* powder.

The obtained results in **Table(5)** showed a marked significant increase in serum total cholesterol, triglycerides, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol levels and a significant reduced in serum high-density lipoprotein cholesterol levels in positive rats compared to normal rats. Comparing untreated rats with those treated with (5, 7.5, 10%) bread fortified with MP revealed a marked significant decrease in serum levels of TC, TG, LDL and VLDL and significant increase in serum HDL which nearly returned toward the normal levels. Our results agreed with (**Bolkent et al., 2005**) *Melissa officinalis* L (MO) admin decreased levels of serum total cholesterol, total lipid. Additionally, (**Pereira et al., 2009 & Ashtiyani et al., 2011**) found that reducing cholesterol involves reducing its excretion, and inhibiting its synthesis and absorption. The *officinalis* also suggested that it includes phenolic alkaloids among the materials that can impede the synthesis of cholesterol. MO is a perennial herb of the *Lamiaceae* family that has been shown to modulate the serum lipid profile. (**Parisa Jandaghi et al., 2016**). In another study, confirmed that the present results showed that toxicity rats caused by KBrO₃ have substantial rises in TG, TC, and LDLc serum levels, and decreased HDL-c serum levels (**Moller and Wallin, 1998**). 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 Jandaghi et al., 2016**).

Table (6): Kidney function in normal, intoxicated and treated rats treated with different levels of *Melissa* powder.

Data in **Table(6)** showed that positive control group have significant elevations in serum levels of kidney function compared to normal control rats. In fed contrast, rats fed on bread fortified with MP (5%, 7.5% and 10%) had expressively reduced serum levels of kidney function in comparison to intoxicated positive rats. While there was non significant changes in uric acid and creatinine between treated groups and normal rats. The present result is in agreement with **Khan et al. (2011) & Shelbaya et al. (2014)** who showed that KBrO₃-intoxicated caused substantial changes in serum kidney function levels compared to negative control group. Another study by **Sief et al., (2015)** revealed that In all parameters of kidneys tested, *Melissa officinalis* (MO) resulted in a pronounced ameliorating effect.

Table (7) Liver function in normal, intoxicated and treated rats treated with different levels of *Melissa* powder.

Results of alanine amino transferase, aspartate amine transaminase and alkaline phosphatase enzymes of rats are presented in **Table(7)**. It has been found that ALT, AST and ALP of positive control rats were significant increase, in comparison to negative control group. On the other hand, treated

groups fed on bread fortified with MP (5%, 7.5% and 10%) had significantly decreased in ALT, AST and ALP comparison to KBrO₃- intoxicated group. Our results agree with **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 AI workers. Also **Ali et al., (2014)** cleared that the liver enzyme production in the treatment groups receiving *Melissa officinalis* (MO) had decreased liver enzyme levels. **Schulz et al., (2004)** reported that *m.officinalis* 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 liver function and treating liver disease. Also **Xufeng Tao et al., (2016)** results showed that total flavonoids (TFs) reduced serum AST, ALT and improved hepatic histopathology.

Table (8) Serum levels of SOD, CAT and GPX enzymes in normal, KBrO₃- intoxicated and intoxicated rats treated with different levels of *Melissa* powder.

Table(8) shows a marked significant decrease in serum antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in positive control rats (Kbro₃ intoxicated) compared to negative rats. In comparison to the treated KBrO₃ intoxicated rats with (5%, 7.5% and 10%) bread fortified with MP caused significant increase in serum activity SOD, CAT and GPX enzymes. Our results agree with **Dastmalchi et al., (2008)** who cleared that *Melissa officinalis L* infusion improves catalase plasma levels, SOD, and GPX.

Table (9) Serum levels of MDA, GSH and TAC in normal, intoxicated and treated rats with different levels of *Melissa* powder.

