تأثیر التغذیة بمستخلص القسط الهندی والمیلسیا علی التلیف الربئوی فی فئران التجارب
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INTRODUCTION

Herbal medicine is a growing area of health care that demands attention. Herbal medicines have been widely utilized as effective assistant treatment of multiple health remedies for the prevention and conditions for centuries by almost every known culture. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases (*Srivastav et al., 2011*). Herbal medicinal products have been documented as a significant source for discovering new pharmaceutical molecules that have been used to treat serious diseases. Many plants species have been reported to have pharmacological activities attributable to their phytoconstituents such are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, etc. (*Batiha et al., 2020*).

Melissa officinalis L. (MO), a perennial herbaceous plant from the family Lamiaceae and commonly known as lemon balm, grows worldwide; however, the Eastern Mediterranean region, Western Asia and Southern Europe, Caucasus, and Northern Iran are considered as its origin.

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MO has been traditionally used for different medicinal purposes including central nervous system disorders such as nervous agitation; sleep disturbances, depression, as well as gastrointestinal problems such as indigestion associated with nervous tension and flatulence (Shakeri et al., 2016). Previous studies have demonstrated spasmolytic (Sadraei et al., 2003), immunostimulant (Drozd and Anuszewska, 2003), anti-inflammatory (Bounihi et al., 2013), antioxidant activity (Hossain et al., 2009), anxiolytic, antidepressant, and anti-stress activities of MO (Taiwo et al., 2012). The major chemical constituents of MO are essential oil components (mostly composed of geranial neral), and hydroxycinnamic acid derivatives, flavonoids, and tannins (Bounihi et al., 2013).

Proximate analysis determinate by *(Tomescu et al., 2015)*, revealed that the leaves of *Melissa officinalis L.* contained carbohydrate (68.18 %), protein (7.54%), ash (8.44%), Moisture (9.64%) and fat (5.85%). Also, recorded that macro and microelements composition of *Melissa officinalis* L. contained Ca (17522), K (14602), Mg (793), Fe (166.5) and Zn (4.21).

Pulmonary fibrosis (PF) is a chronic, progressive lung disease characterised by the excessive proliferation of fibroblasts and deposition of collagen (fibrosis) in the pulmonary interstitium (*Zhou et al., 2016*). There are several clinical studies using phytochemicals for the management of PF. PF is identified by a disrupted pulmonary redox balance linked to inflammation. To restore this balance, antioxidants and antiinflammatory components such as phytochemicals

are frequently suggested as therapy for PF (*Veith et al.*, *2017*) and many preclinical studies have shown promising results for PF therapy. This study aims to show the impact of feeding with *Melissa*officinalis extract on pulmonary fibrosis in experimental rats.

Materials and Methods

Materials

Plant materials

One kg of lemon balm (*Melissa officinalis*) was purchased from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt).

Chemicals

Bleomycin, ethanol and Kits were obtained from Sigma Aldrich (St. Louis, MO, USA).

Experimental animals

Sixty adult male Wistar albino rats ($Rattus\ norvegicus$), weighting 180 ± 10 g, were obtained from Animal House Colony, National Research Center, Giza, Egypt.

Methods

Preparation of melissa ethanolic extract

Preparation of melissa ethanolic extract (MEE) according to (Ashry et al., 2021).

Chemical Methods

Determination of chemical composition of melissa

Moisture, ash, protein, crude fat and crude fiber were determined according to the method outlined by (A.O.A.C., 2010).

Carbohydrate content

The total carbohydrate content of the studied melissa extract samples was calculated by difference 100 – (moisture +ash + protein+ fat + fiber) according to the method described in (A.O.A.C., 2010). The caloric value was calculation according to the methods of (Seleet, 2010).

Determination of minerals analysis of melissa

and Calcium (Ca), Were The minerals, Zink (Zn), Iron (Fe), determined according to *(Isaac and Johnson, 2002)*. Sodium (Na) content was determined according to procedure reported by *(A.O.A.C., 2010)*.

Antioxidant of melissa extract

Estimation of total extract yield determined according to (Ashry et al., 2021).

Determination of total phenolic content (TPC) of melissa extract

The content of total phenolic compounds in the extract was estimated according to (Jayaprakasha et al., 2003).

Determination of melissa extract radical scavenging activity (RSA)

RSA was determined using the method of *(Nogala-Kalucka et al., 2005)*.

