Biochemical Evaluation of Anti-inflammatory Effect of Parsley Oil in Biliary Colic

Authors

Hussein A. Abd El-Maksoud¹, Lobna M. Salem², Mohammed Abdullah³
Mohamed G. El-Harrif²*

¹, ⁴Department of Biochemistry, Benha University, Egypt
²Department of Zoonosis, Benha University, Egypt
³Department of Biochemistry, October 6 University, Egypt

Abstract

Biliary colic is an abdominal pain usually resulted from cholelithiasis and gallstones. Parsley (Petroselinum crispum) derived from the Umbelliferae family is one of the most consumed herbs due to its aromatic properties. The aim of this study was to evaluate the anti-inflammatory effect of parsley in biliary colic. In order to achieve this aim 40 male albino rats was divided into three groups, healthy control group, non-treated induced biliary colic group and parsley oil treated biliary colic group. The result of the present study showed a significant increase of inflammatory biomarkers IL2, IL6, TNF, histamine and cortisol in biliary colic group. The result also showed a significant decrease in inflammatory biomarkers IL2, IL6, TNF, histamine and cortisol in biliary colic group after administration of parsley oil. In conclusion we suggested that parsley can relief the pain resulted from cholelithiasis and gallstones through its anti-inflammatory properties.

Keywords: Biliary colic, Parsley, Anti-inflammatory.

Introduction

Biliary colic is the term used for gallbladder pain usually a rise from contraction of gallbladder against obstruction or passage of the stones through the biliary duct. Pain is usually perceived in the right upper quadrant or epigastrium. (Johnsto et al.,2014) The abdominal pain lasts minutes to hours, usually followed by nausea and vomiting.(Philip, 2010)

The most susceptible people to gallstones formation are female gender, elders and a family history for gallstone disease. Obesity is another risk factor contributing to the metabolic syndrome such as dyslipidemia (in particular hyperlipoproteinemia type IV with hypertriglyceridemia and low HDL cholesterol), for the development of gallstones. Estrogen treatment enhances the risk, both in women when used for anti conception or hormone replacement and in men with prostatic cancer. Among specific dietary factors, short-time high cholesterol as well as high-carbohydrate diets were associated with increased risk for gallstones. (Marschall et al., 2007)

Eighty percent of gallstones are cholesterol stones while about ten percent are brown pigment stone and the remaining are black pigment stone. Three main mechanisms involved in stone formation
which are cholesterol super saturation of bile, gallbladder hypo motility and kinetic, pro nucleating protein factors. Gururaja et al., 2014

Acute cholecystitis is an inflammation of the gallbladder that is due to biliary obstruction of the cystic duct by gallstones. This obstruction causes intraluminal pressure within the gallbladder and triggers an acute inflammatory response. Pei et al., 2013

Parsley (Petroselinum crispum) from the Umbelliferae family is one of the most consumed herbs due to its aromatic properties. (Catunescu et al., 2017). It contains many biologically active compounds such as phenolic compounds, especially flavonoids (e.g., apigenin, apiin), coumarins, and essential oil compound (mainly myristicin and apiol). Moreover, ascorbic acid, tocopherols, and carotenoids also present in parsley. (Abdellatief et al., 2017)

Parsley exhibit many pharmacological properties, including antioxidant, hepato protective, and cardio protective effects, in addition to nephroprotective, antidiabetic, analgesic, spasmylytic, antiplatelet, laxative, diuretic, antibacterial, anti fungal and anti-inflammatory activities. (Farzaei et al., 2013)

Objective of the Research
The main objective of the present study was to evaluate anti-inflammatory effect of parsley oil in biliary colic.

Material and Methods
Healthy adult male albino rats, 8-10 weeks old, and average body weight 150 - 200 gm were used in the experimental investigation of this study. Rats were obtained from the Laboratory Animals Research Center, Benha University and used in accordance with the local ethics committee of Benha University for the use and care of animals in accordance with the NIH recommendations. Animals were housed in separate metal cages, exposed to good ventilation, humidity and to a 12hr. light/dark cycle. Fresh and clean drinking water was supplied ad-libtium. Constant supplies of standard pellet diet, fresh and clean drinking water were supplied ad-libtium.