Results in **Table(9)** showed significant decrease in serum glutathione (GSH) and total antioxidant capacity (TAC) levels compared with those of the normal rats. Treated Kbro₃ intoxicated groups (G₃, G₄ and G₅) have significant increase and ameliorate in serum of GSH and TAC levels compared with those of the untreated Kbro₃ intoxicated rats. While, results show a marked significant increase in serum malondialdehyde (MDA) level comparing with normal rats. In contrast, groups fed on supplemented diet with (5%, 7.5% and 10%) MP had significantly decreased serum levels of (MDA) when compared to positive rats. Our results agree with **Davood Fazli et al., (2012)** who stated that using *Melissa officinalis* infusion resulted in a significant elevation in (TAC) serum levels. Also **Xufeng Tao et al., (2016)** said that total

flavonoids TFs significantly reduced malondialdehyde (MDA). (Xufeng Tao *et al.*,2016) showed that complete flavonoids (TFs) improved Glutathione S-Transferase (GST) protein levels to boost oxidative stress.

Table (10) activity of SOD ,CAT and GPX enzymes in liver tissues of negative, intoxicated and treated groups with different levels of *Melissa* powder.

Table(10) shows a marked significant reduced in the levels of antioxidant SOD, CAT and GPX enzymes in liver tissues of positive control group compared to the normal control group. Also, there was a significant increase in levels of antioxidant enzymes in treated groups fed on pan bread fortified with(5%, 7.5% and 10%)MP compared with those of positive intoxicated rats.Our results is in agreement with Xufeng Tao *et al.*,(2016) who reported that Total flavonoids TFs significantly increased enzymes of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), indicating that oxidative stress was reduced by TFs.

Table (11) levels of MDA and GSH in liver tissues of negative,intoxicated and treated groups with different levels of *Melissa* powder.

Comparing positive control group with negative control group the present results showed a marked significant elevations in MDA and reduced in GSH contents in liver tissues homogenates(**Table 11**).Treated groups fed on pan bread fortified with(5%, 7.5% and 10%)MP caused significant reduced in MDA and elevations in GSH content in liver tissues homogenates compared to those of positive intoxicated control group. Our results agreed with **Bolkent *et al.*,(2005)**who stated that *officinalis* admin increased levels of glutathione in the the liver tissue .Additionally, **Vasques *et al.*,(2016)** found that total flavonoids(TFs) were found to increase Glutathione S-Transferase (GST) levels and liver tissue levels.Another study, confirmed that the most important antioxidants are the polyphenolic compounds (**Pyo *et al.*, 2004**). antioxidants compounds, particularly flavonoids, have a protective effects on hepatic damage from free radicals and liver toxins (**Yoshikava *et al.*,2003**).

Histopathological Examinations

Examination of liver Histopathologically:

Rat's liver at **negative rats (G₁)** showing normal histological architecture of hepatic lobule from central vein and hepatocytes (**Fig. 1**). On the other hand, examined sections from **Control Positive (G₂)** revealed cytoplasmic vacuolization of hepatocytes , focal hepatic necrosis associated with inflammatory cells infiltration (**Fig. 2**) and portal inflammatory cells infiltration. However, rat's liver of **Bread fortified with(5%) MP (G₃)** revealed improved picture, examined sections showed slight cytoplasmic

vacuolization of hepatocytes , and small focal hepatic necrosis associated with inflammatory cells infiltration (**Fig. 3**). Moreover, rat's liver of **Bread fortified with (7.5%) MP (G₄)** showed regression of the histopathological changes, examined sections revealed only cytoplasmic vacuolization of hepatocytes (**Fig. 4**). Meanwhile, marked improved changes was observed in liver of rats fed **Bread fortified with(10%) MP (G₅)**, the liver restored the normal histological structure without histopathological alterations (**Fig. 5**). Our results agree with (**Gokcimen et al.,2007**)who stated that the liver portions of animals treated with *Melissa officinalis*(MO) showed changes in histological picture.Additionally, (**Rosety et al.,2005**) revealed that antioxidants are the foremost system of defense that limits the toxicity associated with free radicals. The cellular antioxidant status also determines the susceptibility to oxidative damage and is usually altered to oxidative stress. (**Sief et al.,2015**) also found that a photomicrograph showing normal central vein and normal hepatocytes in control rat's liver. In the rats treated with *Melissa officinalis*(MO) the liver sections showed normal lymphocyte parenchyma and normal focal aggregation.. (**Shah et al.,2013**)reported that total flavonoids (TFs) were defensive by decreasing coagulation necrosis with significant infiltration of inflammatory cells in the liver.Another study by(**Medina and Moreno-Otero, 2005**) revealed that the findings obtained from the histopathological study, which showed that the injection of KBrO₃ caused morphopathological changes in the liver including inflammation of hepatic sinusoid, vacuolization of hepatocytes and necrosis of scattered hepatocytes, were verified.

