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Exploring Herbal Anti-inflammatory drugs.

Harnessing Natures Remedies for inflammation management

Prof. Parbhane M. B, Kakade Sanika Satish, Wagh Tejas Balu.

Sitabai Thite College Of Pharmacy,Shirur.

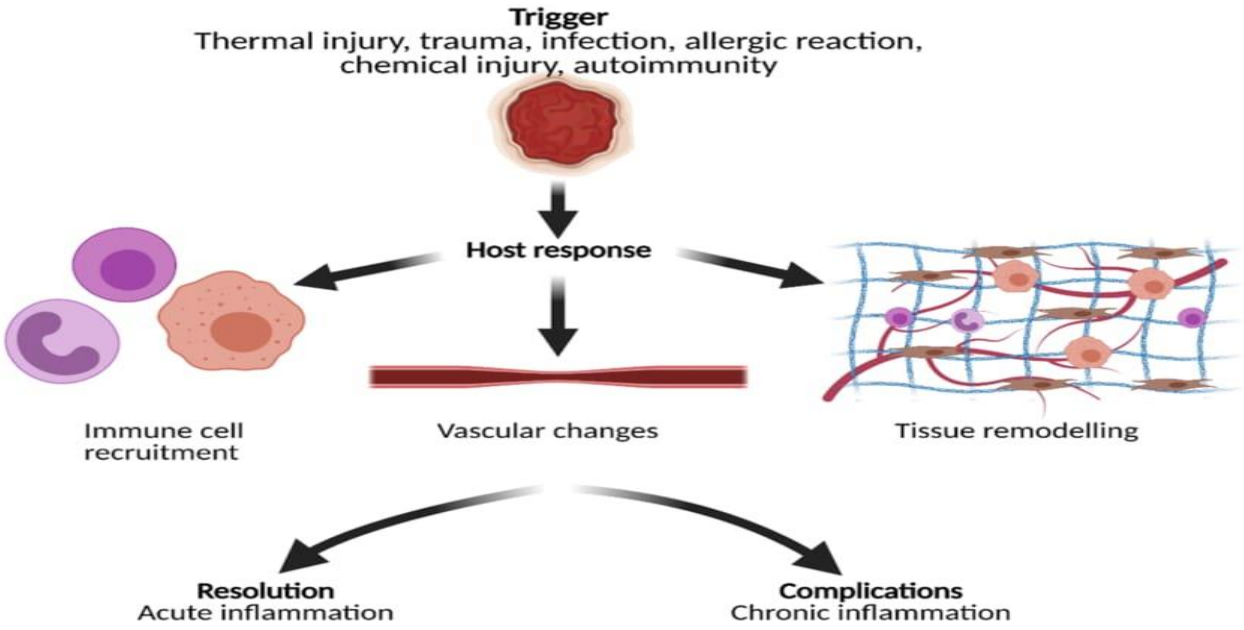
ABSTRACT:

This research paper investigates the anti-inflammatory properties of a novel herbal emulsion formulated with renowned botanicals, namely Ashwagandha, Cinnamon, Turmeric, Ginger, and Stevia. These herbs are known for their individual therapeutic benefits, and their synergistic effects within an emulsion are explored in this study. The methodology involves in vitro assays to assess the emulsion's anti-inflammatory efficacy. Preliminary findings suggest a promising reduction in inflammatory markers, supporting the potential of this herbal blend as a natural anti-inflammatory solution**.** The emulsion's formulation involves a careful combination of standardized extracts of Ashwagandha, Cinnamon, Turmeric, and Ginger, balanced with the natural sweetness of Stevia. In vitro experiments assess the emulsion's impact on pro-inflammatory cytokines, oxidative stress markers, and cellular pathways associated with inflammation. Research suggests that herbal emulsions for anti-inflammatory effects may offer advantages over some commercially available formulations. These benefits could include natural ingredients with potentially fewer side effects and holistic properties**.**

INTRODUCTION :

Diseases for the treatment of which the use of herbal drug delivery for anti-inflammatory drugs is considered are the result of chronic in-flammation or autoimmune diseases (e.g., allergies, atopic dermatitis, psoria-sis, asthma, chronic obstructive pulmonary disease, arthritis (osteoarthritis, rheumatoid arthritis), inflammatory bowel diseases (ulcerative colitis, Crohn's disease), celiac disease, auto inflammatory syndrome, or inflammation accompanying transplant rejection). Chronic inflammatory diseases are one of the most common reductions in quality of life and are among the most common causes of death. This is mainly a problem of Western countries associated the way of life stress and environmental burden. Inflammatory diseases, whether caused by excessive stress on certain tissues/parts of the body or arising from infections accompanying autoimmune or secondary diseases, have become a problem, especially in the Western world to- day. Whether these are inflammations of visceral organs, joints, bones, or the like, they are always a physiological reaction of the body, which always tries to eradicate noxious agents and restore tissue hemostasis. Unfortunately, this often results in damage, often irreversible, to the affected tissues. There are various medicines for controlling and suppressing inflammatory crisis; steroids, non-steroid anti-inflammatory drugs, and immunosuppressant are the practical examples of these medications which are associated with adverse effects while in practice our goal is to apply minimum effective dose by the highest efficacy with the least adverse effects. Thus, we need to apply natural anti-inflammatory factors to minimize the advers effect . Herbal medicines are promoting subjects in medicine and, of course, we have to increase our knowledge about them. Complementary, alternative, and traditional medicines are the pivotal source of herbal medication guidance, but surely modern medicine must prove these guidelines through scientific methods before using them in practice.