The animals were left for 15 days for acclimatization prior to the beginning of the experiment, and kept at constant environmental and nutritional conditions throughout the period of the experiment.

Rats are randomly divided into three main groups, placed in individual cages and classified as follow:

- **First group (Control group):** consist of 10 male rats, were fed on normal diet and fresh, clean drinking water and kept as control group.
- **Second group (biliary colic group):** consist of 15 male rats were fed on normal diet supplemented with 0.5% cholesterol (w/w), 0.15% lecithin and 0.5% sodium desoxy cholate (w/w) for 6 weeks for induction of biliary colic and development of biliary stones and kept as positive control group.
- **Third group (Parsley treated group):** consist of 15 rats with biliary colic treated with parsley oil (0.5ml/ kg) for 6 weeks.

Blood Sampling
Blood samples were collected from medial canthus of the eye of all animal groups in dry, clean screw capped tube, separated and centrifuged at 2500 r.p.m for 15 mints. The clean, clear serum was separated by Pasteur pipette and kept in a deep freeze at -20 C until used for determination of Tumor necrosis factor - α (TNF-α), Interlukin-1 (IL-1), Interlukin-6 (IL-6), Histamine, Cortisol.

Statistical Analysis
All values were expressed as mean ± standard error (SE). All statistical analyses were performed using SPSS (version 19). Statistical differences among the experimental groups were assessed by ANOVA. Duncan’s test was used as a follow-up test and significance was defined at p<0.05.

Results and Discussion
The mean value of serum cortisol level in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group. The
The mean value of serum cortisol level of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group. Table (1)

The mean value of serum histamine level in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group. The mean value of serum histamine level of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group. Table (2)

The mean value of serum IL2 level in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group. The mean value of serum IL2 level of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group. Table (3)

The mean value of serum IL6 level in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group. The mean value of serum IL6 level of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group. Table (4)

The mean value of serum TNFα level in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group. The mean value of serum TNFα level of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group. Table (5)

**Table (1):** The mean values±S.E of serum (Cortisol) in healthy control, biliary colic, biliary colic with parsley groups

| Group          | Period (week) | Mean        |
|----------------|---------------|-------------|
|                | 1             | 2           | 3           | 4           | 5           | 6           |
| G1             | 7.36±0.37  a  | 7.25±0.40  a| 7.41±0.33  a| 7.05±0.39  a| 7.62±0.31  a| 7.35±0.93  a|
| G2             | 25.85±3.11 b  | 35.33±3.97 b| 43.39±5.32 b| 49.95±4.73 b| 52.87±6.28 b| 62.99±3.76 b|
| G3             | 23.00±2.79 b  | 28.56±4.31 b| 31.81±3.16 b| 38.93±3.13 b| 38.30±2.52 b| 42.22±2.30 b|

G1: Control healthy group  
G2: Control biliary colic group  
G3: Biliary colic + parsley treated group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.  
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

**Table (2):** The mean values±S.E of serum (Histamine) in healthy control, biliary colic, biliary colic with parsley groups

| Group          | Period (week) | Mean        |
|----------------|---------------|-------------|
|                | 1             | 2           | 3           | 4           | 5           | 6           |
| G1             | 2.11±0.26  a b| 2.19±0.36  a b| 2.89±0.19  a b| 2.57±0.24  a b| 1.79±0.55  a b| 1.42±0.30  a b|
| G2             | 9.47±1.03  a b| 11.53±0.47  a b| 14.71±2.75  a b| 19.56±1.84  a b| 20.84±4.89  a b| 36.17±4.43  a b|
| G3             | 7.56±0.82  a b| 7.69±0.57  a b| 7.59±0.88  a b| 11.04±0.64  a b| 11.08±0.61  a b| 11.42±0.48  a b|

G1: Control healthy group  
G2: Control biliary colic group  
G3: Biliary colic + parsley treated group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.  
A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.
Cytokines are small protein mediators involved in inflammatory, metabolic, and immune modulatory functions. They are not only produced by monocytes, lymphocytes, fibroblasts, and endothelial cells, but also in hepatocytes and the biliary epithelium. Secreted inflammatory molecules, such as pro-inflammatory cytokines, are among the critical mediators of the altered processes implicated in hepato-biliary disease. 

