Annals of Agri-Bio Research **28** (2): 367-371, 2023

Impact Score : 0.28 (Scopus)

Histological Study of the Chilli Pepper (Capsicum annuum L.) Extract on Adipose Tissue in White Mice (Mus musculus)

ZAHRAA SALIM JABAR* AND BASSIM A. JASSIM[1]

College of Science, Al-Muthanna University, Al-Muthanna, Iraq
**(e-mail: zahraa.salim.sci@mu.edu.iq; Mobile: +96 78300 66783)*

(Received: March 01, 2023; Accepted: April, 9, 2023)

ABSTRACT

The aim of present study was to investigate the effect of chilli pepper (*Capsicum annuum* L.) extract on adipose tissue, total cholesterol and some of histological characterizes of tissues. The current study used 60 white mice, split into three treatment groups with control group, including 15 mice in each group. The treated groups received three doses that varied in concentration of watery hot chilli pepper extract; Group A was the normal control group. Group B, C and D received orally 0.5 ml of chilli pepper for 30 days with concentrations of 0.4, 0.8 and 1.6%, respectively. The adipose tissue showed significant differences in the biometric dimensions of adipose tissue nuclei after being treated with moderate (0.8%) and high (1.6%) doses of water hot chilli pepper extract. In conclusion, the high concentration of watery chilli pepper extract displayed tissue structure with many histological alterations, in contrast to the prior groups that received the moderate and lower doses.

Key words: Adipose tissue, chilli pepper, functional food, medicinal plants, spices

INTRODUCTION

Medicinal herbs have been discovered and used in traditional, therapeutic practices since prehistoric times. The Sumerian civilization left behind the earliest written records of herbs, which mention hundreds of medicinal plants, including opium, on clay tablets (Alamgir and Alamgir, 2017). In non-industrialized communities, medicinal plants are frequently employed, mostly because they are more accessible and affordable than contemporary medications (Awuchi, 2019). Traditional medicine is widely used in many nations, although the World Health Organization maintains a network to promote its safe and responsible use as alternative therapy (Ung et al., 2017). In hot/tropic areas, especially, spices have been utilized in part to combat food spoilage microorganisms (Ahn, 2017).

Spices can prevent both acute and chronic disorders in addition to improving the flavour, aroma and colour of food and beverages. Spices have cognitive and mood-affecting qualities in addition to antioxidant, anti-inflammatory, anti-tumorigenic and glucose and cholesterol-lowering characteristics (Embuscado, 2015; Jiang, 2019).

All of the species and cultivars of chilli peppers are extremely fiery and pungent peppers from the family (Solanaceae) Jaiswal *et al.*, 2021). Chilli peppers are grown all over the world in warm areas, but they are native to the Americas. The cayenne, jalapeo, serrano and Thai chilli peppers are only a few of the varieties of *Capsicum annuum* that are among the most popular chili peppers (Hernández-Pérez *et al.*, 2020). Although tabasco is a cultivar of *C. frutescent*, some of the hottest chilli peppers, such as the habanero, Carolina reaper and ghost chilli pepper (or bhut joloki), are cultivars of *C. chinese*. Chilli peppers are used to flavour barbeque, fiery curries and other savory sauces and can be consumed fresh or dried (Smith, 2015).

Adipose tissue, body fat, or simply fat is a loose connective tissue composed mostly of adipocytes. The stromal vascular fraction (SVF), which is made up of immune cells like pre-adipocytes, fibroblasts and vascular endothelial cells, is found in adipose tissue in addition to adipocytes (Ye *et al.*, 2022). Eliminating or shrinking adipose tissue naturally is one of the biggest medical challenges and even one of the general challenges among people. Meanwhile, herbal supplements and spices are widely used, which

[1]College of Veterinary Medicine, Al-Muthanna University, Al-Muthanna, Iraq.

are sometimes not associated with success (Sun *et al.*, 2016; Azlan *et al.*, 2022). So, aim of the present study was to histologically investigate the effects of the watery chilli pepper (*Capsicum annuum* L.) extract on adipose tissue in white mice.

MATERIALS AND METHODS

Sixty adult white mice weighing 30-32 g were housed at a temperature of 25-28°C and a relative humidity of 40-45% while being fed regular pellets and water. Before beginning the trial, mice spent two weeks getting used to the lab environment in animal house of Science College in AL-Muthanna University.