Conclusion

In conclusion The effects of diet that include fortified pan bread with 10% MP had a good effect and exhibited improvement in kidney and liver functions against induced by potassium bromate(KBrO₃).The consisting of 10% fortified bread MP provide the best improvement in biochemical measurements and histological structure.Hence,regular intake of MP for enriching food product as functional foods may help to improve health status.

Table (1): Chemical analysis of un-fortified and fortified bread with 5% *Melissa* powder.

Content (%) Samples	Moisture	Protein	Fat	Ash	Crude fibers	Total Carbohydrates	Total
un-fortified bread Control	8.449	11.190	6.669	0.970	0.69	72.032	100
fortified bread with 5% <i>Melissa</i> powder	7.917	11.369	6.099	1.445	1.10	72.07	100

Table (2): Active Component of un-fortified and fortified bread with (5,7.5 and 10%) *Melissa* powder.

Samples \ Content (%)	Total phenolic (mg/g)	Total flavonoid (mg/g)
un-fortified bread Control	1.561	0.16
fortified bread with (5%) <i>Melissa</i> powder	1.701	1.29
fortified bread with (7.5%) <i>Melissa</i> powder	2.021	1.84
fortified bread with (10%) <i>Melissa</i> powder	2.456	1.96
<i>Melissa</i> powder	44.674	14.01

Table (3): Sensory evaluation of pan bread un-fortified and fortified with different levels of *Melissa* powder.

Characters \ Samples	External Color 5	Internal Color 5	Taste 10	Odor 5	Volume 5	Texture 5	Tenderness 5	General Acceptability 10
Control (un-fortified bread)	4.83 ^a ± 0.41	4.83 ^a ± 0.41	8.41 ^a ± 2.20	4.75 ^a ± 0.41	4.83 ^a ± 0.40	4.91 ^a ± 0.20	4.75 ^a ± 0.41	9.50 ^a ± 0.83
Bread fortified with (5%) MP	3.70 ^b ± 0.40	3.36 ^b ± 0.43	7.33 ^{ab} ± 2.16	4.00 ^{ab} ± 0.31	4.66 ^a ± 0.51	4.40 ^a ± 0.64	4.15 ^a ± 0.36	8.25 ^a ± 1.08
Bread fortified with (7.5%) MP	2.83 ^c ± 0.41	2.66 ^c ± 0.51	6.25 ^{ab} ± 1.75	3.33 ^{bc} ± 0.51	2.83 ^b ± 0.40	3.00 ^b ± 0.89	3.16 ^b ± 0.75	6.25 ^b ± 1.17
Bread fortified with (10%) MP	2.08 ^d ± 0.66	1.75 ^d ± 0.61	5.41 ^b ± 1.46	3.00 ^c ± 1.09	2.41 ^b ± 1.02	2.50 ^b ± 1.09	2.50 ^b ± 0.89	5.30 ^b ± 1.42
LSD	0.58	0.60	2.31	0.79	0.77	0.94	0.78	1.38

Means with different letters in each column are significantly different at $p < 0.05$.

SD: Standard Deviation

Table (4). nutritional parameters in normal, intoxicated and treated rats with different levels of *Melissa* powder.

