HISTOLOGICAL EFFECTS OF AQUEOUS EXTRACT OF *Mentha spicata* ON LIVER IN ALBINO MICE

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ABSTRACT

This study was conducted to investigate the effects of high concentration of *Mentha spicata* on liver tissues. Fourteen mature male albino mice were used in this study and divided into two groups. Control group received 1 ml normal saline daily for 7days through oro- gastric tube. The remained animals received 1ml of aqueous extract of *Mentha spicata* daily for 7 days through oro-gastric tube. At the end of experiment, all animals were dissected. Liver Samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin. The slides were examined macroscopically. The results showed that coagulative degeneration, degeneration of hepatocytes with necrosis, inflammation vacuolization, inflammatory cell infiltration and fatty degenerative changes as also hemorrhage and increased size of Kupffer cells and vacuolated cytoplasm. From this study it has been concluded that high concentration of aqueous extract of *Mentha spicata* have a side effect on the structure and function of liver tissue.

Key word: hepatocytes, degeneration, infiltration, nercrosis, kupffer cells

التأثيرات النسيجية من المستخلص المائي لنبات النعناع على انسجة كبد الفئران المختبرية

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المستخلص

طبقت هذه الدراسة لمعرفة تاثير التراكيز العالية من نبات النعناع *Mentha spicata* على انسجة الخلايا الكبدية. استخدمت في هذه الدراسة 14 ذكر من الفئران المختبرية وقسمت الى مجموعتين. مجموعة السيطرة جرعت ب 1 مل من المحلول الملحي خلال 7 يوم بواسطة انبوبة التجريع الفموي. المجموعة الثانية جرعت ب 1مل من المستخلص المائي من نبات النعناع ولمدة 7 يوم بواسطة انبوبة التجريع الفموي. في نهاية التجربة تم قتل الحيوانات وتم تشريحها وعملت مقاطع نسيجية للكبد وفحصت تحت المجهر الضوئي. اوضحت النتائج ظهور عمليات تنكس للخلايا الكبدية مع حدوث تنخر للخلايا والتهاب وعائي وارتشاح في الخلايا الالتهابية مع حدوث تنكس دهني وايضا نزف مع زيادة في اعداد خلايا كوبر وتفجي السايتوبلازم. نستنتج من هذه الدراسة ان التراكيز العالية من المستخلص المائي لنبات النعناع له تأثيرات جانبية على التركيب النسيجي والوظيفي لنسيج الكبد.

كلمات مفتاحية: الخلايا الكبدية، التنكس، الارتشاح، التنخر، خلايا كوبر

INTRODUCTION

Medicinal plants have always played an important role in the treatment of human diseases all over the world (16, 31). About 80% of the world's population relies on plant-derived medicines for their healthcare (21). Plants contain a variety of organic compounds as secondary metabolites, and a large number of them display pharmacological properties. However, most medicinal plants are used as self medication without knowing their possible side effects. Therefore, traditional plants need further scientific investigation on their toxic side effects (22). It was reported that Iraq has many diverse herbal plants and traditional medicine is widely used for treatment of many diseases in this country (5). *Mentha* (Family, Lamiaceae) is a genus of 6 species in the flora of Iraq (7, 8). These species have been used in folk medicine and are available in traditional medicinal plant stores and local markets (9). *Mentha spicata*, commonly known as spearmint, is one of these species. It is a perennial herb with a characteristic spearmint odor with a good flavor and fragrance (6, 18). The plant traditionally used worldwide in pharmaceutical preparations, confectionery and food industries, and also in hygiene and cosmetic products (11, 24). The leaves are well recognized for making herbal tea (1, 14). Some studies have been shown that spearmint oil has anti-fungal, anti-microbial, anti-inflammatory, anti-tumor and antioxidant activity (19, 27, 32, 33). These activities are due to the presence of active constituents like menthone, menthol, rosmaninric acid and carvone (4, 15). However, there is some evidence which show that despite its beneficial effects, spearmint has some toxic and adverse effects. Severe histopathological changes in kidney, liver and uterus tissue (3, 20). The data of the toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals. This study was therefore undertaken to evaluate the possible toxicological effect of the *Mentha spicata* extract on liver tissue of mice.

MATERIAL AND METHODS

Preparation of aqueous extract of peppermint: Leaves of *Mentha spicata* were cleaned, and standardized in "Iraqi National Herbarium". Peppermint leaves were washed and left for air-dry and ground into powder using an electric grinder. 10 gm of powder was macerated in 100ml boiling distilled water, mixed by a glass rod, and was left in steam bath for 60 minutes. The aqueous extract was filtrated by using filter paper (28).

