The Effect of Vitamin K and Turmeric on Histamine Levels: An Arthritis Study

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2018

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Acknowledgments

First and foremost, I would like to thank Dr.Mondira Sengupta. She has helped me fully understand what Rheumatoid Arthritis is and has provided me with information on how one is affected with this disease. She has also helped me in getting assistance and guidance from Dr.Jolly and Dr.Malfait, who are researchers studying RA at Rush Hospital.

I would also like to thank Mr. Thielsen, who was always there to provide substantial advice with the project and who has taught me to use various scientific equipment, such as the microplate reader.

Last but not the least, I would like to thank my parents, especially my mother, who has RA. Their continued support has helped me obtain more knowledge on this disease.

Purpose, Hypothesis, Rationale:

Purpose: The purpose of this experiment is to determine whether Mephyton (vitamin K) or Turmeric powder will either help increase or decrease the levels of histamine in the Drosophila flies, which could correspond to the levels of inflammation in the flies.

Hypothesis: It is predicted that Turmeric powder and Mephyton(Vitamin K) will increase the levels of histamine because both of these substances carry anti-inflammatory inputs, which will then result in less inflammation all over the bodies of the Drosophila flies.

Rationale: Mephyton(Vitamin K) and Turmeric are reported to be involved in reducing inflammation. Vitamin K has been involved in both cardiovascular and bone health, and recently in the movement of pro-inflammatory cytokines. Turmeric,on the other hand, can help get rid of Eicosanoids, which are molecules that promote inflammation in the body. Turmeric contains a compound called Curcumin that could help eradicate the Eicosanoids from the body.

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Review Of Literature

Rheumatoid arthritis(RA) and related diseases have been around since organisms developed joints. In rheumatoid arthritis, the immune system which usually defends the body from foreign substances, such as viruses and bacteria, in turn, attacks the joints, turning it into an autoimmune disease.Some methods for reducing inflammation throughout the body is to control weight. This is extremely vital if one has the disease, on the grounds that putting on weight can put a considerable measure of power onto the knees, which could result in extremely terrible pain. Another arrangement is to work out, in light of the fact that not exclusively does it remove the stress from the aggravated piece of the body, yet it additionally makes the bones more stronger. The use of mephyton(Vitamin K), and turmeric powder as a preventative measure against RA, could assist in yielding solutions that may help eradicate RA from a person's life.

Histamine has a key part in unfavorably susceptible conditions. Harmed tissue mast cells discharge histamine, causing the surrounding veins to enlarge and increment in permeability. This allows liquid and cells of the immune system, for example, leukocytes (white platelets) and blood plasma proteins, to leak from the bloodstream through the vessel walls and move to the site of tissue damage or disease, where they start to battle the contamination and feed and recuperate the harmed tissues (Histamine, 2017). These fiery reactions coming about because of the freedom of histamine have been believed to be intervened by the histamine H1 receptor — ordinarily known as antihistamines — which have been utilized to treat hypersensitivities for a long time (Thurmond, Gelfand, & Dunford, 2008). Mast cells are found in the skin and in every mucosal tissue at homeostasis.A mast cell (otherwise called a mastocyte or a labrocyte) is a kind

of white platelet. In particular, it is a kind of granulocyte got from the myeloid stem cell that is a part of the immune and neuroimmune frameworks and contains numerous granules rich in histamine and heparin (Mast Cells, 2017). The mast cells were distinguished as a mesenchymal cell which, recognized several years later that these cells contain in their granules most of the body's histamine. Mast cells assume a focal part in inflammatory and immediate allergic reactions. They can discharge powerful provocative inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid that follow-up on the vasculature, smooth muscle, connective muscle, mucous glands and inflammatory cells. Histamine is not just released when the body experiences a poisonous substance, it is additionally released when mast cells recognize damage(Amin, 2011).

Rheumatoid joint pain is an immune system illness that can influence the whole body. Indications can include morning stiffness, extreme weariness, and sore joints.Rheumatoid Arthritis is an immune system ailment, which implies your body attacks itself. Individuals who exhibit Rheumatoid Arthritis, their body translates the synovium, the delicate coating around the joints, as a danger like an infection or microscopic organisms and attacks it. This attack makes liquid aggregate inside the joint. The liquid development causes swelling, agony, firmness, and irritation around your joints.

Osteoarthritis is fundamentally a degenerative disease. Your indications will revolve around the joint where the cartilage has been worn away. Low levels of inflammation might be present in these areas(Amin 2011). Individuals with Osteoarthritis encounter a breakdown of the cartilage that pads the joints. The wearing out of cartilage makes your bones rub against each other uncovering little nerves, which causes pain. Osteoarthritis does not include an immune

system process like Rheumatoid Arthritis does, but rather mellow irritation additionally happens(Roth, 2015).