Experimental design

Forty adult male Wistar albino rats (Rattus norvegicus), weighing (180±10g) were obtained from Animal House Colony, National Research Center, Giza, Egypt; the rats were kept in suitable plastic cages and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to ethical committee of Faculty of Science, Al-Azhar University, Assuit, Egypt; however, this study was approved by the same ethical committee. After the rats being acclimatized with experimental room conditions, they were divided randomly into four groups (10 rats each). The groups of rats were divided as follow: Group (1): Comprised of normal healthy rats as control groups, Group (2): Comprised of rats those were subjected to oral administration of melissa ethanolic extract (MEE) at a dose (450 mg/kg/day) for six weeks, Group (3): Comprised of rats those were subjected to intraperitoneal (IP) injection at a dose (15 mg/kg twice weekly) of **Bleomycin**-induced pulmonary fibrosis for 4 weeks, and Group (4): Comprise of **Bleomycin**-induced pulmonary fibrosis rats those were treated with oral of (450 mg/kg/day) MEE for six weeks.

Blood sampling

At the end of the treatment period, rats were fasted overnight 3-5 ml, non-heparinized blood and following diethyl ether anesthesia specimens (3-7 ml from each animal) were drawn from the retro-orbital plexus using sterile glass capillary (single draw vacutainer

needle) into open vacutainer collecting tubes (*Paget and Thosmon*, 1979). The non-heparinized blood specimens were left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated at once by micro pipette, divided into aliquots and stored at -80°C until biochemical measurements could be completed as soon as possible.

Body weight changes:

At the beginning and the end of experimental study, each rats was weighted; and the changes in body weight (body gain) was calculated according to (Ashry et al., 2021).

Biochemical determinations

Serum T.C, T.G, HDL-c and LDL-c were determined according to (Cole et al., 1997; Artiss and Zak, 1997; Lopes-Virella et al., 1977 and Wieland & Seidel, 1983); respectively. Serum glucose was determined according to the method described by (Young, 2001). Serum (ALT), (AST), (GGT) and (ALP), were determined according to (Schumann and Klauke, 2003; Moss and Henderson, 1999; IFCC, 1983 and Trinder, 1969); respectively. Serum urea, creatinine and uric acid were determined according to the method described by Husdan and Rupoport, (1969), Trinder, (1969) and Chaney et al., (1960); respectively.

Statistical analysis

Statistical analysis was carried out according to *(Steel and Torrie, 1960)*.

Results and discussion

Gross chemical composition and caloric values of melissa on dry weight basis

Table (1): Gross chemical composition and caloric values of melissa on dry weight basis (mg/100g).

Sample	Moisture %	Ash %	Protein %	Crude Fat %	Crude fiber	Total carbohydr %ates	Caloric value (K.cal/100)
Melissa	9.32±0.33	9.19±0.75	14.03±6.77	6.62±0.70	9.62±0.84	60.54±0.57	357.86±2.30

- Mean of three replicates

The data in **Table** (1) revealed that the chemical composition of The chemical composition of melissa in the present study are melissa. agreement with *(Tomescu et al., 2015)* they reported that moisture, ash, proteins, crude fat, and total carbohydrates in melissa were 9.64%, 8.44%, 7.54, 5.85, 68.18; respectively. Also, *(Doğan et al., 2021)* reported that moisture, ash and proteins were 8.99%, 9.94% and 13.50%, respectively. Variations in moisture contents, ash, protein, crude fiber, crude fat and carbohydrate due to several factors such as climate and growing and postharvest management and processing conditions.

Minerals content of melissa

100g) /Table (2): Minerals content of melissa (mg

Sample	Na	Са	Fe	Zn
Melissa	88.55±1.02	2295.37±543.15	142.13±8.83	5.15±0.24

- Mean of three replicates

The data in **Table** (2) revealed that the minerals content of melissa. These results agreement with *Ghosh et al.*, (2019) they stated that potassium was more common (17.275 ppm), followed by Ca (5698 ppm), Mg (5550 ppm), Fe (119.4 ppm), Na (83.34 ppm), and Zn (29.163 ppm). While, these results disagreement with (*Abdellatif et al.*, 2021) they reported that the most common mineral was K (16,412), followed by iron (282), Rb (75), Ba (58), Na (51), Zn (28), in melissa. The difference in the concentration of these minerals among various studies is due to the plant's ability to absorb nutrients from the soil, the soil's mineral content, sample preparation, irradiation and the counting system (van der Ploeg et al., 1999).