INFLAMATION:



Inflammation is an essential part of your body's healing process. It occurs when inflammatory cells travel to the place of an injury or foreign body like bacteria. If inflammatory cells stay too long, it may lead to chronic inflammation. Chronic inflammation is a symptom of other health conditions, such as rheumatoid arthritis. Your healthcare provider may recommend medication or at-home management .You can reduce inflammation by eating anti-inflammatory foods and managing stress. Inflammation is a process by which you body's white blood cells and the things they make protect you from infection from outside invaders, such as bacteria and viruses. But in some diseases, like arthritis, you body's defense system your immunesystem triggers inflammation when there are no invaders to fight off. In the autoimmune diseases, your immune system acts as if regular tissues are infected or somehow unusual, causing damage. Inflammation can be either short-lived (acute) or long-lasting (chronic). Acute inflammation goes away within hours or days. Chronic inflammation can last months or years, even after the first trigger is gone. Conditions linked to chronic inflammation include:

• Cancer

• Heart disease

• Diabetes

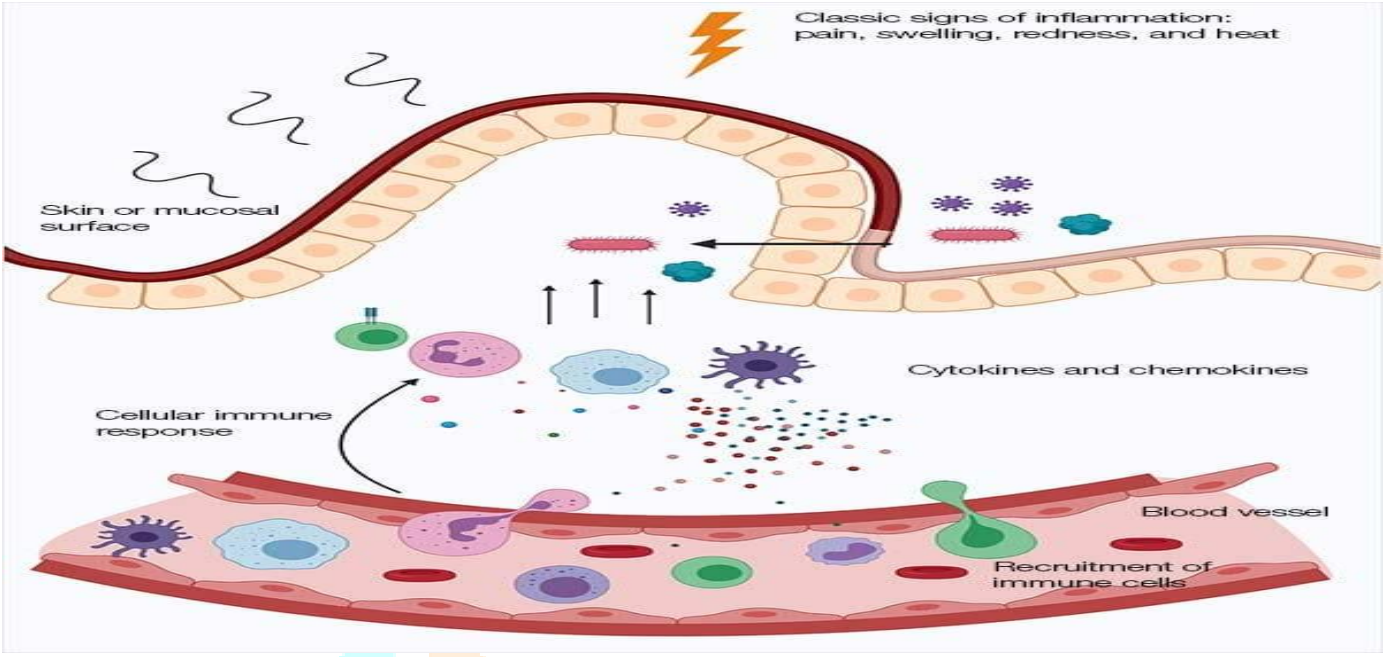
• Asthma

• Alzheimer's disease

Symptoms of inflammation include:

Redness, A swollen joint that may be warm to the touch, Joint pain, Joint stiffness, A joint that doesn't work as well as it should. Often, you'll have only a few of these symptoms.

Inflammation may also cause flu-like symptoms including:



Fever, Chills, Fatigue/loss of energy, Headaches, Loss of appetite

When inflammation happens, chemicals from your body's white blood cells enter your blood or tissues to protect your body from invaders. This raises the blood flow to the area of injury or infection. It can cause redness and warmth. Some of the chemicals cause fluid to leak into your tissues, resulting in swelling. This protective process may trigger nerves and cause pain. Treatment for inflammatory diseases may include: medications, rest, exercise, and surgery to correct joint damage. Your treatment plan will depend on several things, including your type of disease, your age, the medications you're taking, your overall health, and how severe the symptoms are.

The goals of treatment are to:

- Correct, control, or slow down the disease process
- Avoid or change activities that aggravate pain
- Ease pain through pain medications and anti-inflammatory drugs
- Keep joint movement and muscle strength through physical therapy
- Lower stress on joints by using braces, splints, or canes as needed

Some ways to ease long-term inflammation include:

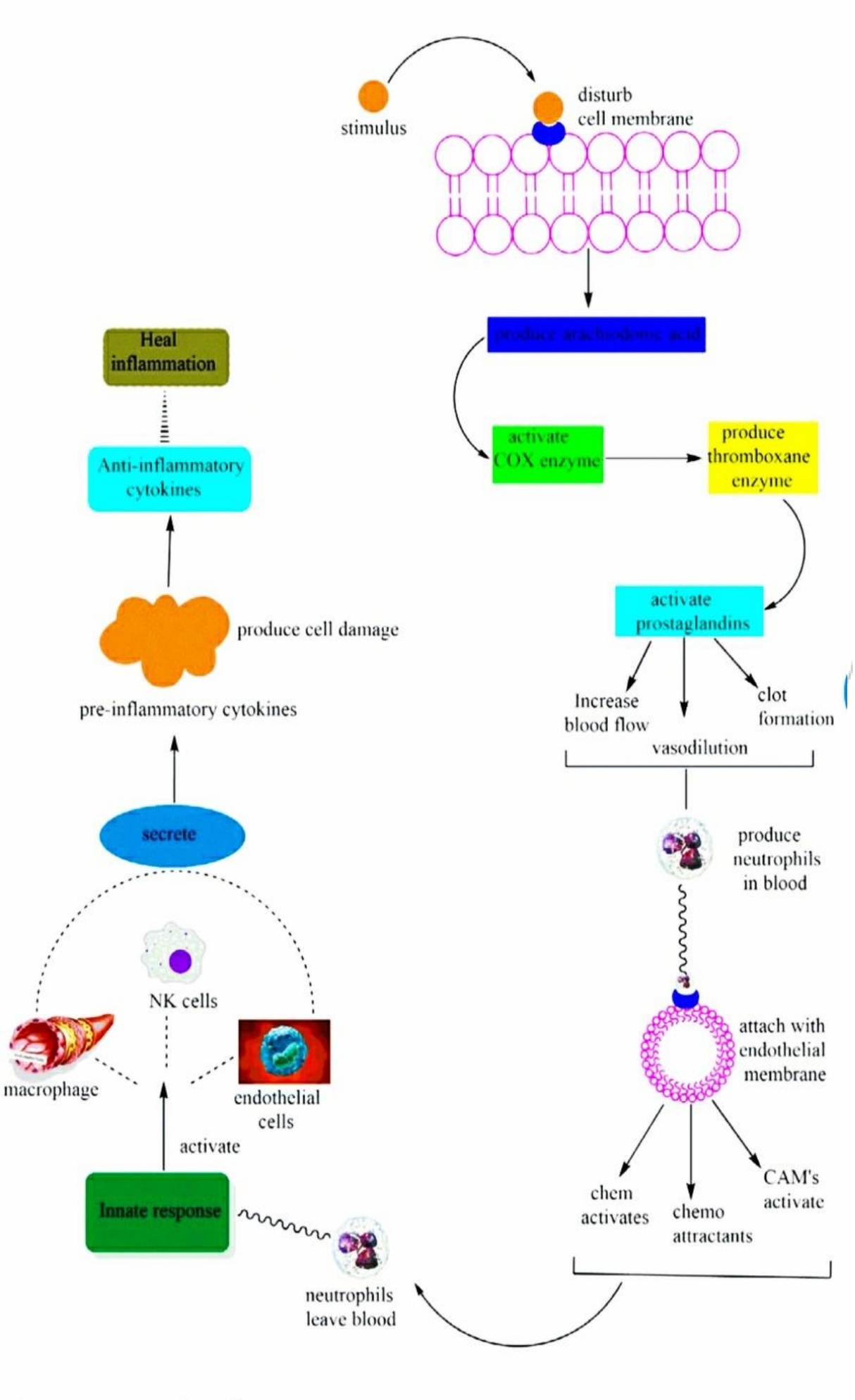
- Quit smoking.
- Limit how much alcohol you drink.
- Keep a healthy weight.
- Manage stress.
- Get regular physical activity.
- Try supplements such as omega-3 fatty acids, white willow bark, curcumin, green tea, or capsaicin. Magnesium and vitamins B6, C, D, and E also have some anti- inflammatory effects. Talk with your doctor before starting any supplement.

Anti-Inflammatory Diet:

The things you eat and drink can also play a role in inflammation. For an anti- inflammatory diet, include foods like: Tomatoes, Olive oil, Leafy green vegetables (spinach, collards), Nuts (almonds, walnuts), Fatty fish (salmon, tuna, sardines), Fruits (berries, oranges).

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage.

MECHANISUM OF INFLAMATION :



HERBS

Herbs are potentially good for pain and inflammation different types of herbs are used. Ashwagandha, Cinnamon, Turmeric, Ginger, Stevia.

Ashwagandha:(*Withaniasomnifera,)*

It is the one of the most important herb in Ayurveda, which is traditional form of medicinal based on Indian principles of natural healing. It is a small shrub with yellow flowers that's native to India and south Asia. The powder or extract fron the plant roots are use to treat various condition. Ashwagandha contains compounds, including WA, that may help reduce inflammation in the body. Animal studies have shown that WA may also help reduce levels of inflammatory proteins such as interleukin-10 (IL-10), and there's some evidence ashwagandha may help reduce inflammatory markers in humans too. In a 2021 study, researchers gave people with COVID-19 an Ayurvedic drug containing 0.5 grams of ashwagandha and other herbs twice per day for 7 days. This reduced participants' levels of inflammatory markers CRP, IL-6, and TNF-a compared with a placebo.

Cinnamon :

Cinnamon is a delicious spice made from the barks of trees from the *Cinnamomum* family.The two main types of cinnamon are *Ceylon cinnamon*, also called "true" cinnamon, and *Cassia cinnamon*, which is the most commonly available type 50.People have prized cinnamon for its health properties for thousands of years. An analysis of 12 studies in over 690 participants found that taking 1,500-4,000 mg of cinnamon daily for 10-110 days significantly reduced the inflammatory markers CRP and MDA, compared with a placebo. Also, cinnamon raised the body's antioxidant levels. Interestingly, the analysis found that only Cassia cinnamon, the more common variety of cinnamon, reduced both CRP and MDA levels. Ceylon cinnamon only reduced MDA levels .Similarly, an analysis of 6 studies in 285 people found that taking 1,200-3,000 mg of cinnamon daily for 8-24 weeks significantly reduced CRP levels .This effect was especially apparent in conditions in which CRP levels were high, such as NAFLD, type 2 diabetes, and rheumatoid arthritis. Notably, while cinnamon is safe in small amounts, too much cinnamon can be dangerous. Cinnamon, especially the more common Cassia variety, has high levels of coumarin. This compound has been linked to liver damage when people consume too much of it. Cinnamon's tolerable daily intake is 0.05 mg per pound (0.1 mg per kg) of body weight. One teaspoon (2.5 grams) of Cassia cinnamon contains 7-18 mg of coumarin this means the average adult should consume no more than 1

teaspoon (2.5 grams) of cinnamon per day. It's best to season with cinnamon sparingly to avoid its side effects.

Turmeric:

Turmeric (*Curcuma longa*) is a spice popular in Indian cuisine that people have used since ancient times. It's packed with over 300 active compounds. The main one is an antioxidant called curcumin, which has powerful anti-inflammatory properties. Numerous studies have shown that curcumin can block the activation of NF- kB , a molecule that activates genes that promote inflammation. An analysis of 15 high quality studies followed 1,223 people who took 112-4,000 mg of curcumin daily for periods of 3 days to 36 weeks. Taking curcumin significantly reduced inflammatory markers compared with taking a placebo. Markers included interleukin 6 (IL - 6), high-sensitivity C- reactive protein (hs-CRP), and malondialdehyde (MDA). Studies in people with osteoarthritis have found that taking curcumin supplements provided pain relief similar to that of the common nonsteroidal anti-inflammatory drugs (NSAIDs) ibuprofen and diclofenac. Unfortunately, turmeric only contains 3% curcumin by weight, and your body doesn't absorb it well. It's best to take curcumin with black pepper, as the latter contains a compound called piperine, which can increase curcumin absorption by up to 2,000% If you're looking to take curcumin for its anti-inflammatory properties, it's best to purchase curcumin supplements, ideally ones that also contain black pepper extract or piperine. You can purchase them from health food stores and online.