Fifteen animals were used in Group A as the control group. Group B of 15 animals received a low dose in concentration (0.4%) of chili pepper (0.5 ml) daily for 30 days. Fifteen mice in Group C received 0.5 ml concentration (0.8%) of a medium dose of chilli peppers orally, and 15 mice in Group D received 0.5 ml of concentration (1.6%) high dose of a watery chilli pepper extract daily for 30 days.

The chilli peppers were bought from a neighborhood market in AL- Samawah city. Every day the fresh chilli pepper fruits were washed, allowed to dry at room temperature, and then blended. Ten grams of plant powder were added to 200 ml of distilled water in a sterile glass beaker, shaken for 24 h, and then used (Arabi *et al.*, 2020). For creating the extract, the material was dried in an electric oven at 40°C after being run through layers of sterile soft cotton to determine its candidacy. The filtrate was then separated using a centrifuge (3000 rpm). After that, the extract was gathered, placed in a clean bottle and stored in the freezer until required.

Formalin was used to preserve the tissue samples for 48 h. The samples were dehydrated in graded ethanol concentrations. After cleaning with xylene, these were imbedded in paraffin wax for cutting. The tissue sections with a 5 m thickness were mounted on glass slides and stained for light microscopic examination with hematoxylin and eosin (Fereidouni *et al.*, 2017).

RESULTS AND DISCUSSION

The cholesterol value in treated mice with low dose of watery chilli pepper extract after 30 days was 104.16±4.30 mg/dl, while the cholesterol value in the group that treated with middle dose was 88.83±8.10 mg/dl, and the cholesterol values in the group that treated with high dose was 83.16±4.18 mg/dl, whereas in control group it was 151.66±2.33 mg/dl (Table 1). Thus, the level of cholesterol in group D had significant decrease when compared with control, B and C groups. It was as the spices red pepper, ginger and mustard can stimulate the conversion of cholesterol to bile acids by effects of active compound in spices foods, an important pathway of elimination of cholesterol from the body.

Table 1. Levels of serum cholesterol in mice (mg /dl)

Treatment / Parameter	Cholesterol (Mean±S. E.)
Control group	151.66±2.33[a]
Low dose	104.16±4.30[b]
Middle dose	88.83±8.10[bc]
High dose	83.16±4.18[c]
p value	0.001

*Different letters denote the significant differences at P>0.05.

The tissue arises when body has an excessive amount of cholesterol, which has two sources. It is mostly made in the liver of the body and is present in dairy products and other non-vegetarian foods. Twenty and 30% of it comes from the food we eat. Actually, a tiny amount of cholesterol is necessary for body to operate effectively. Cholesterol is transported throughout the body via blood which is known as blood cholesterol (Luo *et al.*, 2020). Because cholesterol cannot dissolve in blood, it travels through blood vessels by attaching to lipo-proteins, which are big globular particles that carry components of plasma such as cholesterol and triglycerides. The risk of heart attack and cardiovascular disease increases as blood cholesterol levels rise (Alenghat *et al.*, 2019). It is possible that the active ingredient (capsaicin) in chilli peppers, which causes these physiological differences in cholesterol values, is the reason for the variations in the experimental groups. This outcome is consistent with Kenig *et al.* (2018) who reported that consuming chilli peppers decreased blood cholesterol.

Like all other tissues, adipose tissue is composed of cells and extracellular matrix. The majority of structural elements in this tissue

are cells, which outnumber the sparse extracellular matrix. The main cells in adipose tissue are called adipocytes. In addition to adipocytes, diverse cell types also include preadipocytes, fibroblasts, capillary endothelial cells, macrophages and stem cells. The stromal vascular fraction is made up of all of these non-adipocyte cells, and their main function is to nourish and safeguard the adipose tissue.

The adipocytes were large and filled with lipid droplets, according to the histological findings of adipose tissue sections, and they were distributed normally within the tissue field (Fig. 1), which was in agreement with findings of Nogueira *et al.* (2022). There were no pathological lesions visible in the tissue section, and there were no interstitial spaces throughout the tissue field. All of the adipocytes had peripherally located sight nuclei (Fig. 2). The nuclei of adipose tissue were 6.23±0.01 µm in diameter (Table 2).

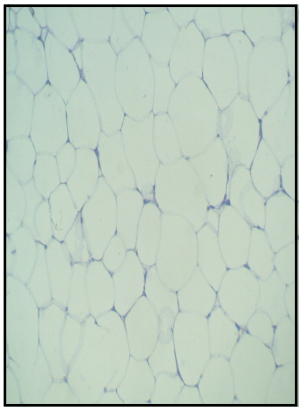


Fig. 1. Transverse section of adipose tissue in control group which showed adipocytes were normally H & E stain 20x.