Parameters \ Groups	FI (g/day)	FER(g)	BWG (%)
Control Negative G ₁	18.51 ^a ± 0.51	0.04 ^a ± 0.02	15.01 ^a ± 5.43
Control Positive G ₂	18.81 ^a ± 0.44	0.06 ^a ± 0.01	23.23 ^a ± 6.23
Bread fortified with (5%) MP G ₃	18.79 ^a ± 0.37	0.04 ^a ± 0.02	15.94 ^a ± 6.51
Bread fortified with (7.5%) MP G ₄	18.72 ^a ± 0.36	0.05 ^a ± 0.03	16.66 ^a ± 10.71
Bread fortified with (10%) MP G ₅	18.55 ^a ± 0.61	0.03 ^a ± 0.01	14.24 ^a ± 3.64
LSD	0.71	0.03	10.41

Means with different letters in each column are significantly different at $p < 0.05$

SD: Standard Deviation.

Table (5): Serum levels of Triglycerides, Total cholesterol, High density lipoprotein Cholesterol, Low density lipoprotein cholesterol, Very low density lipoprotein Cholesterol in normal, intoxicated and treated rats with different levels of *Melissa* powder.

Variables Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control NegativeG ₁	101.49 ^d ±23.87	113.35 ^c ±11.09	52.46 ^a ± 1.51	26.36 ^d ± 23.54	22.67 ^c ± 2.21
Control PositiveG ₂	282.10 ^a ±18.14	222.92 ^a ±25.74	45.67 ^c ± 2.65	191.83 ^a ± 16.85	44.60 ^a ± 5.17
Bread fortified with (5%) MP G ₃	252.40 ^{ab} ±18.42	211.90 ^{ab} ±13.93	48.79 ^b ±1.68	161.22 ^b ±19.39	42.38 ^{ab} ±2.78
Bread fortified with (7.5%) MP G ₄	222.02 ^b ±19.48	189.87 ^b ±25.95	50.62 ^{ab} ± 0.81	133.43 ^b ± 16.20	37.97 ^b ± 5.19
Bread fortified with (10%) MP G ₅	152.60 ^c ±23.70	121.85 ^c ±21.58	51.46 ^a ± 0.93	76.76 ^c ± 21.33	24.37 ^c ± 4.31
LSD	31.47	31.03	2.49	29.63	6.22

Means with different letters in each column are significantly different at $p < 0.05$

SD:Standard Deviation.

Table (6): Kidney function in normal, intoxicated and treated rats with various levels from *Melissa* powder.

Variables Groups	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control NegativeG ₁	4.34 ^d ± 0.81	30.45 ^d ± 4.62	0.55 ^d ± 0.04
Control PositiveG ₂	10.41 ^a ± 1.97	91.60 ^a ± 13.41	1.20 ^a ± 0.13
Bread fortified with (5%) MP G ₃	7.96 ^b ± 0.25	66.37 ^b ± 4.40	0.92 ^b ± 0.10
Bread fortified with (7.5%) MP G ₄	6.74 ^{bc} ± 0.28	55.09 ^c ± 3.67	0.73 ^c ± 0.07
Bread fortified with (10%) MP G ₅	5.35 ^{cd} ± 0.40	45.04 ^c ± 2.82	0.60 ^{cd} ± 0.03
LSD	1.48	10.48	0.13

Means with different letters in each column are significantly different at $p < 0.05$.

SD:Standard Deviation.

Table (7) Serum levels of Alanine Amine Transaminase, Aspartate Amine Transaminase, Alkaline Phosphatase in normal, intoxicated and treated rats with different levels of *Melissa* powder.

Parameters Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control NegativeG ₁	33.07 ^c ± 3.36	107.03 ^d ± 13.65	194.50 ^e ± 16.54
Control PositiveG ₂	130.07 ^a ± 11.99	242.67 ^a ± 14.55	584.25 ^a ± 27.65
Bread fortified with (5%) MP G ₃	93.49 ^b ± 10.92	188.07 ^b ± 20.24	445.50 ^b ± 28.75
Bread fortified with (7.5%) MP G ₄	72.77 ^c ± 7.63	171.65 ^b ± 7.74	366.50 ^c ± 13.47
Bread fortified with (10%) MP G ₅	59.88 ^d ± 3.75	136.17 ^c ± 11.50	283.50 ^d ± 22.94
LSD	12.55	21.32	34.19

Means with different letters in each column are significantly different at $p < 0.05$.

