

Comparative Evaluation of the Anti-Inflammatory Potential of Three Different Anti Diabetic Natural Compounds (Piperine, Lupeol, Beta Sitosterol)

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ABSTRACT

Background: Anti-inflammatory drugs interfere in the pathophysiological process of inflammation, to minimize tissue damage and provide greater comfort to the patient. The anti-inflammatory drugs in the market today cause a lot of side effects. Hence, it is important to develop new naturally occurring anti-inflammatory drugs.

Aim: To evaluate and compare anti-inflammatory potential of Lupeol, Piperine and Beta Sitosterol.

Materials and methods: Protein denaturation inhibition Assay was performed. All the information and reported studies concerning lupeol, piperine and beta sitosterol were summarized from library and digital databases (e.g. Google Scholar, Sci-finder, PubMed, Springer, Elsevier, MDPI, Web of Science, etc.). Statistical analysis was done using ONE-WAY-ANOVA and Duncan Multiple Range Tests. Data were analysed to check the statistical significance between the groups.

Results: The results have shown that the protein denaturation inhibition of lupeol, piperine and beta sitosterol happened in a dose dependent manner with concentrations ranging from 100-500 µg/ml. It is also observed that beta sitosterol has the maximum anti-inflammatory potential followed by piperine and then lupeol.

Conclusion: Hence lupeol, piperine and beta sitosterol have good anti-inflammatory potentials with beta sitosterol having the best anti-inflammatory potential in comparison to Lupeol and Piperine. After further in vivo studies and clinical trials, they can be recommended as potential anti-inflammatory drugs in the market.

Key words: Innovative technology, Anti-inflammatory, Beta sitosterol, Protein denaturation inhibition, Protease inhibition, Lupeol, Piperine, Novel method

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INTRODUCTION

Inflammation is the immune response to dangerous stimuli, like pathogens, damaged cells, toxic substances or irradiation [1]. They act by removing injurious stimuli and initiating the process of healing [2]. Inflammatory response is the coordinated activation of signalling pathways which regulate the inflammatory mediator levels in tissues and inflammatory cells recruited from the bloodstream [3]. Anti-Inflammatory property refers to the ability of a substance to reduce inflammation or any of its 5 cardinal signs [4].

Through years of ingenious synthesis, many non-steroidal anti-inflammatory agents (NSAIDs) have been prepared and marketed. However, these drugs are known to provoke adverse effects such as gastrointestinal irritations, headaches, drowsiness and nausea [5]. Their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention [6]. Hence, the search for alternative anti-inflammatory drugs mainly from

natural herbs is required as they don't have side effects and are cost-effective as well as easily accessible [7].

Mangoes, White cabbage, Lupin seeds, Strawberries, Figs and Olives are rich sources of lupeol [8]. It is a natural triterpenoids with immense anti-inflammatory potential targeting key molecular pathways which involves nuclear factor kappa B (NFκB), cFLIP, Fas, Kras, phosphatidylinositol-3-kinase (PI3K)/Akt and Wnt/β-catenin in a variety of cells [9].

Black peppers, white pepper and long peppers are good sources of piperine. Many laboratory studies suggest that piperine the main active compound in black pepper may effectively fight inflammation. For example in arthritis induced animal studies treatment with piperine resulted in less joint swelling and fewer blood markers of inflammation [10] and piperine also suppressed inflammation in the airways caused by asthma and seasonal allergies [11].

Beta sitosterol (BS) which is found abundantly in sesame seeds, flax seeds, almonds and cashews [12] is suggested to modulate the immune function, inflammation, and pain levels by controlling the production of inflammatory cytokines [13]. Beta Sitosterol lowers cholesterol levels

and is precursor to an anabolic steroid [14]. Many research papers have shown that Beta Sitosterol has anti-inflammatory effects on microglia and found that BS exerts anti-inflammatory effects mainly by inhibiting the activation of P38, ERK, and NF- κ B pathways [15].

Though, the anti-inflammatory potentials of Lupeol, Piperine and Beta Sitosterol have been established in the past, this is the first ever research that compares the anti-inflammatory of Lupeol, Piperine and Beta Sitosterol to each other as well as to the standard drug Aspirin conducted in vivo. Our team has extensive knowledge and research experience that has translate into high quality publications [16-35].

The aim of the current study was to evaluate and compare anti-inflammatory potential of Lupeol, Piperine and Beta sitosterol.

MATERIALS AND METHODS

Chemicals

All the reagents and chemicals used in the present study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. Glyphosate was procured from Sigma Chemical Company St. Piperine, Lupeol and beta sitosterol purchased from Sigma Chemical Company St. Louis, MO, USA.