Antioxidant of melissa extract Melissa extract of yield (%), total phenolic compounds (TPC) and radical scavenging activity (RSA)

Table (3): Melissa extract yield (%), (TPC) and (RSA)

Parameter	Yield (%)	TPC (mg/g)	RSA (%)	
Melissa ethanolic extract	13.1±0.42	1.39±0.25	62.4±20.55	

The yield was 13.1%, total phenolic content (TPC) 1. 39%, and radical scavenging activity (RSA) 62.4% of the melissa ethanolic extract (MEE); are shown in **Table** (3). These results in this study were in disagreement with that reported by (*Doğan et al., 2021*) they stated that MEE exhibited the highest scavenging activity by inhibiting 62.83. The reason is due to the total phenolic acids are capable of removing free radicals, chelating metal catalysts; activate antioxidant enzymes, and inhibiting oxidases. Mostly a direct relationship has

Parameter Groups	Body weight gain (g/100g)
Group (1) Control (-)	16.6±1.7 ^A
Group (2) MEE (450 mg/kg)	17.4±3.8 ^A
Group (3) Bleomycin (+) (15 mg/kg)	9.46±0.98 ^c
Group (4) Bleomycin+ MEE (450	15.4±0.46 ^E
mg/kg)	13.4±0.40

been found between total phenolics and antioxidant activity of plant extracts and fruits indicating that the phenolic compounds are the major contributor towards imparting antioxidant attributes to plants. The multiple biological activities of medicinal plants indicate their potential as a source of functional foods and nutraceuticals (Singh et al., 2009).

Body Weight gain

Table (4): Effect of melissa extract on body weight gain in rats.

The same column, means with different superscript letters are significantly different at (p \le 0.05.)

The current results revealed that bleomycin had a significant decrease in body weight; while rats treated with melissa ethanolic extract (MEE) showed a significant increase in body weight.

These results agreement with *Lieshchova and Brygadyrenko (2021)* they reported that Male rats that consumed shoots of M. officinalis, to the 30^{th} days of the experiment, had the weight of 134.5% of their individual initial weight

Effect of melissa extracts on serum total cholesterol (T.C), triglycerides (T.G), high density lipoprotein-

cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and glucose in rats

Table (5): Effect of melissa extract on serum (T.C), (TG), (HDL-c), (LDL-c) and glucose in rats.

The same column, means with different superscript letters are significantly different at ($p \le 0.05$).

The current results revealed that bleomycin disrupted the lipids metabolism by increasing T.C, T.G, LDL-c, and lowering HDL-c, while MEE extract improve the lipids metabolism by decreasing T.C, T.G, LDL-c, and increasing HDL-c, and these findings match with previous work of *(Yang et al., 2022)* they found that the formation of PF induced by bleomycin in rats was accompanied by dramatic metabolic disturbances in the serum, with downregulation of a large number of lipids metabolites and abnormal fuctuations in pathways such as glycerolipid metabolism and glycerophospholipid metabolism.

Liver function

Effect of melissa extract on serum ALT, AST, GGT and ALP activities (U/L)

Table (6): Effect of melissa extract on serum ALT, AST, GGT and ALP activities (U/L) in rats.

The same column, means with different superscript letters are significantly different at (p \le 0.05).

The obtained data revealed that bleomycin disrupted the liver enzymes by increasing ALT, AST, GGT and ALP activity, while MEE extract improve the liver enzymes by decreasing ALT, AST, GGT and ALP activity. These results agreement with *Lieshchova and Brygadyrenko (2021)* they reported that serum AST, ALT, ALP, total

bilirubin were lower after 30th days intake of both 5% *M. officinalis, L. angustifolia*, or *V. angus-castus*, in high-fat diet rats. While, *Opyd et al.*, *(2018)* found that, the serum ALT activity was not influenced by experimental feeding, whereas the serum AST activity was significantly increased after native linseed supplementation.

Kidney functions Effect of melissa extract on serum urea, creatinine, uric acid levels (mg/dl)

Parameters	Urea	Creatinine	Uric acid
Groups	(mg/dl)	(mg/dl)	(mg/dl)
Group (1) Control (-)	60.3±1.6 ^A	0.66±0.02 ^A	4.06±0.67 ^A
Group (2) MEE (450 mg/kg)	55.5±1.7 ^A	0.58±0.02 ^A	4.30±1.3 ^A
Group (3) Bleomycin (+) (15 mg/kg)	80.3±5.2 ^c	1.8±0.10 ^c	6.20±0.29 ^c
Group (4) Bleomycin + MEE (450 mg/kg)	62.7±3.3 ^E	1.15±0.05 ^E	4.11±0.63 ^E

Table (8): Effect of melissa extract on serum urea, creatinine and uric acid levels (mg/dl) in rats.