SD:Standard Deviation.

Table (8) Serum levels of superoxide dismutase , catalase and glutathione peroxidase enzymes in normal, intoxicated and in different treated groups of *Melissa* powder.

Parameters Groups	SOD (u/ml)	CAT (ng/ml)	GPX (u/ml)
Control NegativeG ₁	154.50 ^a ± 6.65	10.37 ^a ± 0.72	375.75 ^a ± 30.44
Control PositiveG ₂	28.50 ^c ± 4.43	0.64 ^c ± 0.21	60.25 ^c ± 17.46
Bread fortified with (5%) MP G ₃	46.00 ^d ± 4.54	4.25 ^d ± 0.54	140.50 ^d ± 7.85
Bread fortified with (7.5%) MP G ₄	66.00 ^c ± 5.77	6.97 ^c ± 0.43	210.50 ^c ± 16.54
Bread fortified with (10%) MP G ₅	110.25 ^b ± 18.11	8.02 ^b ± 0.21	285.00 ^b ± 9.09
LSD	14.23	0.71	27.37

Means with different letters in each column are significantly different at $p < 0.05$.

SD:Standard Deviation.

Table (9): Serum levels of malondialdehyde, glutathione and total antioxidant capacity in normal, intoxicated and treated rats with different levels of *Melissa* powder.

Parameters Groups	MDA (nmol/ml)	GSH (ng/ml)	TAC (ng/ml)
Control NegativeG ₁	1.64 ^e ± 0.92	355.00 ^a ± 36.39	6.93 ^a ± 0.87
Control PositiveG ₂	26.37 ^a ± 1.83	32.25 ^e ± 12.61	0.58 ^e ± 0.14
Bread fortified with (5%) MP G ₃	19.25 ^b ± 1.55	92.00 ^d ± 8.64	1.37 ^d ± 0.32
Bread fortified with (7.5%) MP G ₄	14.06 ^c ± 2.87	137.00 ^c ± 14.31	2.43 ^c ± 0.42
Bread fortified with (10%) MP G ₅	7.50 ^d ± 0.93	234.25 ^b ± 39.11	5.83 ^b ± 0.51
LSD	2.67	38.67	0.77

Means with different letters in each column are significantly different at $p < 0.05$.

SD: Standard Deviation.

Table (10) Activity of superoxide dismutase , catalase and glutathione peroxidase enzymes in liver tissues of control negative, intoxicated and treated groups with different levels of *Melissa* powder.

Parameters Groups	SOD (u/mg)	CAT (ng/mg)	GPX (u/mg)
Control NegativeG ₁	132.50 ^a ± 10.34	9.42 ^a ± 0.76	270.25 ^a ± 23.08
Control PositiveG ₂	28.00 ^e ± 4.54	0.85 ^e ± 0.29	60.00 ^e ± 20.83
Bread fortified with (5%) MP G ₃	42.50 ^d ± 4.20	2.86 ^d ± 1.12	119.50 ^d ± 14.93
Bread fortified with (7.5%) MP G ₄	57.25 ^c ± 5.31	5.71 ^c ± 0.45	166.75 ^c ± 13.84
Bread fortified with (10%) MP G ₅	78.25 ^b ± 5.43	8.08 ^b ± 0.31	244.25 ^b ± 9.81
LSD	9.61	1.01	25.91

Means with different letters in each column are significantly different at $p < 0.05$.

SD: Standard Deviation.