Laboratory animals

Fourteen Swiss albino mice of either sex (10-12 weeks old) weighing 25-30 grams were used in this study. Mice were kept in the University's animal house under standard environmental conditions (temperature: 22 ± 2°C and 12/12 h (light/dark) period) and fed with standard pellets and water *ad libitum* throughout the experiments. Mice were divided randomly into two groups:

A- Control group: animals received 1ml distilled water daily for 7 days through oro-gastric tube.

B- Experimental mice: animals administered with 1ml of aqueous extract of *Mentha spicata* daily for 7 days through oro- gastric tube (23). The animals were observed daily for abnormal clinical signs and death. Body weights were measured and recorded at the beginning and the end of the experiment. At the end of the study, all animals were sacrificed by spinal dislocation and dissected.

Histological Examination

For histological examination, the liver samples were removed and fixed in 10% of buffered formalin solution for 24 hour. The tissues were processed (dehydration, cleaning and infiltration). Then, they were embedded in paraffin wax and sectioned with microtome to produce 5 µm paraffin wax tissue sections. After that, the sections were stained with Haematoxylin & Eosin followed by mounting with DPX mounting media. Next, the sections were examined using light microscope (12).

Statistical Analyses

Statistical analysis of data was performed using the Statistical Package for Social Sciences (SPSS for Windows, V. 13.0, Chicago, USA). Statistical significance was determined by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Results were presented as mean ±SD. p values < 0.05 were regarded as statistically significant (17).

RESULT AND DISCUSSION

1- Body weight: The weights of mice were not significantly different between control and treated group following administration of the extract

Table 1.Indicate the body weight

Groups	Mean of body weight (gm)±SE	
	Before treatment	After7-days treatment
Control	14.3±0.65	17.60± 0.55
Treated	14.5±0.70	18.2±0.57

Histological Examination of Liver Tissue

Histological examination of the liver tissues showed varied damage when compared with the baseline-control group (Figure 1).

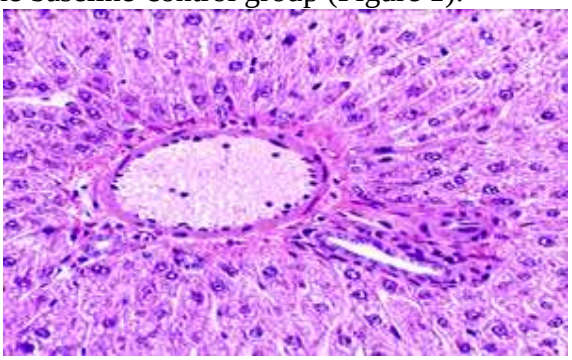

Figure 1. Histological section of liver tissue in normal group show central vein, column of hepatocytes radiating from the central vein, liver sinusoids. (H a& E stain) (200X).

The effects of *mentha spicta* are shown coagulative degeneration, degeneration of hepatocytes with necrosis, , inflammatory cell infiltration and fatty degenerative changes as also hemorrhage and increased size of Kupffer cells and vacuolated cytoplasm. Mice that were treated with *Mentha spicata* (group 2) experienced the greatest damage which was mainly irreversible and included nuclear changes (pyknosis, karyolysis and), cytolysis and massive congestion, Inflammatory cellular infiltration was abundant around the central vein in group2. In recent years, an increasing percentage of people from developed countries have been using complementary and alternative medicines .Peppermint *(Mentha spicata)* is widely used in food, cosmetics and medicines and pharmaceutical industries (10). In this study the administration of aqueous extract of *Mentha spicata* affects the structure of hepatocytes in liver. Liver is the gateway of body as well as the largest metabolic organ, containing an enzymatic system. This allows the metabolism of protein, fat and carbohydrate and maximum excretion of all foreign substances from the body (11). Cytotoxicity of some medicinal plants has been well known (2). According to the results of this study, the aqueous extract of *M. spicata* leaves has cytotoxic effects on liver tissues. These changes might due to high concentration of menthol which is the principle components of the *Mentha* extracts (30). The cytotoxic activity of *Mentha* essential oils could be due to the major compositions including menthol, limonene, carvone. Mentha arvensis had major component of menthol (92.38%), and *Mentha spicata* of limonene (44.12%) and carvone (41.31%)(29, 36) Once at all, this concentration of *Mentha* extract caused several changes in the plasma membrane and these changes could be explained due to the effect of essential oils which act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels . The volatile oils saturated the membranes and can interact by means of their physiochemical properties and molecular shapes, and can influence their enzymes, carrier, ion channels and receptors (13). Several toxicological effects have been associated with the oil components of *Mentha arvensis* and *Mentha spicata*. In particular, menthol has been found to interact with cytosolic Ca_2, probably through an intracellular Ca_2 store release and Ca_2 channel blocking (30). Menthone has been reported to be a growth inhibitor, whereas pulegone, a potent abortifacient, is metabolized by hepatic microsomal monooxygenases to a series of hepatotoxins that cause liver cancer(30). Similarly, Spindler and Madsen(34) reported that all hematological and biochemical parameters as well as absolute and relative weights of the organs were within normal range, whereas the histopathological examination revealed alteration in the rat brain following the administration of peppermint oil (oil from *Mentha piperita* for a 86-day experimental period). Also the pathological changes may lead to impaired liver function which interferes with the secretion of plasma proteins, This leads to decreased blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the oedema and congestion observed in the different tissue (25,26).