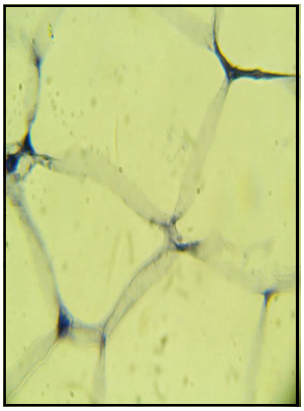


Fig. 2. Transverse section of adipose tissue in control group which showed all adipocyte had sight nuclei peripherally in location H & E stain 100x.

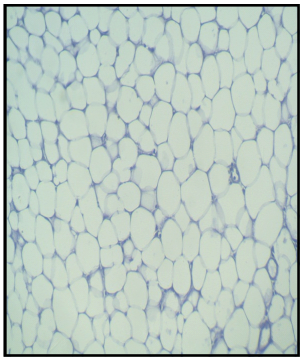
Table 2. Diameter of adipose tissue nuclei in mice (µm)

Parameter \ Treatment	Adipose tissue nuclei (mean±S.E)
Control group	6.23±0.01[a]
Low dose of chilli pepper	5.02±0. 08[b]
Middle dose of chilli pepper	3.34±0.06[c]
High dose of chilli pepper	2.05±0.07[d]
Total p value	0.001

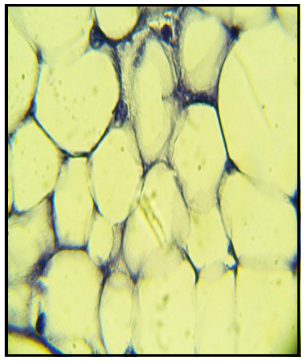
*Different letters denote the significant differences at P<0.05.

The histological changes observed in the adipose tissue sections after treatment with a watery extract of hot pepper for a month increased the inter-statial spaces between adipocyte, in some locations showing fibrous aggregation as spots in shape and dark in colour (Fig. 3), and the diameter of adipose tissue nuclei (5.02±0.08 µm) was significantly reduced compared with control group (Table 1).

Adipocyte size was reduced in the tissue segment, and some cells had been destroyed in various places (Fig. 4). When opposed to the control group, the internal septa were clearly noticeable through the adipose tissue because the majority of adipocytes had lost their nuclei. These adipose tissue histology findings were in line with Nirengi *et al.* (2016), who showed that the effects of chilli peppers on adipose tissue led to hot pepper extract and capsaicin effectively inhibiting abiogenesis, increasing adipocyte lipolysis and causing fat cells to shrink.



Fig, 3. Transverse section of adipose tissue treated group with low dose of chilli pepper which showed fibrous aggregation as spots in shape with dark in colour H & E stain, 20x.



Fig, 4. Transverse section of adipose tissue ttreated group with low dose of chilli pepper which showed reduced in adipocyte size and some cells destruction H & E stain,100x.

Most tissue fields of adipose tissue were normal in structure as in the control group, but there were some histological differences in the shape and size of adipocytes nucleus, no histo-pathological lesions. The tissue sections in the mice group that received low doses of hot pepper extract were similar to the tissue sections in the control group, which is in accordance to Varghese *et al.* (2017).

The tissue section of C group, the adipose tissue divided by thick trabecular which

appeared as lobes, the result noted prominent spaces between lobes filled by fluid, the adipocyte nuclei was 3.34±0.06 µm in diameter which had significant decrease compared with both control and B group (Fig. 5; Table 1).

Some of adipocytes were aggregation which formed tiny clusters inside the same lobe of adipose tissue. The tissue section revealed extensive areas of adipose tissue, the cells of which appeared progressive and empty (Fig. 6), the tissue-related results showed numerous fibrotic lesions. Due to capsaicin reduced obesity-induced glucose intolerance (Wang *et al.*, 2021) by not only suppressing inflammatory responses but also enhancing fatty acid oxidation in adipose tissue and liver, both of which are important peripheral tissues affecting insulin resistance.

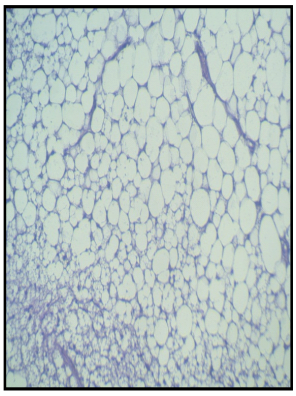


Fig. 5. Transverse section of adipose tissue treated group with middle dose of chilli pepper which showed prominent spaces between lobes that filled with secretions and inflammatory cells H & E stain, 20x.