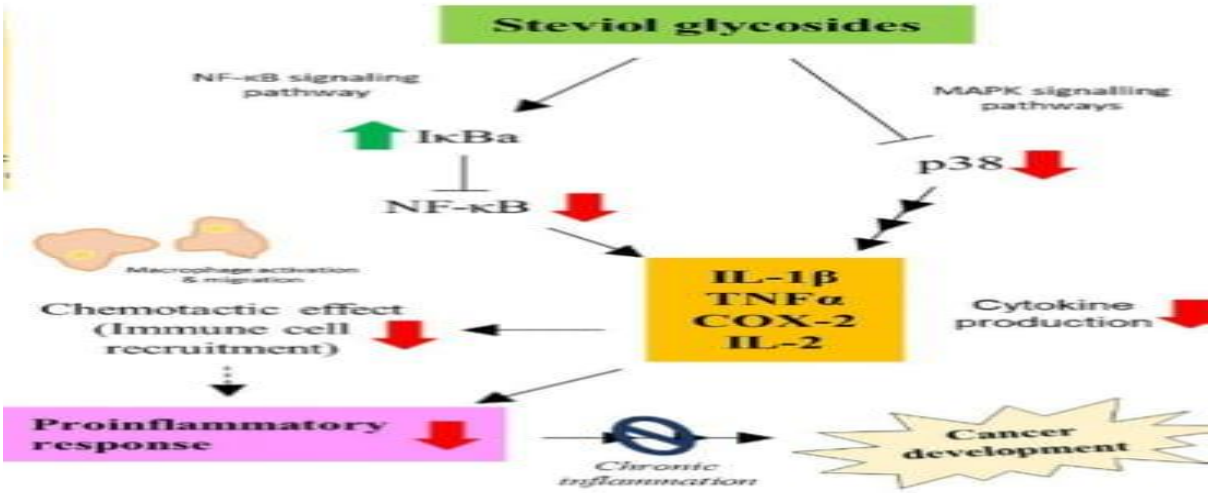
Ginger:

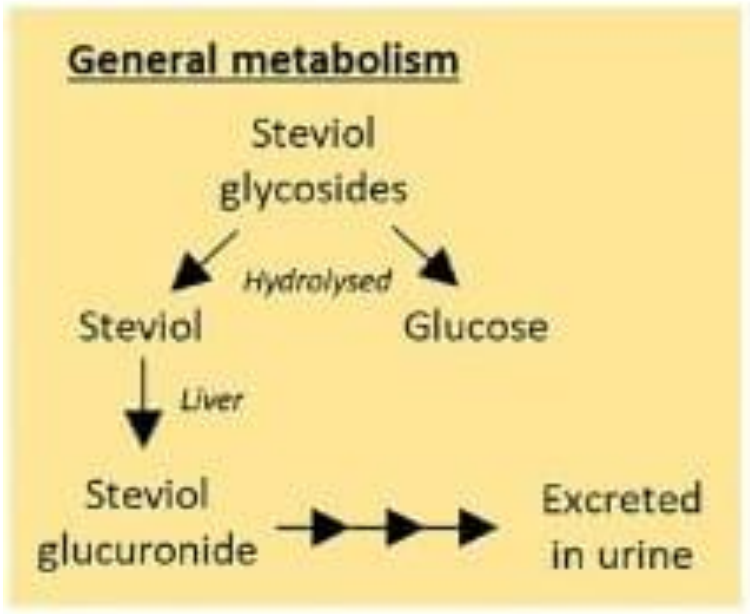
(*Zingiber officinale*) is a delicious spice with a peppery yet sweet flavor. You can enjoy this spice in various ways, such as fresh, dried, or powdered. Outside of ginger's culinary uses, people have used it for thousands of years in traditional medicine to heal numerous conditions. These include colds, migraines, nausea, arthritis, and high blood pressure. Ginger contains more than 100 active compounds, such as gingerol, shogaols, zingiberene, and zingerone, to name a few. These are likely responsible for its health effects, including helping reduce inflammation in the body. An analysis of 16 studies in 1,010 participants found that taking 1,000-3,000 mg of ginger daily over 4-12 weeks significantly reduced markers of inflammation compared with a placebo. These markers included C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-a). Other research looked at

the effects of taking 500-1,000 mg of ginger daily in people with osteoarthritis, a degenerative condition involving joint inflammation. The studies found ginger may reduce inflammatory markers such as TNF-a and interleukin 1 beta (IL-1ß), as well as reduce joint pain and increase joint mobility. Ginger is also incredibly versatile and easy to incorporate into many dishes, such as stir-fries, stews, and salads.

Stevia:

rebaudiana is a unique plant with anti-inflammatory and bactericidal properties. Stevia extract inhibits the activity of many pathogenic bacteria, can be used to treat immune diseases and reduce edema. Nowadays the demand of medicinal plants is increasing due to the growing scientific evidence confirming the health benefits of extracts and phytochemicals isolated from plants. Many of the phytoconstituents with biological activity found in plants, including alkaloids, flavonoids, tannins and phenolic compounds are known to possess potential antioxidant activities. It is noteworthy that many of these biologically active substances have been considered relevant in medicine in the prevention of chronic diseases such as cancer, cardiovascular and neurodegenerative diseases. This may be related to their antioxidant, antibacterial, anticancer, antifungal, and antiviral activities as well their ability to regulate cellular activities of inflammation-related cells (mast cells, macrophages, lymphocytes and neutrophils). Due to the sweetness and supposed therapeutic properties of its leaf, Stevia (Stevia *rebaudiana Bertoni)* has been used as a natural sweetener and in traditional medicine. 2009). Stevia's sweet taste is attributed to several glycosides such as stevioside, rebaudioside A, B, C, D, E and dulcoside A. These natural sweeteners possess therapeutic potential against several diseases such as diabetes mellitus, candidiasis, hypertension, inflammation, obesity and cancer, among others. In addition, other metabolites, such as flavonoids, alkaloids, water-soluble chlorophylls, xanthophylls, hydroxycynnamoyl derivatives (caffeoyl and chlorogenic acid derivatives), neutral water- soluble oligosaccharides, free sugars, amino acids, lipids, essential oils and trace elements present in Stevia. The phytochemicals content of fresh Stevia leaves and dried by FD, CD, VD, MW, IR, SD and SH methods is shown in Fig. 1. The total phenolics (TPC) and flavonoids (TFC) content of fresh Stevia leaves aqueous extracts was 2.58 g .