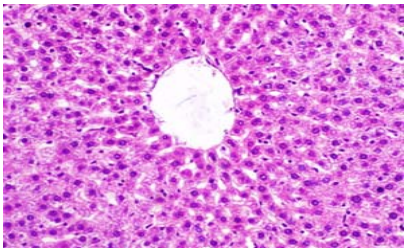
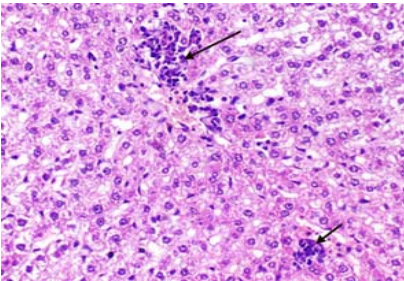
Table (11) Concentration of malondialdehyde, and glutathione in liver tissues of normal ,positive control and treated rats with different levels of *Melissa* powder.

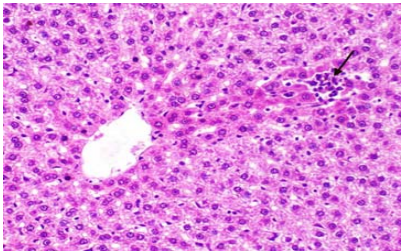
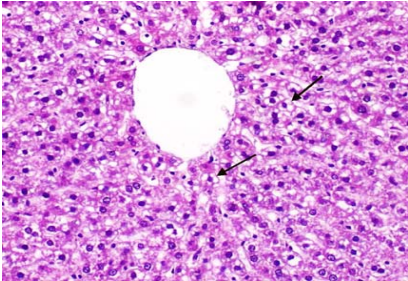
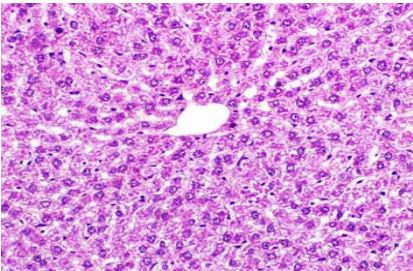
Parameters Groups	MDA (nmol/mg)	GSH (ng/mg)
Control NegativeG₁	2.50 ^e ± 1.21	258.75 ^a ± 28.21
Control PositiveG₂	22.81 ^a ± 1.21	32.50 ^e ± 16.90
Bread fortified with (5%) MP G₃	19.68 ^b ± 1.24	72.50 ^d ± 6.75
Bread fortified with (7.5%) MP G₄	12.60 ^c ± 2.89	107.50 ^c ± 6.45
Bread fortified with (10%) MP G₅	7.26 ^d ± 0.91	166.25 ^b ± 3.40
LSD	2.49	23.16

Means with different letters in each column are significantly different at $p < 0.05$.

SD:Standard Deviation.

Examination of liver Histopathologically:

liver	Results
	(Fig. 1). Rat's liver at negative control showing the normal histological architecture of hepatic lobule (H & E X 200).
	(Fig. 2).Rat's liver at positive control showing focal hepatic necrosis associated with inflammatory cells infiltration (short arrow) and portal inflammatory cells infiltration (long arrow) (H & E X 200).

	<p>(Fig. 3).Rat's liver at rats treated with 5% showing small focal hepatic necrosis associated with inflammatory cells infiltration (arrow) (H & E X 200).</p>
	<p>(Fig. 4).Rat's liver at rats treated with 7.5% showing cytoplasmic vacuolization of hepatocytes (arrow) (H & E X 200).</p>
	<p>(Fig. 5).Rat's liver at rats treated with 10% showing no histopathological alterations. Note normal hepatic lobule (H & E X 200).</p>