The same column, means with different superscript letters are significantly different at (p \le 0.05).

The current results revealed that bleomycin disrupted the kidney enzymes by increasing serum creatinine, urea and uric acid, while MEE extract improve the kidney enzymes by decreasing serum creatinine, urea and uric acid. These results agree with (Abdel-Moneim et al., 2011) who suggested that medication of linseed oil reduced the grades of serum creatinine, blood urea nitrogen and uric acid. While, the present results disagree with (Opyd et al., 2018)

they found that, the serum concentration of creatinine and urea was not affected by the linseed dietary treatments

Conclusion

In conclusion, this study demonstrates the effect of feeding with MEE on pulmonary fibrosis in experimental rats. Serum glucose level, lipids profile, liver and kidney functions of rats feeding on MEE was investigated. The obtained results observed that MEE caused a reduction in serum glucose levels and significantly decreased in serum lipids profile. Also, treatment with MEE caused improvement in both, liver and kidney functions compared with the control group. Therefore, it is recommended to take it daily.

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تأثیر التغذیة بمستخلص المیلسیا علی التلیف الرئوی فی فئران التجارب سعاد محمد عمر ' ، هند محمد علی ' ، محمود عشری '، لیلی محمد سید سالم "

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الملخص

التليف الرئوي هو مرض تنفسي تتشكل فيه ندبات في أنسجة الرئة، مما يؤدي إلى مشاكل خطيرة في التنفس. تهدف هذه الدراسة إلى معرفة تأثير مستخلص الميلسيا على التليف الرئوي الناجم عن البليوميسين في ذكور الفئران البالغين. تم تقسيم ذكور فئران الويستر البيضاء

البالغة والتي تزن (١٨٠ ± ١٠ جم) بشكل عشوائي إلى أربع مجموعات (١٠ فئران لكل مجموعه) على النحو التالي: المجموعة (١) فئران سليمة طبيعية تعمل كمجموعة ضابطة ، المجموعة (٢) فئران تتناول عن طريق الفم مستخلص المليسا الإيثانولي (بجرعة ٤٥٠ مجم / كجم / يوم) لمدة ستة أسابيع ، المجموعة (٣) فئران تعرضت للحقن بالبطن بجرعه (١٥ مجم / كجم مرتين أسبوعيا) من البليوميسين لمدة ٤ أسابيع ، والمجموعة (٤) فئران التليف الرئوي الناجم عن البليوميسين والتي عولجت عن طريق الفم (٤٥٠ مجم / كجم / يوم) من مستخلص الميلسيا لمدة ستة أسابيع. أظهرت النتائج أن مستخلص الميلسيا نجح في تقليل تليف الرئة الناجم عن البليوميسين. وقد تجلى ذلك من خلال الانخفاض الكبير في وظائف الكبد والكلى ، والكوليسترول الكلي ، والدهون الثلاثية ، و الكولسترول منخفض الكثافة، والجلوكوز إلى جانب التحسن الملحوظ في الكولسترول مرتفع الكثافة. لذلك أوصت هذه الدراسة باستخدام الميلسيا في الغذاء لما لها من فوائد عديدة.

الكلمات المفتاحية

المليسيا، البليوميسين، وظائف الكبد والكلي، التليف الرئوي، دهون الدم، الفئران.

Effect of Feeding with Melissa officinalis Extract on . Pulmonary Fibrosis in Experimental Rats.

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Abstract

Pulmonary fibrosis is a respiratory disease in which scars are formed in the lung tissues, leading to serious breathing problems. The objective of this study was to determine the effect of MEE against lung fibrosis induced by Bleomycin in adult male Wistar albino rats

weighting (180±10g) were randomly divided into four groups (10 rats each) as follows: group (1) normal healthy rats as control groups, group (2) rats those were subjected to oral administration of MEE at a dose (450 mg/kg/day) for six weeks, group (3) rats those were subjected to intraperitoneal (IP) injection at a dose (15 mg/kg twice weekly) of Bleomycin-induced pulmonary fibrosis for 4 weeks, and group (4) Bleomycin-induced pulmonary fibrosis rats those were treated with oral of (450 mg/kg/day) MEE for six weeks. The results revealed that MEE succeeded to decline the lung fibrosis-induced by Bleomycin; this was evidenced by the significant reduction of liver and kidney function, total cholesterol, triglycerides, LDL, and glucose coupled with marked improvement in HDL. So this study recommended to use melissa in diets for its many benefits.

Keywords: *Melissa officinalis*, Bleomycin, Liver and kidney functions, lung fibrosis, Lipids profile, Rats.