ACTIVE CONSTITUENTS :

Sr No.	Drug name	Botanical Name	Active constituents
1	Ashwagandha	Withania somnifera	Alkaloid, steroids, saponins, flavonoids, phytophenols, glycosides.
2	Cinnamon	Cinnamon cassia	Cinnamaldehyde, essential oils, eugenol, linalool, cinnamyl acetate.
3	Turmeric	Curcuma longa	Curcumin, volatile oil, curcuminoids.
4	Ginger	Zingiber officinale	Phenolic &terpene, gingerols, shogaols, polyphenols.
5	Stevia	Rebaudiana bertoni	Stevioside, steviolbioside,steviol glycoside.

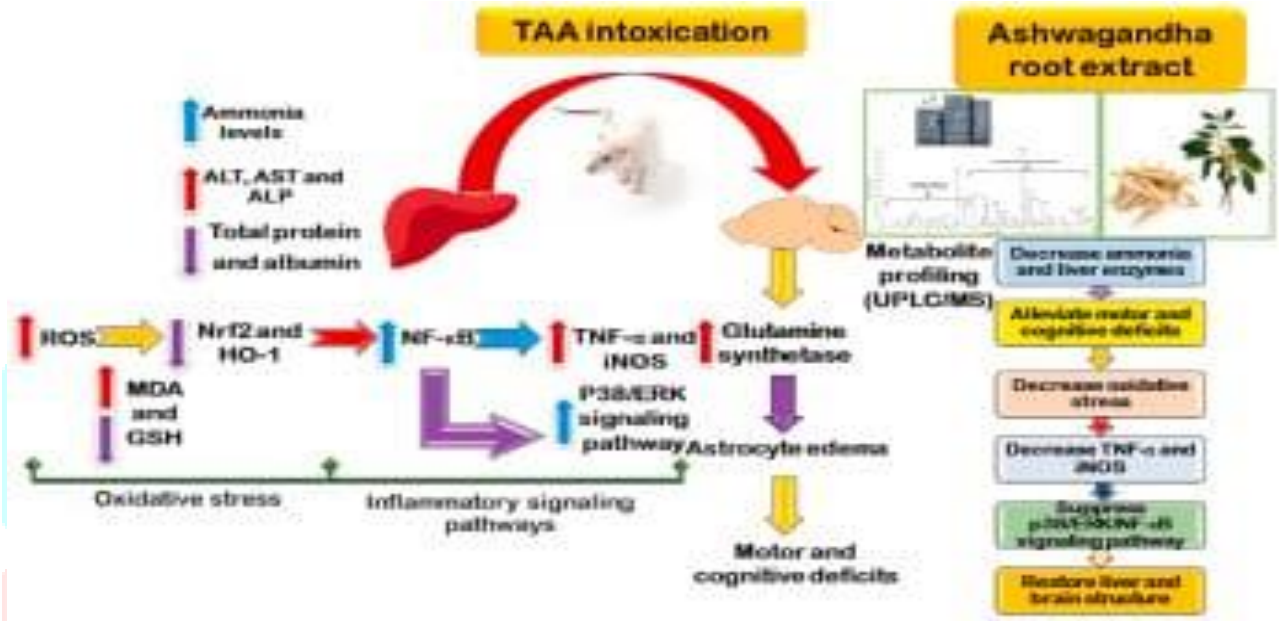
MATERIAL & METHOD :

For Anti-inflammatory activity following extract used:

1. Ashwagandha
2. Cinnamon
3. Turmeric
4. Ginger
5. Stevia

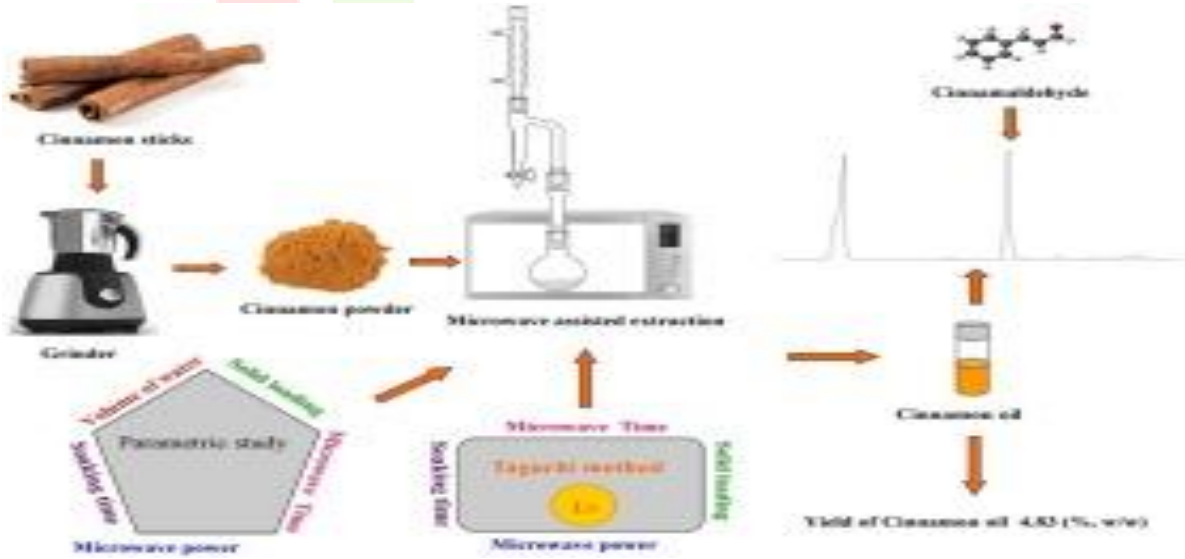
Methods of extraction:

1. Ashwagandha :



Ashwagandha roots are cut into small pieces and spread on SS sieve of 10 mesh to 30 mesh. Grind it into a powder using a blender or food procecer . place root in glass jar . pour the alcohol into jar make sure it's completely covered . Use a ratio of 1:5 (1 part ashwagandha root to 5 parts alcohol) for standard tincture.