Inhibition of albumin denaturation

The anti-inflammatory activity of 3 different antidiabetic natural compounds was studied by using inhibition of albumin denaturation technique which was studied according to the methods of Mizushima, followed by minor modifications. The reaction mixture consisted of the test extracts and 1% aqueous solution of bovine serum albumin. The pH of the mixture was adjusted by using a small amount of 1N HCl for dissolving the mixture. The plant compounds (Piperine, Lupeol, beta sitosterol) with increase in concentration (100, 200,300,400, and 500 μ g/ml) were incubated at 37 $^{\circ}$ C for 20 min and then later were heated to 51 $^{\circ}$ C for 20 min, once the samples cooled down, their turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment had been performed in triplicate. In this study, Aspirin was used as the standard anti-inflammatory drug.

Calculation: % Inhibition= $100 - ((A1 - A2)/A0) * 100$

Statistical analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyse the statistical significance between groups. P value of <0.05 was considered to be significant.

DISCUSSION

In vitro anti-inflammatory activity of lupeol by protein denaturation inhibition

On comparing the anti-inflammatory property of standard (Aspirin) and lupeol, the protein denaturation inhibition by lupeol increases in a dose dependent manner ranging from 20% to 75% with an increasing concentration from 100 μ g/ml to 500 μ g/ml (Figure 1).

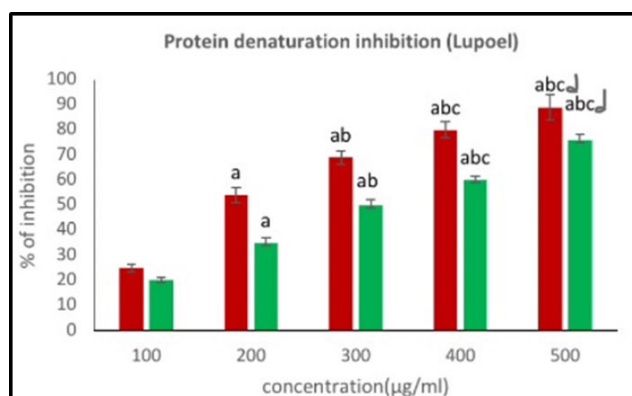


Figure 1: Showing the in vitro anti-inflammatory activity of lupeol. Each bar represents the mean \pm SD of 6 observations. The X axis represents the concentration of lupeol that increases from 100 μ g/ml to 500 μ g/ml. The Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%.The red bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The green bar represents the percentage inhibition of protein denaturation by Lupeol . Significance was considered at the levels of $p < 0.05$. A-compared with 100 μ g; b-compared with 200 μ g; c-compared with 300 μ g.

In vitro anti-inflammatory activity of piperine by protein denaturation inhibition. On comparing the anti-inflammatory property of standard (Aspirin) and piperine, The Protein Denaturation Inhibition by piperine increases in a dose dependent manner ranging from 20% to 85% in a concentration range of 100 μ g/ml to 500 μ g/ml (Figure 2).

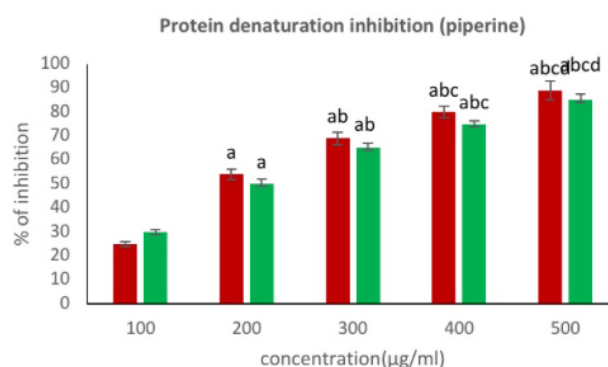


Figure 2: Showing the in vitro anti-inflammatory activity of piperine. The X axis represents the concentration of piperine that increases from 100 μ g/ml to 500 μ g/ml. The Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%.The red bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The green bar represents the

percentage inhibition of protein denaturation by piperine. Significance was considered at the levels of $p < 0.05$. A-compared with 100 μg ; b-compared with 200 μg ; c-compared with 300 μg . d-compared with 400 μg .