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الملخص العربي

تأثير الخبز المدعم بالمليسيا ضد الأجهاد التأكسدي المحدث في الجرذان

تعرف نبات المليسيا أيضًا باسم بلسم النحل ، بلسم الليمون ، بلسم العسل ، وهو عشب معمر. وهو عضو في عائلة (النباتات المزهرة) التي غالباً ما يشار إليها باسم (عائلة النعناع)، أجريت هذه الدراسة لمعرفة تأثير النظام الغذائي المحتوي على الخبز المدعم بنسبة (٥ ، ٧.٥ ، ١٠٪) من مسحوق المليسيا (MP) على الفئران التي تعاني من الإجهاد التأكسدي ، تم استخدام مستويات مختلفة من النظام الغذائي المحتوي على الخبز المدعم بنسبة (٥ ، ٧.٥ ، ١٠٪) تم استخدام مسحوق المليسيا في تحضير الخبز المختبرة التي خضعت للتقييم الحسي ، وكان أفضل مستوى للخبز المدعم بعد التقييم الحسي ٥٪ ولكن أفضل مستوى للخبز المدعم بعد الاختبارات البيولوجية كان ١٠٪ ، وتم تقدير التركيب الكيميائي لنسبة ٥٪ بسبب حصوله على احسن قبول في التقييم الحسي ، استخدمت هذه الدراسة خمسة وعشرون جرذاً من ذكور الألبينو تم تقسيمهم بشكل عشوائي الى خمس مجموعات تحتوي كل منها على خمس فئران. المجموعة الأولى تم تغذيتها على النظام الغذائي الأساسي كمجموعة ضابطة سالبة وتم حقن باقي الفئران الأخرى ٢٠ فأر بواسطة برومات البوتاسيوم تم حقنها داخل الغشاء البريتوني بجرعة

واحدة (١٢٥ ملجم / كجم من وزن الجسم) لأحداث الإجهاد التأكسدي ، بعد ذلك تم تقسيم المجموعات التي تم حقنها إلى ٤ مجموعات فرعية على النحو التالي ، المجموعة الفرعية ٢ التي تم تغذيتها على النظام الغذائي الأساسي تحتوي على خبز غير مدعم كمجموعة كنترول موجبة ، المجموعات الفرعية ٣ و ٤ و ٥ تم التغذية على حمية تحتوي على خبز مدعم بمسحوق المليسيا بنسبة (٥ ، ٧.٥ ، ١٠٪) على التوالي ، وأظهرت النتائج المتحصل عليها أن المجموعه التي تغذت بمستوى ١٠٪ من الخبز المدعم بمسحوق المليسيا قد أحدثت انخفاض معنوي بشكل ملحوظ في مستويات مصل الدم من الكوليسترول الكلي ، الدهون الثلاثية ، الليبوبروتينات منخفضة الكثافة LDL-C ، الليبوبروتينات متوسطة الكثافة VLDL-C ، اليوريا ، حمض البوليك ، الكرياتينين ، ووظائف الكبد (AST،ALT،MDA و ALP) وزيادة ملحوظة في مستويات المصل من الليبوبروتينات عالية الكثافة HDL-C ، والقدرة الإجمالية لمضادات الأكسدة (TAC) ، والجلوتاثيون (GSH) ، ونشاط أنزيمات الجلوتاثيون بيروكسيداز (GPX) ، (SOD) والإنزيمات (CAT). كما أظهرت النتائج حدوث انخفاض في MDA وزيادة في محتوى GSH ونشاط إنزيمات مضادات الأكسدة (GPX و SOD و CAT) في أنسجة الكبد بالإضافة إلى التحسينات الجزئية في أنسجة الكبد مقارنة بتلك المجموعة الضابطة الموجبة ، وعدم وجود فروق معنوية تذكر بالنسبة للطعام المأخوذ والنسبة المئوية للزيادة في وزن الجسم ومدى الاستفادة من الغذاء المتناول والتي كانت تميل نحو نتائج المجموعة الضابطة السليمة ، وكانت أفضل النتائج لجميع المؤشرات البيوكيميائية والهستوباثولوجية لأنسجة الكبد والتي كانت قريبة من النتائج الطبيعية كانت للمجموعة التي تغذت على الخبز المدعم ب ١٠٪ من مسحوق المليسيا في النهاية تشير النتائج الحالية الى أن الاستخدام المنتظم من الخبز المدعم ب ١٠٪ من مسحوق المليسيا له تأثير مفيد في تحسين وظائف الكبد والكلية ويعد عامل وقائي ضد الأجهاد التأكسدي المحدث ببرومات البوتاسيوم في الفئران.

الكلمات المفتاحية: أنزيمات مضادات الأكسدة ، وظائف الكبد ، وظائف الكلية ، دهون الدم، مليسيا ، الأجهاد التأكسدي ، الفئران.