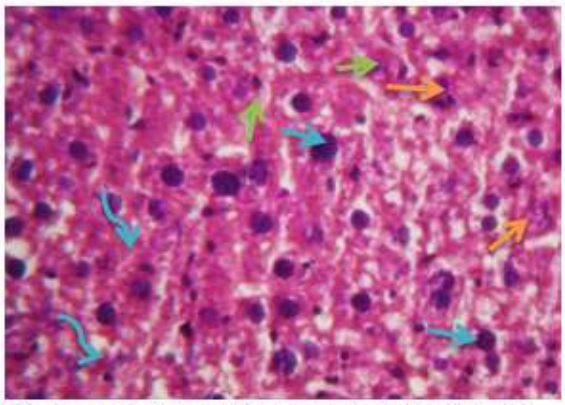

Figure (2) Histological section of liver tissue in second mice group show degeneration of some nuclei , fragmentation of nuclei , karyolysis , inflammatory Infiltration (H a& E stain) (200X).

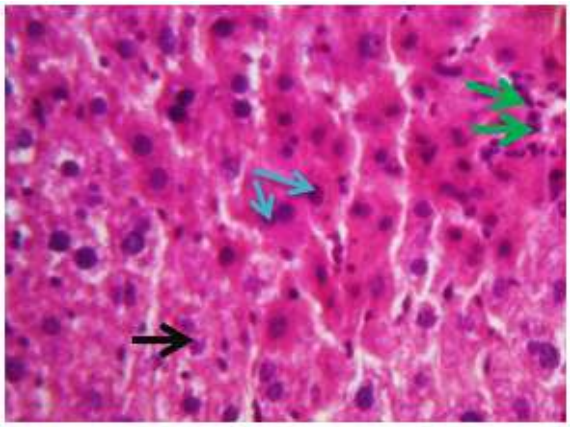

Figure (3) Histological section of liver tissue In mice second group show coagulative degeneration , degeneration of some nuclei , inflammatory infiltration (H a& E stain) (200X).

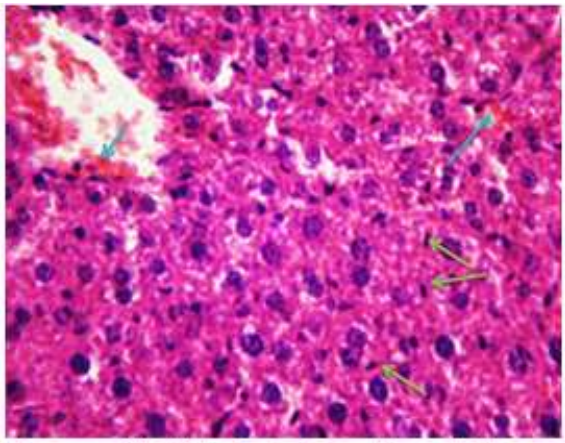

Figure (4) Histological section of liver tissue in mice second group show hemorrhage , increase of kuffper cell (H a& E stain) (200X).

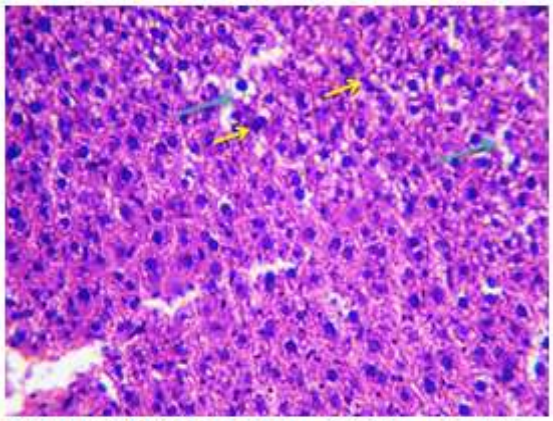

Figure (5) Histological section of liver tissue in mice second group show degeneration of some nuclei , pyknosis fatty infiltration in cytoplasm (H a& E stain) (200X).

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