Histamine is a natural nitrogenous compound which normally occurs in the human body, where it acts as neurotransmitter and is engaged with resistant reactions and the regulation of physiological functions. In an unfavorably susceptible response, the resistant framework(immune system) discharges an abnormal state of histamine, prompting provocative responses which are in charge of hypersensitive side effects. The individuals who are intolerant to histamine will experience the ill effects of comparable responses from an oral admission of histamine. Histamine is delivered by the deterioration of histidine(Thurmond, Gelfand, & Dunford, 2008).

To determine the levels of histamine in the Drosophila fruit flies, a Histamine ELISA Kit will be used. This protein connected immunosorbent examine, is a test that distinguishes and measures antibodies in the blood. This test can be utilized to decide whether one has antibodies identified with certain irresistible conditions. Antibodies are proteins that the body creates in light of unsafe substances called antigens(Kinman, 2017).

The development of the synovial coating of joints in rheumatoid joint inflammation (RA) requires an expansion in the vascular supply to the synovium, to adapt to the increased requirement for oxygen and supplements. New blood vessel arrangement – 'angiogenesis'– is perceived as a key event in the development and maintenance of the pannus in RA, suggesting that focusing on veins in RA might be a challenging future remedial methodology. Although, some expert angiogenic factors have been shown to be expressed in RA synovium, vascular endothelial growth factor (VEGF) has been exhibited to a have a central involvement in the angiogenic process in RA(Paleolong, 2009). To further this understanding, there has been a study

on dogs that were infused with histamine. Histamine was injected in anesthetized dogs during control conditions, after H2-receptor barricade with metiamide, after H1-receptor was blocked with chlorpheniramine, and after joined H1-and H2-receptor blockade. Histamine implantation, alone, induced marked foundational vasodilatation, aspiratory vasoconstriction, and transient increments in cardiovascular yield and heart rate(Tucker,Weir,Reeves,Grover, 1975). Hence, H2-receptors seem to intercede the vasodilatation, tachycardia, and expanded cardiovascular yield injected by histamine, though H1-receptors seem to intervene the vasoconstrictor and the negligible heart depressant activities of histamine.

Vitamins K is a fat-soluble vitamin that have been involved in both cardiovascular and bone health, and recently in the movement of proinflammatory cytokines. Vitamin K is cofactor in the γ-carboxylation of vitamin K dependent proteins. Two vitamin K-dependent proteins, osteocalcin and matrix-gla protein, are available in skeletal and vascular tissue (Shea, Booth, Massaro, Jacques, D'Agostino, Dawson-Hughes, Benjamin 2008). Inside cells, there are a few essential pathways that are vital in conveying the introduction to an torment or damage. Early work on avian retroviruses, and specifically reticuloendotheliosis infection strain T (Rev-T), recognized an oncogene (v-rel) capable for changing avian lymphoid cells. In a study conducted with Drosophila fruit flies, the Drosophila dorsal gene is known to assume a basic part in dorsal-ventral improvement of the fly larvae, going about as a translocation factor in the cell core to control gene expression. Around a similar time, another atomic translocation factor, with sequencing homology to these flagging variables, was found, which ended up being known as atomic factor kappa-B(NF-κB) . This is significant because if these flies have arthritic behaviours, then it would be best to say that these flies are in common with the arthritic

behaviours that patients with rheumatoid and osteoarthritis arthritis because they exhibit and mimic the same behaviours as compared to one another (Hodges,Pitsillides,Ytrebø, L. M., 2017).

Turmeric powder has a lot of great effects. One of them can be reducing inflammation at a certain area. The anti-inflammatory component of turmeric originates from one compound specifically called curcumin. The advantages from consuming curcumin are impactful. Studies have demonstrated curcumin to have against tumor and hostile to oxidant movement notwithstanding its intense calming impacts. The extenuating activity of curcumin originates from its capacity to hinder the body's generation of professional incendiary flagging mixes called eicosanoids.Eicosanoids are signaling molecules made by the enzymatic or non-enzymatic oxidation of arachidonic acid or other polyunsaturated fatty acids.They have many effects on your body, including inflammation, fever promotion, blood pressure regulation, and blood clotting. Utilizing turmeric to moderate the body's generation of eicosanoids gets their levels in the body back to ordinary levels and subsequently, chronic systemic inflammation in the body diminishes essentially (Goel, Boland, & Chauhan, 2001).