In vitro anti-inflammatory activity of beta Sitosterol by protein denaturation inhibition. On comparing the anti-inflammatory property of standard and beta sitosterol, The Protein Denaturation Inhibition by beta sitosterol happens in a dose dependent manner ranging from 20% to 87% in a dose dependent manner as the concentration of beta sitosterol increases from 100 $\mu\text{g}/\text{ml}$ to 500 $\mu\text{g}/\text{ml}$ (Figure 3).

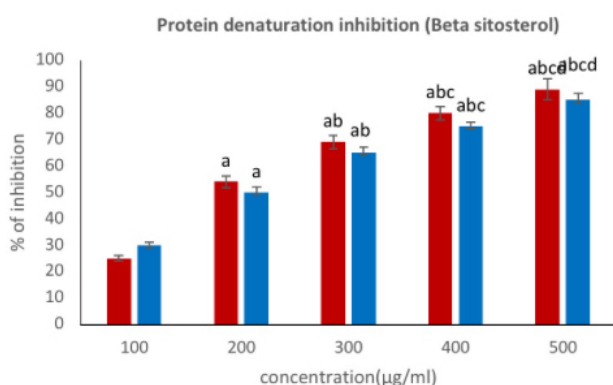


Figure 3: Showing the in vitro anti-inflammatory activity of β -sitosterol. Each bar represents the mean \pm SD of 6 observations. The X axis represents the concentration of beta sitosterol that increases from 100 $\mu\text{g}/\text{ml}$ to 500 $\mu\text{g}/\text{ml}$. The Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%.The red bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The blue bar represents the percentage inhibition of protein denaturation by beta sitosterol. Significance was considered at the levels of $p < 0.05$. A-Compared with 100 μg ; B-compared with 200 μg ; C-Compared with 300 μg . D-Compared with 400 μg .

The results have shown that the protein denaturation inhibition of lupeol, piperine and beta sitosterol occurs in a dose dependent manner with concentrations ranging from 100-500 $\mu\text{g}/\text{ml}$. In comparison beta sitosterol exhibited a potent anti-inflammatory potential with an Ic_{50} of 210 $\mu\text{g}/\text{ml}$ followed by piperine with an Ic_{50} of 240 $\mu\text{g}/\text{ml}$ and then lupeol with an Ic_{50} of 320 $\mu\text{g}/\text{ml}$

DISCUSSION

The purpose of our study was to determine and compare the Anti-inflammatory potential of three different anti-diabetic plant derived compounds (Piperine, Lupeol, beta sitosterol). Since NSAIDS (Non-Steroidal Anti-Inflammatory Drugs) are synthetic, they tend to have side effects like heart burns, vomiting, drowsiness and gastrointestinal problems. Hence, there is a need for developing natural, plant based products in the market as they have least to no side effects and are cheaper in comparison to NSAIDS. In the present study, it has been

observed that beta sitosterol has shown maximum protein denaturation inhibition which makes it the best anti-inflammatory compound followed by piperine and then lupeol. With further studies and trials Lupeol, Piperine and Beta Sitosterol can be recommended as potential anti-inflammatory drugs in the market.

From the results of the present study, it is observed that Lupeol has a good anti-inflammatory potential. In a previous study it was observed that, Lupeol and lupeol linoleate have shown a significant reduction in paw swelling by 39% and 58%, respectively, in adjuvant arthritis induced in rats which proved anti-inflammatory properties of lupeol. Paw edema had been induced by carrageenan and dextran in male swiss mice, and the inflammation reduced 30minutes after the administration of Lupeol Acetate [36,37]. In the chronic model of granuloma pouch in rats, lupeol exhibited 33% reduction in granuloma weight. In the arthritis model, lupeol exhibited 29% inhibition after 21 days. This showed that lupeol isolated from *Strobilanthes callosus* exhibited significant anti-inflammatory and antiarthritic activities [36].

In the present study, it was observed that Piperine has a very good anti-inflammatory potential. Similarly in another previous study, Piperine inhibited the expression of IL6 and MMP13 and also reduced the PGE2 production in a dose dependant manner at concentrations from 10 to 100 $\mu\text{g}/\text{ml}$. This suggested that piperine has anti-inflammatory, and antiarthritic effects in an arthritis induced animal model [38]. Also, piperine acts by mast cell-stabilizing activity, exhibits anti-inflammatory activity, thereby providing an effective treatment for Allergic Rhinitis [39].