**Table (3):** The mean values±S.E of serum (IL2) in healthy control, biliary colic, biliary colic with parsley groups.

| Group                  | Period (week) | Mean     |
|------------------------|---------------|----------|
|                        | 1             | 2        | 3        | 4        | 5        | 6        |
| G1                     | 0.51±0.14a    | 0.28±0.06a | 0.36±0.11a | 0.46±0.08a | 0.38±0.14a | 0.54±0.16a | 0.42±0.05a |
| G2                     | 0.71±0.12b    | 1.32±0.28c | 1.57±0.20ab | 2.05±0.45a | 2.44±0.17b | 2.97±0.46a | 1.84±0.19a |
| G3                     | 0.46±0.13     | 0.72±0.13  | 0.83±0.06   | 0.79±0.052  | 0.78±0.111  | 0.90±0.04  | 0.75±0.04c |

G1: Control healthy group  G2: Control biliary colic group  G3: Biliary colic + parsley treated group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P<0.05) between any two means, within the same column have the same superscript letter.

**Table (4):** The mean values ±S.E of serum (IL6) in healthy control, biliary colic, biliary colic with parsley groups.

| Group                  | Period (week) | Mean     |
|------------------------|---------------|----------|
|                        | 1             | 2        | 3        | 4        | 5        | 6        |
| G1                     | 6.4±±0.46a    | 5.88±0.86a | 5.82±0.55a | 6.13±0.52a | 6.38±1.03a | 6.04±0.27a | 6.12±0.25a |
| G2                     | 15.46±0.24b   | 28.2±2.68c | 30.08±3.74c | 35.8±2.26b | 39.16±2.55b | 47.47±6.79b | 32.71±2.48b |
| G3                     | 9.25±0.67c    | 17.98±1.68b | 18.55±2.79b | 24.68±3.66c | 32.46±4.87b | 40.22±4.43b | 23.86±2.44b |

G1: Control healthy group  G2: Control biliary colic group  G3: Biliary colic + parsley treated group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

**Table (5):** The mean values±S.E of serum (TNFα) in healthy control, biliary colic, biliary colic with parsley groups.

| Group                  | Period (week) | Mean     |
|------------------------|---------------|----------|
|                        | 1             | 2        | 3        | 4        | 5        | 6        |
| G1                     | 31.21±2.17a   | 33.19±2.15a | 30.20±5.02a | 28.61±4.21a | 27.56±3.37a | 27.92±1.22a | 29.78±1.26a |
| G2                     | 63.42±12.03a  | 65.36±10.79ab | 80.85±14.10b | 114.99±6.57ab | 121.03±4.22ab | 132.87±7.80ab | 96.42±6.77a  |
| G3                     | 44.54±8.05a   | 47.81±10.35a | 42.80±7.70a  | 55.33±8.00ab  | 57.33±4.60bc  | 72.67±5.18bc  | 53.41±3.45a  |

G1: Control healthy group  G2: Control biliary colic group  G3: Biliary colic + parsley treated group

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

**Discussion**

Gallstone disease is one of the most prevalent gastrointestinal problems with over load on healthcare providers that is supposed to increase in old peoples (Marschall et al., 2007). Our data showed that the mean value of serum histamine, IL2, IL6, cortisol and TNFα levels in biliary colic group was significantly increased (P<0.05) on comparison with healthy control group.

Cytokines are small protein mediators involved in inflammatory, metabolic, and immune modulatory functions. They are not only produced by monocytes, lymphocytes, fibroblasts, and endothelial cells, but also in hepatocytes and the biliary epithelium. Secreted inflammatory molecules, such as pro-inflammatory cytokines, are among the critical mediators of the altered processes implicated in hepato-biliary disease. (Pei et al., 2013)
different from that in bacterial infection in vitro suggesting that IL-8 and IL-1β have roles in the development of acute cholecystitis in humans. Also, several serum cytokine concentration (TNFα, IL-6, IL-8, and IL-10) were increased in patients with acute cholangitis compared to a normal group, this indicate its relation to the systemic inflammatory response The elevation of cytokines may directly reflect the local inflammatory status of the gallbladder. In addition, IL-8 and IL-1β consistently remained at high levels during the entire period of acute cholecystitis. This suggests that IL-8 and IL-1β, could serve as constant and reliable indicators for patients with acute cholecystitis. Pei et al., 2013 In addition, (Hoogerwerf et al., 2005) found that, about ten folds increasing in lamina propria and muscular is mucosa mast cells in children with biliary dyskinesia and symptomatic gallstones in the patients with gallstone and biliary type pain. It is possible that mast cells are associated with pain and would be less in asymptomatic gallstone patients