Rheumatoid Arthritis, in today’s society affect a great deal of people and many die from it every year.Going back to where the aggravation happens in the body, and how the histamine is conveyed to those specific parts, we could make the conclusion that the purpose behind the inflammation happening in the body is either the expansion or diminishing of histamine that is let out in the body. The results from this experimentation will show a way to eradicate the development of Rheumatoid Arthritis, so that it does not permanently reside and affect the other joints as well as the bones.

Materials:

Safety Note: Glassware should not be used for extraction purposes or for any other containment purposes. Histamine may adhere to the glass, and using glassware may affect test results.

- ELISA Kit
- Disposable Plastic Tips
- Mephyton (Vitamin K)
- Drosophila Fruit Flies
- Reagent boats
- Microplate Reader with 650nm filter
- Precision Pipettes that range from 10-1000 Mu/Liter
- Deionized water
- Graduated Cylinders
- Plate cover or plastic film to cover plate during incubation.

Procedure:

Part 1: Preparing the food with Turmeric

1. In a weigh-boat, measure out 5.13 grams Fruit Fly food.
2. In a separate weigh-boat measure out 0.45 grams of Turmeric and mix it with the Fruit Fly food. Transfer the food into vials and add water until the mixture absorbs it and makes a paste.Mark these vials as Turmeric.
3. To transfer the flies into the vials, put them in the freezer for about 3-5 mins. This process the flies to go into a sleep mode. Carefully transfer the flies into the vials with the turmeric.

Part 2: Preparing the food with Mephyton(Vitamin K)

1. In a weigh-boat, measure out 5.13 grams of Fruit Fly Food.
2. Take a beaker and fill it up with 10 ml of water.
3. Take a syringe and withdraw 1 ml of Mephyton(Vitamin K), and insert it into the beaker. Mix well until the Vitamin K dissolves into the water.
4. Mix this solution with water until it turns into a paste-like consistency.
5. To transfer the flies into the vials, put them in the freezer for about 3-5 mins. This process the flies to go into a sleep mode. Carefully transfer the flies into the vials with the turmeric.

Part 3: Reagent,Wash Buffer, and Dilution Buffer Preparation

1. Bring all reagents, plate wells to be used samples and calibrators to room temperature(20-25 degrees Celsius) before use.
2. Prepare the necessary volume of wash buffer by mixing 1 part **25X Wash Buffer** with 24 Deionized water. Label as **Working Wash Buffer.**
3. Mix entire contents of foil pouch in1 liter deionized water. Label as **Dilution Buffer.**

Part 4: Assay Protocol

1. Add 50 ul of standards or diluted (if necessary) sample to the appropriate wells.
2. Mix each reagent by inverting the reagent bottle prior to use.
3. Add 50 Mu/L of the **Histamine-HRP Conjugate** to each well. Use a multi-channel or repeater pipette if appropriate.
4. Mix by gently shaking the plate. A microplate shaker may be used if available.
5. Cover the plate with plastic film and incubate at room temperature (18-30 degrees Celsius) for 45 minutes.
6. Empty out the contents of wells into sink and blot on paper towel to remove as much fluid as possible.
7. Add 300Mu/L **Working Wash Buffer** per well.
8. Shake plate slightly during soak period for best wash results.
9. Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.
10. Repeat steps 7-9 for 3 times.

11. Add 150 Mu/L of substrate to each well. Be careful not to touch the inner well walls. Use a multichannel pipette for best results. Mix by shaking plate gently.
12. Incubate at room temperature (18-30 degrees Celsius) for 30 minutes.
13. To ensure uniform color development, before recording absorbance gently shake the plate by sliding it back and forth on a flat surface or, if available, use the shaker function on the reader.
14. Measure the absorbance at 650 nm if not using Stop Solution else, add 75 Mu/L Stop Solution (1N HCL).... to each well then measure the absorbance at 450 nm.

Variables:

Independent Variable: The amount of histamine standards put into each vial.

Dependent Variable: The increase or decrease of the histamine levels with the addition of Turmeric and Mephyton(Vitamin K).

Control group: The vials of flies that have not been affected by either Turmeric or Mephyton(Vitamin K).