In the present study, it is observed that Beta Sitosterol has an excellent anti-inflammatory potential. Similarly, it had been observed that Beta-sitosterol inhibited the expression of vascular adhesion molecule 1 and intracellular adhesion molecule 1 in TNF-alpha-stimulated Hirschsprung-associated enterocolitis as well as the binding of U937 cells to TNF-alpha-stimulated Hirschsprung-associated enterocolitis and reduces the phosphorylation of nuclear factor-kB p65. In the rat paw edema test previously, an inhibitory effect was found which goes from 50-70% which had been exhibited by beta-sitosterol [40]. In the same line with our findings, it was found that beta sitosterol decreased the activation of NFkB transcription factor in PMA-stimulated macrophage cells which suggest the anti-inflammatory action is mediated [41].

Since the extracts were natural, they could easily be destroyed and also lost in culture. But as they were all plant derived compounds. They are easily accessible and are cheaper than synthetic chemical compounds. With further in-vitro, in-vivo and clinical studies, these plant derived compounds could quench the thirst of herbal products in the market. The anti inflam properties of lupeol, piperine and beta sitosterol could be attributed to the factors that they are triterpenoids, alkaloids and

phytosterols respectively that have proven to have anti-inflammatory properties in previous findings.

Limitations-Since they are all natural products, they might not be found everywhere and may get damaged during culture as well. Future scope-All these three drugs may be recommended for the treatment of inflammation-mediated development of diabetes and associated complications. However, among three drugs analysed beta sitosterol can be prioritized as it proved to possess better efficacy.

CONCLUSION

Our present study clearly indicates that all the three natural compounds have ability in inhibiting the protein denaturation among which the beta sitosterol exhibited the maximum protective effect followed by piperine and lupeol. This study concludes that all these three drugs may be recommended for the treatment of inflammation-mediated development of diabetes and associated complications. However, among three drugs analyzed beta sitosterol can be prioritized as it proved to possess better efficacy.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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AUTHOR CONTRIBUTION

Hooriyah: Contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

V Vishnupriya: Contributed in study design, guiding the research work, manuscript correction.

J Selvaraj, Gayathri R, Kavitha S: Study design, statistical analysis, manuscript proofreading and correction.

REFERENCES

1. Medzhitov R. Inflammation 2010: New Adventures of An Old Flame. *Cell* 2010; 140:771-6.
2. Ferrero-Miliani L, Nielsen OH, Andersen PS, et al. Chronic inflammation: Importance of NOD2 and NALP3 In Interleukin-1 β Generation. *Clin Expe Immunol* 2007; 147:227-35.
3. Lawrence T. The nuclear factor NF-Kb pathway in inflammation. *Cold Spring Harbor Perspectives Biol* 2009; 1:001651.
4. Sun Y, Blieden C, Merritt BY, et al. Hemophagocytic lymphohistiocytosis and myelodysplastic syndrome: A case report and review of the literature. *J Med Case Rep* 2021; 15:98.
5. Chan J, Haroon N. Treatment: NSAIDs . *Oxford Med Online* 2016.
6. Saxena RS, Gupta B, Saxena KK, et al. Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor tristis* Linn: An Indian medicinal plant. *J Ethnopharmac* 1984; 11:319-330.
7. Wong SH, Chan FK. Adverse Effects of NSAIDs in the gastrointestinal tract: Risk factors of gastrointestinal toxicity with NSAIDs. *Springer Cham* 2016; 45-59.
8. Lerma-Torres JM, Navarro-Ocaña A, Calderón-Santoyo M, et al. Preparative scale extraction of mangiferin and lupeol from mango (*Mangifera indica* L.) leaves and bark by different extraction methods. *J Food Sci Techno* 2019; 56:4625-31.
9. Sankaran M. Lupeol: An ubiquitous compound for multiple ailments. *RA J Applied Res* 2017.
10. Rinkunaite I, Simoliunas E, Alksne M, et al. Anti-inflammatory effect of different curcumin preparations on adjuvant-induced arthritis in rats. *BMC Compl Med Therapies* 2021; 21:1-2.
11. Wang Y, Sun H, Zhang J, et al. Streptozotocin-induced diabetic cardiomyopathy in rats: Ameliorative effect of PIPERINE via Bcl2, Bax/Bcl2, and caspase-3 pathways. *Biosci Biotech Biochem* 2020; 84:2533-44.
12. Pacheco DF, González Ceballos L, Castro AZ, et al. Synthesis of new steroidal carbamates with plant-growth-promoting activity: Theoretical and experimental evidence. *Int J Mol Sci* 2021; 22.
13. Liao PC, Lai MH, Hsu KP, et al. Identification of β -sitosterol as in vitro anti-inflammatory constituent in *Moringa oleifera*. *J Agri Food Chem* 2018; 66:10748-59.
14. Saeidnia S. The story of beta-sitosterol- A review. *Euro J Medi Plants* 2014; 4:590-609.
15. Sun Y, Gao L, Hou W, et al. β -Sitosterol alleviates inflammatory response via inhibiting the activation of ERK/p38 and NF- κ B pathways in LPS-Exposed BV2 Cells. *BioMed Res Int* 2020; 1-10.
16. Wu F, Zhu J, Li G, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). *Artif Cells Nanomed Biotechnol* 2019; 47:3297-305.
17. Chen F, Tang Y, Sun Y, et al. 6-shogaol, a active constituents of ginger prevents UVB radiation

- mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol* 2019; 197:111-518.
18. Li Z, Veeraraghavan VP, Mohan SK, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway. *J Photochem Photobiol* 2020; 203:111-773.
 19. Babu S, Jayaraman S. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother* 2020; 131:110-702.
 20. Malaikolundhan H, Mookkan G, Krishnamoorthi G, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbek* on HCT-116 colon cancer cell lines. *Artif Cells Nanomed Biotechnol* 2020; 48:1206-1213.
 21. Han X, Jiang X, Guo L, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol* 2019; 47:3577-84.
 22. Gothai S, Muniandy K, Gnanaraj C, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed Pharmacother* 2018; 107:1514-1522.
 23. Veeraraghavan VP, Hussain S, Balakrishna JP, et al. A comprehensive and critical review on ethnopharmacological importance of desert truffles: *Terfezia clavaryi*, *terfezia boudieri*, and *tirmania nivea*. *Food Reviews Int* 2021; 1-20.
 24. Sathya S, Ragul V, Veeraraghavan VP, et al. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. *Environ Nanotechnol Monitoring Management* 2020; 14:100333.
 25. Yang Z, Pu M, Dong X, et al. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. *Ara J Chem* 2020; 13:5501-16.
 26. Rajendran P, Alzahrani AM, Rengarajan T, et al. Consumption of reused vegetable oil intensifies BRCA1 mutations. *Crit Rev Food Sci Nutr* 2020; 1-8.
 27. Barma MD, Muthupandiyani I, Samuel SR, et al. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol* 2021; 126:105-132.
 28. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent* 2021; 31:285-286.
 29. Samuel SR, Kuduruthullah S, Khair AMB, et al. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent* 2021; 31:436-41.
 30. Tang Y, Rajendran P, Veeraraghavan VP, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells. *Materials Sci Eng* 2021; 114:111-656.
 31. Yin Z, Yang Y, Guo T, et al. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. *Environ Toxicol* 2021; 36:1011-20.
 32. Veeraraghavan VP, Periadurai ND, Karunakaran T, et al. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci* 2021; 28:3633-40.
 33. Mickymaray S, Alfaiz FA, Paramasivam A, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. *Saudi J Biol Sci* 2021; 28:3641-3649.
 34. Teja KV, Ramesh S. Is a filled lateral canal-A sign of superiority? *J Den Sci* 2020; 15:562-3.
 35. Kadanakuppe S, Hiremath S. Social and behavioural factors associated with dental caries experience among adolescent school children in Bengaluru City, India. *Bri J Med Medi Res* 2016; 14:1-10.
 36. Sarpate R, Tupkari S. Evaluation of anti-inflammatory activity of *Strobilanthes callosus* Nees and *Strobilanthes ixiocephala* Benth. *Int J Green Phar* 2012; 16:199.
 37. Geetha T, Varalakshmi P. Anti-inflammatory activity of lupeol and lupeol linoleate in rats. *J Ethnopharmacol*. 2001; 15:77-80.
 38. Sabina EP, Nagar S, Rasool M. A role of piperine on monosodium urate crystal-induced inflammation—An experimental model of gouty arthritis. *Inflammation* 2011; 34:184-92.
 39. Settupane RA. Other Causes of rhinitis: Mixed rhinitis, rhinitis medicamentosa, hormonal rhinitis, rhinitis of the elderly, and gustatory rhinitis. *Immuno Aller Clin North Am* 2011; 31:457-67.
 40. Ribeiro AEAS, Soares JMD, Silva HAL, et al. Inhibitory effects of *Morus nigra* L. (Moraceae) against local paw edema and mechanical hypernociception induced by *Bothrops jararacussu* snake venom in mice. *Biomed Pharmacother* 2019; 111:1046-56.
 41. Lewit RA, Veras LV, Cowles RA, et al. Reducing underdiagnosis of hirschsprung-associated enterocolitis: A novel scoring system. *J Surg Res* 2021; 261:253-260.