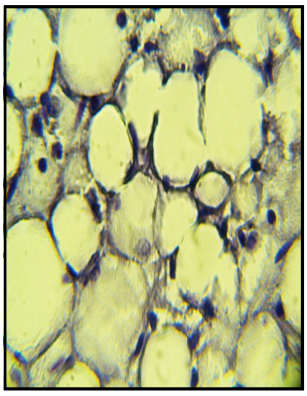


Fig. 6. Transverse section of adipose tissue treated group with middle dose of chilli pepper which showed cells were empty and progressive, with many lesion of fibrosis H & E stain, 100x.

The tissue findings of adipose tissue were observed most adipose tissue lobes became dark in colour, most cells were empty and without lipid droplets, progressive in size and most adipocytes were without nuclei after being treated with a high dose of hot pepper extract for 30 days. The adipose tissue lobe was surrounded by a strong connective tissue capsule in the tissue results (Fig. 7), and the diameter of the adipose tissue nuclei 2.05±0.07 µm was significantly smaller than in the prior groups, due to the active ingredient especially capsaicin in high value stimulated adipocytes to release chemicals that attracted immune cells and led to inflammation, these findings are consistent with those of Baskaran *et al.* (2016).

The tissue section showed notable adipocyte degeneration with prominent inflammatory cells. The results revealed some of adipocyte destruction in many regions of the adipose lobe. Progression adipocytes aggregated in certain locations with abnormal shapes because oxidative process resulted from effect of active compounds of chilli pepper when exposed to high dose of extract (Fig. 8). Since the majority of developing adipocytes lacked nuclei, there were numerous irregular gaps that were filled with viscous secretion (Fig. 8). These findings are consistent with those of Ohyama *et al.* (2015), who showed that the chilli pepper caused secretion and adipocyte cell reduction.

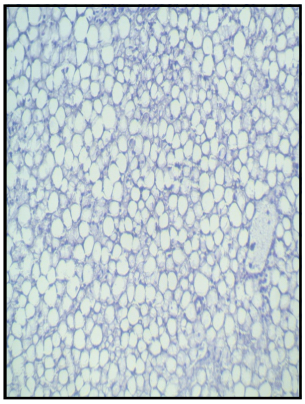


Fig. 7. Transverse section of adipose tissue treated group with high dose of chilli pepper which showed most adipose tissue lobes became dark in colour and most adipocytes were without nuclei, inflammatory cells aggregation around dilation H & E stain, 20x.

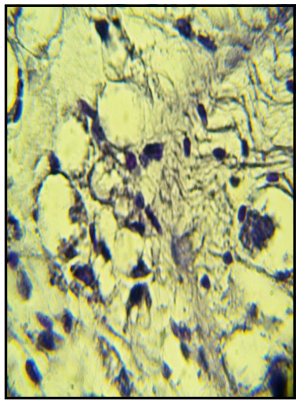


Fig. 8. Transverse section of adipose tissue treated group with high dose of chilli pepper which showed prominent adipocyte degeneration and spaces that filled with thick secretion H & E stain, 100x.

CONCLUSION

In conclusion, based on histological investigations, the adipose tissue showed significant differences in the biometric dimensions of adipose tissue nuclei after being treated with moderate and high doses of water hot chilli pepper extract, in rat model. The high concentration of watery chilli pepper extract displayed tissue structure with many

histological alterations, in contrast to the prior groups that received the moderate and lower doses.

REFERENCES

Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports* **50**: 111-116.

Alamgir, A. N. M. and Alamgir, A. N. M. (2017). Origin, definition, scope and area, subject matter, importance and history of development of pharmacognosy. Therapeutic Use of Medicinal Plants and their Extracts: Vol. 1: Pharmacognosy, Springer, Cham. pp. 19-60. *https://link.springer.com/chapter/10.1007/978-3-319-63862-1_2.*

Alenghat, Francis J. and Andrew, M. Davis (2019). Management of blood cholesterol. *JAMA* **321**: 800-801.

Arabi, M., Ostovan, A., Bagheri, A. R., Guo, X., Li, J., Ma, J. and Chen, L. (2020). Hydrophilic molecularly imprinted nanospheres for the extraction of rhodamine B followed by HPLC analysis: A green approach and hazardous waste elimination. *Talanta* **215**: 120933.

Awuchi, C. G. (2019). Medicinal plants: The medical, food and nutritional biochemistry and uses. *Int. J. Adv. Acad. Res.* **5**: 220-241.