Results: Table and graphs

Dark Blue: absence of Histamine

Light Blue: Presence of Histamine

Absorbance Readings:

	1	2	3	4	5	6	7	8	9	10	11	12	Average
A-Standard	1.858	1.547	1.068	1.121	0.707	0.825	0.582	0.583	0.414	0.409	0.259	0.25	
B-Control	0.382	0.339	0.876	0.321	0.33	0.48	0.336	0.404	0.304	0.331	0.333	0.319	0.3526
C-Vitamin K	0.302	0.284	0.305	0.269	0.283	0.208	0.289	0.294	0.269	0.281	0.281	0.286	0.2792
D-Turmeric	0.293	0.268	0.262	0.25	0.261	0.255	0.249	0.254	0.277	0.261	0.27	0.23	0.2608

Data Table showing the average histamine concentration for the Standard;

Standard Concentration (ng/mL)	%B/Bo
0	100
2.5	64
5.0	45
10	34
20	24
50	14

Graph exhibiting the Histamine in Standard Buffer

Results: Data Analysis & Calculations

In order to get the standard curve from the absorbance reading, what I needed to find was the maximal binding value,which would be the averages of the standard concentration which was run in duplicates. Since the first standard concentration was 0ng/mL, I took the absorbance values from that set, added them, and divided them by 2. The number is then to be divided by itself, knowing that that would be the initial standard value for 0ng/mL. Then, in order to get the percent value, the number was multiplied by 100. This was calculated as follows:

Percent of Maximal Binding for **0ng/mL:**
Values from the set: 1.878 + 1.547 = 3.425
3.425 / 2 = 1.7
1.7 / 1.7 = 1 * 100 = 100%
This process was then repeated for all the other standard concentrations.

After getting the Percent of maximal binding for the standards, what I had to figure out next was the maximal binding for the average values of the Control, Turmeric, and Vitamin K that I got from the plate reader. So, the way that I calculated the %B/BO was to first divide 0.3526, which was the average value for the Control and divide it by 1.7. After that, in order for it to yield the percentage, I simply multiplied the number by 100; to get 21%. The calculations goes as follows:

Average Value from plate reader for Control: 0.3526
0.3256 / 1.7 = 0.2074
0.2074 * 100 = 21% == %B/BO
This process of calculation was then repeated again with the average values of the Turmeric and Vitamin K.

Last, I needed to figure out, for what standard concentration was the maximal binding at. For this particular calculation, I needed to use the equation of the line of best fit. The %B/BO for the Control, Turmeric, and Vitamin K were all y-values in the equation, which meant solving for the x-value to get the standard concentration. The calculation goes as follows:

Equation of the Line = y = -1.25x + 65.1
Plug in 21 (Control) for the y-value and solve for x:
21 = -1.25x + 65.1
- 44.1 = -1.25x
X = 35.28 ng/mL for the maximal binding at 21%
This process of calculation was then repeated for Turmeric and Vitamin K.

According to the data that was received, there is a trend that shows both the Vitamin K and the Turmeric actually increased the histamine levels in the fruit fly, which was indicated as a really light blue color as compared to the standard, therefore resulting the presence of histamine; whereas if histamine wasn't present, it would result in a pigmented blue color.

Experimental Error

There is a possibility of the occurrence of human error in the form of an inaccuracy while transferring the Substrate and the Histamine Enzyme Conjugate into the wells. The scientific procedure and safety precautions were followed throughout the experiment. The materials that were used throughout this experiment have been thoroughly cleaned, sterilized and gloves were worn on at all times. Special care was taken during the incubations and while transferring the certain liquids to the wells. Also, the precaution was taken of not allowing any liquids from the ELISA kit to be transferred into glassware. Also, the experiment that was done was in duplicates, in order to rule out the possibility of inaccurate data, in the form of results.Thus, an extra effort was made to avoid experimental error.

Conclusion

In conclusion, the levels of histamine did increase in both samples of Vitamin K and turmeric. My hypothesis stated, “Turmeric powder and Mephyton (Vitamin K) will increase the levels of histamine because both of these substances carry anti-inflammatory inputs, which will then result in less inflammation all over the bodies of the Drosophila flies.” My hypothesis was proven right because the maximal binding of the Turmeric and Vitamin K displayed the levels of histamine increase as compared to the Standard. But that was not the main objective of this experiment. Although it was previously stated that turmeric and vitamin K carried these anti-inflammatory inputs, these substances do not prove to reduce or lower the inflammation in the flies. On the other hand, RA is an autoimmune disease, which practically means that the system is self-destructive. In this disease, instead of histamine being mass produced, we want to contain the histamine levels in a way such that it does not inflame the sinovial coating around the joint. Like I indicated earlier, figuring out a certain medication to stop the release of histamine and prevent inflammation, we would be able to help millions of people that have been diagnosed with RA cure their disease and maybe provide a safe chance for recovery.

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