Gallbladder mast cells would have the potential relation onto pain generation by two different mechanisms: sensitivity to distension as seen with mast cell degranulation in the gastrointestinal tract and through the generation of gallbladder spasms induced by mast cell products. For example, both histamine and cysteinyl leukotrienes increase the excitability of gallbladder smooth muscle and causing contraction Brian et al., 2006

Cortisol is a glucocorticoid hormone released by the hypothalamic pituitary adrenal (HPA) axis in response to inflammation through activation of the hypothalamic pituitary adrenalaxis by pro inflammatory cytokines, such as IL-6 leading to subsequent increase in plasma concentrations of cortisol. (Andrew et al., 2013)

Our result showed that the mean value of serum histamine, IL2, IL6, cortisol and (TNFα) levels of parsley treated group was significantly increased (P<0.05) on comparison with healthy control and significantly decreased (P<0.05) on comparison with biliary colic group.

parsley oil is rich with myristicin (32.75%), apiole (17.54%), α pinene (16.64%), β pinene (11.54%) and 1-allyl-2,3,4,5-tetramethoxybenzene (10%). (Mejdie et al., 2016) In accordance with our data (David Hoffmann, 2010) revealed that parsley has been used for the treatment of inflammatory condition, liver diseases, constipation, flatulence, jaundice, colic pain, and rheumatism.

In addition (Shahbaa et al., 2015) showed that all the doses of parsley effectively suppressed the edema produced by histamine, which indicates that, parsley provides its anti-inflammatory action by means of either inhibiting the synthesis or inhibiting release of inflammatory mediators. Also, (Jiet al., 2011) found that, myristicin (one of the most important constituent of parsley) at the concentration of 50 μM, significantly inhibit production of some cytokines such as TNF-α, and IL-1α. So, myristicin is suggested to be applied in higher concentrations for evaluation of anti-inflammatory effects concerned with TNF-α, and IL-1α.

Moreover, (Kamatou et al., 2008) revealed that, the mechanisms of action of many phenolic compounds such as flavonoids and curcuminoids occur by free radical scavenging activities and inhibition of pro inflammatory enzymes such as cyclooxygenases and lipoxygenases in the inflammatory process. Furthermore,(Farzaei et al., 2013) showed that, Petroselinum crispum seed hydroalcoholic extract offer analgesic activity in mice by reducing KCl and CaCl2 induced contractions in rats and the analgesic effect depends on blocking voltage gated calcium channels. Different extracts from aerial parts of parsley demonstrated antispasmodic activity on spontaneous and acetylcholine induced contractions of rat isolated ileum.

**Conclusion**

In conclusion, our results demonstrated the biliary colic accompany by increased level of serum inflammatory biomarkers and showed that the parsley can relief the biliary colic resulted from cholelithiasis and gallstones through its...
antiinflammatory properties. Further research is required to clarify specific mechanisms of action of parsley oil in biliary colic.