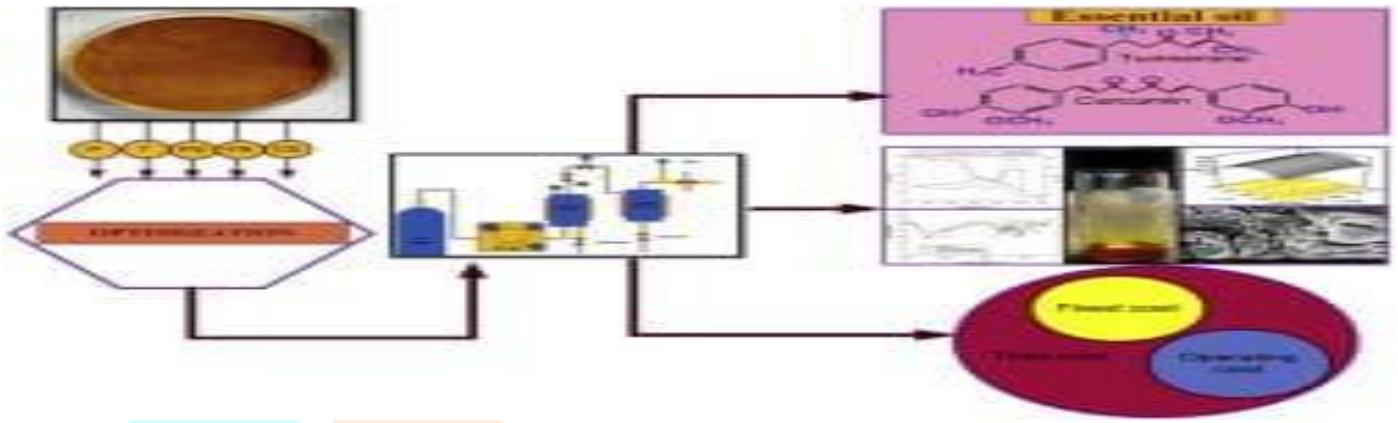
2. Cinnamon :



(Steam distillation method)Amount of 100 to 150 g of mashed cinnamon sticks were introduced into the distillation flask (1L), which was connected to the steam generator via a glass tube and to a condenser to retrieve the oil. The essential oils were volatilized with boiling water at temperature 100 deg * C for 5 and 10 hours. The recovered mixture was allowed to settle and the oil was withdrawn9-

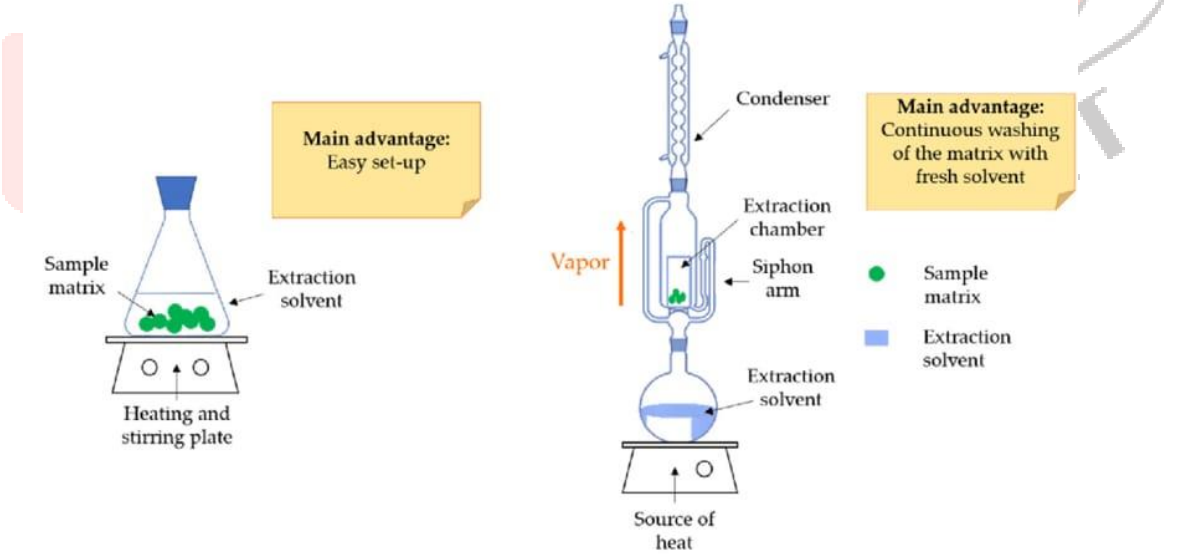
10. After the steam distillation process, the product was collected and separated using separatory funnel. The essential oils settled at the bottom layer of the separatory funnel and were separated several times until no oil was left in the separatory funnel steam distillation. The antibacterial activity of the essential oil was tested using disc agar diffusion method. Nutrient agar plates were swabbed with respective broth cultures of the organisms. Each sterile filter paper disc was impregnated with 10 µl oil. The paper discs were then transferred onto the agar plate and incubated for 24 hours at 37 deg * C All the process was carried out under laminar flow hood.

3. Turmeric (curcumin):

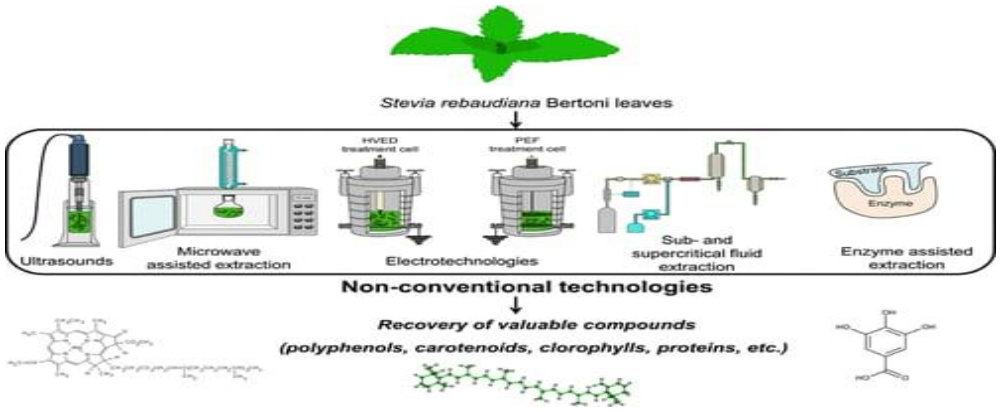


steam distillation is used with volatile solvents to extract turmeric essential oils . Here , an autoclave machine was used to pass through the turmeric ,after which the steam was cooled down using water and the essentialoils were obtained.