Azlan, A., Sultana, S., Huei, C. S. and Razman, M. R. (2022). Antioxidant, anti-obesity, nutritional and other beneficial effects of different chilli pepper: A review. Molecules **28**: 898. *https://doi.org/10.3390/molecules27030898.*

Baskaran, P., Krishnan, V., Ren, J. and Thyagarajan, B. (2016). Capsaicin induces browning of white adipose tissue and counters obesity by activating TRPV1 channel-dependent mechanisms. *British J. Pharm.* **173**: 2369-2389. *10.1111/bph.13514.*

Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants–A mini review. *J. Fun. Foods* **18**: 811-819.

Fereidouni, F., Harmany, Z. T., Tian, M., Todd, A., Kintner, J. A., McPherson, J. D., Borowsky, A. D., Lechpammer, M., Bishop, J. Demos, S. G. and Levenson, R. (2017). Microscopy with ultraviolet surface excitation for rapid slide-free histology. *Nat. Biomed. Eng.* **1**: 957-966.

Hernández-Pérez, T., Gómez-García, M. D. R., Valverde, M. E. and Paredes-López, O. (2020). *Capsicum annuum* (hot pepper): An ancient Latin–American crop with outstanding bioactive compounds and nutraceutical potential. A review. *Comp. Rev. Food Sci. Food Safety* **19**: 2972-2993.

Jaiswal, V., Gahlaut, V., Kumar, N. and Ramchiary, N. (2021). Genetics, genomics and breeding of chilli pepper (*Capsicum frutescens* L.) and other Capsicum species. *Adv. Plant Breed. Strategies: Vegetable Crops: Vol. 9: Fruits and Young Shoots*. Springer, Cham. pp. 59-86.

Jiang, T. A. (2019). Health benefits of culinary herbs and spices. *J. AOAC Int.* **102**: 395-411. *https://doi.org/10.5740/jaoacint.18-0418.*

Kenig, S., Baruca-Arbeiter, A., Mohorko, N., Stubelj, M., cernelic-Bizjak, M., Bandelj, D. and Petelin, A. (2018). Moderate but not high daily intake of chilli pepper sauce improves serum glucose and cholesterol levels. *J. Functional Foods* **44**: 209-217.

Luo Jie, Hongyuan Yang and Bao-Liang Song (2020). Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol.* **21**: 225-245.

Nirengi, S., Homma, T., Inoue, N., Sato, H., Yoneshiro, T., Matsushita, M. and Hamaoka, T. (2016). Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy. *J. Biomed. Optics* **21**: 091305. *doi. 10.1117/1.JBO.21.9.091305.*

Nogueira, S., Garcez, F., Sá, S., Moutinho, L. C., Cardoso, A., Soares, R. and Leal, S. (2022). Early unhealthy eating habits underlie morpho-functional changes in the liver and adipose tissue in male rats. *Histochemistry Cell Biol.* **157**: 657-669.

Ohyama, K., Nogusa, Y., Suzuki, K., Shinoda, K., Kajimura, S. and Bannai, M. (2015). A combination of exercise and capsinoid supplementation additively suppresses diet-induced obesity by increasing energy expenditure in mice. *Am. J. Phys. Endocrin. Metabol.* **308**: E315-E323.

Smith, S. H. (2015). In the shadow of a pepper-centric historiography: Understanding the global diffusion of capsicums in the sixteenth and seventeenth centuries. *J. Ethnopharmacology* **167**: 64-77.

Sun, N. N., Wu, T. Y. and Chau, C. F. (2016). Natural dietary and herbal products in anti-obesity treatment. *Molecules* **21**: 1351.

Ung, C. O. L., Harnett, J. and Hu, H. (2017). Community pharmacist's responsibilities with regards to traditional medicine/complementary medicine products: A systematic literature review. *Res. Social Administ. Pharm.* **13**: 686-716.

Varghese, S., Kubatka, P., Rodrigo, L., Gazdikova, K., Caprnda, M., Fedotova, J. and Büsselberg, D. (2017). Chilli pepper as a body weight-loss food. *Int. J. Food Sci. Nutr.* **68**: 392-401.

Wang, Y., Zhou, Y. and Fu, J. (2021). Advances in antiobesity mechanisms of capsaicin. *Curr. Opinion Pharm.*. **61**: 01-05.

Ye, R. Z., Richard, G., Gévry, N., Tchernof, A. and Carpentier, A. C. (2022). Fat cell size: Measurement methods, pathophysiological origins and relationships with metabolic dysregulations. *Endocrine Rev.* **43**: 35-60.