References
1. Abdellatif S. A., Azza A.A. Galala, Sameh M. Farouk, Mohamed M. Abdel-Daim, 2017 "Ameliorative effect of parsley oil on cisplatin-induced hepato-cardiotoxicity: A biochemical, histopathological, and immunohistochemical study" Biomedicine & Pharmacotherapy 86:482–491
2. Andrew Schrepf a, Lauren Clevery a, Desire Christensen a, Koen DeGeest b, David Bender b, Amina Ahmed b, Michael J. Goodheart c, Laila Dahmoush d, Frank Penedo e, Joseph A. Lucci III f, Parvin Ganjei-Azar g, Luis Mendez h, Kristian Markon a, David M. Lubaroff c, Premal H. Thaker k, George M. Slavich l, Anil K. Soodm, Susan K. Lutgendorf (2011) "Cortisol and inflammatory processes in ovarian cancer patients following primary treatment: Relationships with depression, fatigue, and disability" Brain, Behavior, and Immunity 30 S126–S134
3. Ayman F. Khalil, Haiam O. Elkatry, Hanaa F. El Mehairy (2015) "Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats" Annals of Agricultural Science 60(2), 353–359
4. Brian Rau, Craig A. Friesen, James F. Daniel, Adnan Qadeer, Ding You-Li, Charles C. Roberts, George W. Holcom (2006) "Gallbladder wall inflammatory cells in pediatric patients with biliary dyskinesia and cholelithiasis: a pilot study" Journal of Pediatric Surgery 41, 1545–1548
5. Cătunescu M.G, Ioan Rotar, Roxana Vidican, Florina Bunghez, Ancuța M. Rotar, 2017 "Gamma radiation enhances the bioactivity of fresh parsley (Petroselinum crispum (Mill.) Fuss Var. Neapolitanum)" Radiation Physics and Chemistry 132, 22–29
6. Farzaei M.H., Z. Abbasabadi, M.R. Ardekani, R. Rahimi, F. Farzaei, (2013) "Parsley: a review of ethnopharmacology, phytochemistry and biological activities" J. Tradit. Chin. Med. 33:815–826.
7. Gots JE, Matthay MA. 2016 "Sepsis: pathophysiology and clinical management" BMJ;353:i1585.
8. Gururaja MP, Anusha Raj, Himanshu Joshi, CS ShasrayKalanchoe, 2014 "Pininatum in Treatment of Gallstones" An Ethnopharmacological Review Int.J.PhamTech Res., 6(1), pp 252-261.
9. Hoogerwerf WA, Gondesen K, Xiao S-Y, et al., 2005"The role of mast cells in the pathogenesis of pain in chronic pancreatitis. BMC Gastroenterology;5:8.
10. Ji Young Lee and Wansu Park (2011) Anti-Inflammatory Effect of Myristicin on RAW 264.7 Macrophages Stimulated with Polyinosinic-Polyribidylic Acid. Molecules, 16, 7132-7142
11. Johnsto M.J, n, J.E.F. Fitzgerald b, A. Bhangu, N.S. Greaves, C.L. Prew, I. Fraser (2014) "Outpatient management of biliary colic: A prospective observational study of prescribing habits and analgesia effectiveness" International Journal of Surgery 12 169-176
12. Kamatou, G.P., Makunga, N.P., Ramgola, W.P., Viljoen, A.M., 2008. South African Salvia species: a review of biological activities and phytochemistry" J. Ethnopharmacol. 119 (3), 664–672.
13. Marschall H-U, Einarsson C., 2007"Gallstone disease (Review)”. J Intern Med; 261: 529–542.
14. Mejdi Snoussi, Ameni Dehmani, Emira Noumi, Guido Flamini, Adele Papetti (2016) "Chemical composition and antibiofilm activity of Petroselinum crispum and Ocimum basilicum essential
15. Pei-Yuan Su, Shih-Jen Liu, Yi-Hua Chen, Shun-Sheng Wu, Yao-Li Chen, Jhin-Ran Ke, Cheng-Yuan Peng, Yuh-Pyng Sher 2013 "Increased IL-8 and IL-1β in the bile of acute cholecystitis patients" Bio Medicine 3, 181-185

16. Philip E. Jaffe 2010 "Biliary colic" Decision Making in Medicine (Third Edition), Pages 194-195

17. Savard CE, Blinman TA, Choi HS, Lee SK, Pandol SJ, Lee SP. 2002 Expression of cytokine and chemokine mRNA and secretion of tumor necrosis factor-alpha by gallbladder epithelial cells: response to bacterial lipopolysaccharides. BMC Gastroenterol; 2:23

18. Shahbaa M. Al-khazrajhi (2015), "Studying the Analgesic, Anti-inflammatory and Antipyretic Properties of The Aqueous Extract of Petroselinum crispum in Experimental Animal Models" IOSR Journal Of Pharmacy 5, 9 17-23.