4. Ginger: it extracted using Soxhlet extraction , ginger breakup into small pieces and extracted by soxhlet method.



5. Stevia : stevia leaves are harvested and dried and extracted dried powder of leaves with hot water after which primary clarification is reached by filtration and centrifugation.



IDENTIFICATION TEST :

Ashwagandha:

S. No.	Secondary Metabolites	Aqueous	Ethanol
1	Phenolics	-	+
2.	Alkaloids	-	+
3.	Saponin	+	+
4.	Tannin	-	-
5.	Glycosides	-	+
6.	Carbohydrates	-	-
7.	Flavinoids	+	+
8.	Amino acid	+	-
9.	Terpenoids	-	+
10.	Starch	+	+

Phytochemical Evaluation

The freshly prepared ethanolic and aqueous extracts of Withania somnifera were qualitatively analyzed for the presence of phytochemical constituents using the following standard protocol.

1. **Test for Phenol Ferric Chloride test (Kar, 2004) [4]**
   Take 2 ml of Ashwagandha filtrate in a test tube and then add 2 ml of ferric chloride (1%). The appearance of dark green or bluish green color indicated the presence of phenol.
2. **Test for tannins**
   Add few drops of lead acetate solution in a test tube with 2 ml of filtrate. Yellowish coloration was indication of positive result.
3. **Test for Saponin Froth Formation test (Kokate et al., 1994) [5]**
   1 ml of extract was taken in test tube add 20 ml of distilled water. Take 10 ml of filtrate was taken in a graduated cylinder. Add 5 ml of distilled water and shake vigorously. Formation of persistent froth indicates the presence of saponins**.**
4. **Test for Flavonoids (Harbourne, 1973) [3] Alkaline Reagent Test**

To the 200 mg of extract in a test tube add few drops of Sodium hydroxide solution. Then add few drops of dilute hydrochloric acid, change in the color from deep yellow colour to colourless indicates the presence of flavonoids.

5. **Test for Glycosides :**Kellar-Kiliani Test (Sim, 1968) Add 1 ml of ashwagandha extract in a test tube with 1 ml of glacial acetic acid which contains traces of ferric chloride. Add 1 ml of concentrated sulphuric acid slowly along the sides of the test tubes. The appearance of greenish blue color at the junction of the two liquids indicates the presence of glycosides.

6. **Test for Steroids:** Salkowski Test (Wallis, 1985) To 200 µl of filtrate extract add few drops of conc. H2SO4 carefully along the sides of test tubes. The change in color of lower layer to yellowish and reddish upper layer indicated the presence of steroids.

7. **Test for Alkaloids**

(a.) Wagner's TestTo 200 µl of crude extract add few drops of Wagner's reagent to the inner side of test tube. A reddish brown precipitate was formed which confirmed the presence of alkaloids.

- **Preparation of Wagner's reagent**
  Iodine: 1.27g
  Potassium iodide : 2g Distilled water: 5mlThe solution was further diluted in 100 ml of distilled water for working solution.

(b) Mayer's Test: Equal amount of extract and 1% hydrochloric acid were added and heated gently. Mayer's and Wagner's reagent were added to the mixture. Cream colored of the resulting precipitate was taken as proof for the presence of alkaloids.

- **Preparation of Mayer's reagent**: Mercuric chloride: 13.6 parts Potassium iodide :50 parts Distilled water: 940 parts

Ashwagandha:

S. No.	Secondary Metabolites	Aqueous	Ethanol
1	Phenolics	-	+
2.	Alkaloids	-	+
3.	Saponin	+	+
4.	Tannin	-	-
5.	Glycosides	-	+
6.	Carbohydrates	-	-
7.	Flavinoids	+	+
8.	Amino acid	+	-
9.	Terpenoids	-	+
10.	Starch	+	+

Cinnamon:

Phytochemical tests	Test name
Alkaloids	Wagner's test
Carbohydrates	Molish test
Steroids	Salkowski test
Terpenoids	Chloroform test
Flavonoids	Alkaline reagent test
Reducing sugar	Benedict's test
Amino acids	Ninhydrin test
Glycosides	Keller-kilian test
Phenols	Fecl3 test

Ginger:

Active compounds	Reagent type	Result of warm aqueous extract of *Zingiber officinale*
Alkaloids	Reagent Mayer	+
Turbines	Foam reagent	+
	Mercury chloride reagent	+
Glycosides	mulch reagent	+
Phenolic	Chloride reagent	+
Tannins	Lead acetate reagent	+
Flavonoids	sulfuric acid	+
Potassium	Hydroxide	+
Resins	alcohol ethyl	-

Stevia :

Gas chromatography, Mass spectroscopy(GC-MS)and, Fourier Transform, Infrared Spectroscopy(FTIR). The reproducibility of extraction and of chromatographic analysis was proved.

Tannins are also present in higher concentration followed by alkaloids, glycosides, saponins, sterols, triterpenoils, anthraquinone, and other reducing compounds.

FORMULA:

SR. NO	INGREDIENTS	F1	F2	F3	F4
1	Ashwagandha	5	5	5	5.5
2	Cinnamon	2	2	2	2.5
3	Turmeric	2	2	2.5	3
4	Ginger	2	2.5	2	2.5
5	Stevia	1	1	1	1
6	Lecithin(emulsifier)	1	2	2	1
5	Water	20 ml	20ml	20ml	20ml

Preparation of Emulsion:

Preparation of Emulsion:(By gum method)

Ingredients :

- Water
- Acacia
- Ashwagandha
- Cinnamon
- Turmeric
- Ginger
- Stevia (for sweetness)

Equipment:

- Beaker
- Magnetic stirrer
- pH meter
- Thermometer
- Weighing balance

Procedure:

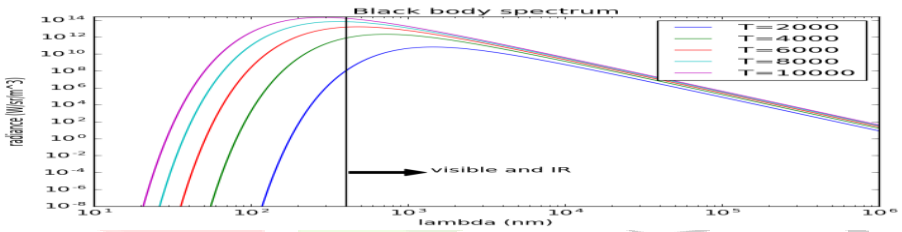
1. 20 ml of water into a beaker.
2. Add a calculated amount of Gum Arabic to the water. Stir well until Gum Arabic dissolves. This forms the gum solution.
3. In another beaker, measure and weigh the required amount of oil.
4. Add the powdered ingredients (ashwagandha, cinnamon, turmeric, ginger) to the oil phase. Mix thoroughly to disperse the powders evenly.
5. Slowly add the gum solution to the oil phase while stirring continuously. This forms the primary emulsion.
6. Use a pH meter to measure and adjust the pH of the emulsion if necessary.
7. If sweetness is desired, add stevia and continue stirring until the emulsion is well combined.
8. Monitor the temperature throughout the process to ensure it remains within the desire range.
9. Optionally, use a homogenizer for finer particle size and better stability.
10. Transfer the emulsion to a storage container and label it appropriately.

Evaluation of Emulsion:

1. Visual Inspection : Visual inspection of emulsions may include appearance, particles and color requirements : not conspicuous, no oil separation, no phase separation, practically free from visible particles no turbidity or precipitation, change of color not more than one degree. Regarding the physical stability and tendency of the prepared formulas of emulsion toward sedimentation and separation, we can summarize the results of this test as follows:
   F1, F2, F3, have failed during the formulation in spite of changing the type & (%) of oil and ratio of surfactant. This indicate the failure of turmeric and ginger oil to give acceptable emulsion formula. This may attributed to HLB values of these oils and the single use of surfactant (tween 20) which hydrophilic one. We may alleviate this problem by use less ratio of oil, or use of combination of surfactant (hydrophilic and lipophilic such combination of tween 20 or 80 with span 20 or 80).F4 (using cinnamon oil) was succeeded (remained stable for longer time) and the separation was reversible (dispersed by simple shaking). So, we can consider it as better formula.F3 and F2 were succeeded during preparation and remained stable for 10 min then separated. The emulsification did not easily return by simple shaking.

Sr no	Formula code	Result
1	F1	Separated
2	F2	Separated
3	F3	Separated
4	F4	Succeeded

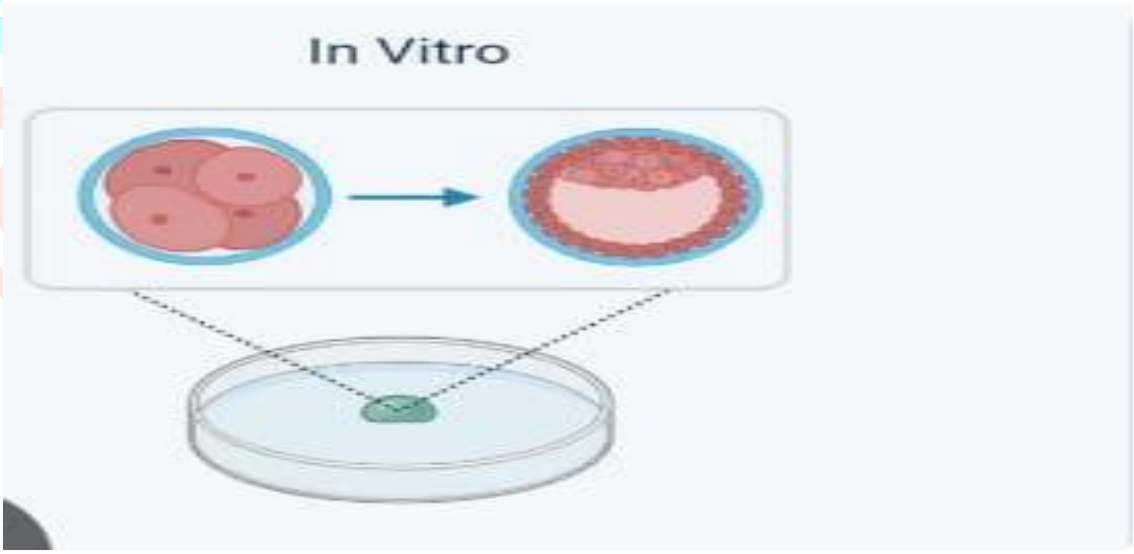
2. Assay of drug loading : Addition of specified volume (5ml) of the selected formula of herbal emulsion in a suitable organic solvent like ethanol followed by sonication, then filtered sample is determined spectrophotometrically at the determined Ymax.



3. Determination of droplet size:
   The droplet size of selected formula was examined using zeta sizer instrument to get idea about the micromeritic properties of the prepared formula.

Figure 9. Zeta Sizer

SCREENING METHODS FOR ANTI-INFLAMATORY ACTIVITY:



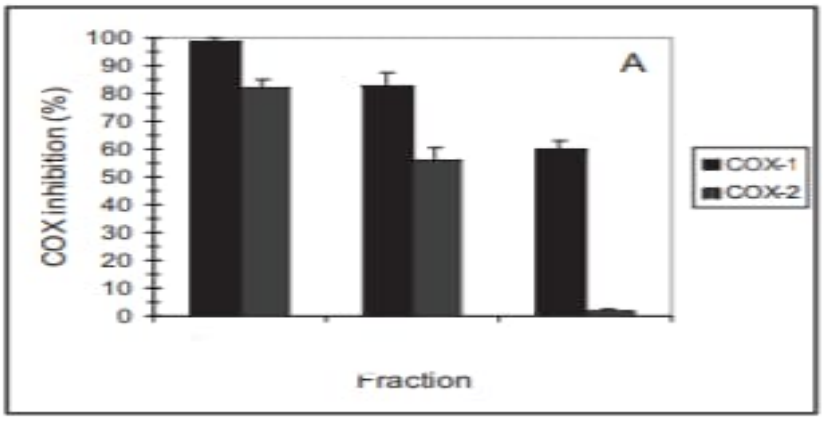
In-VitroMethod:

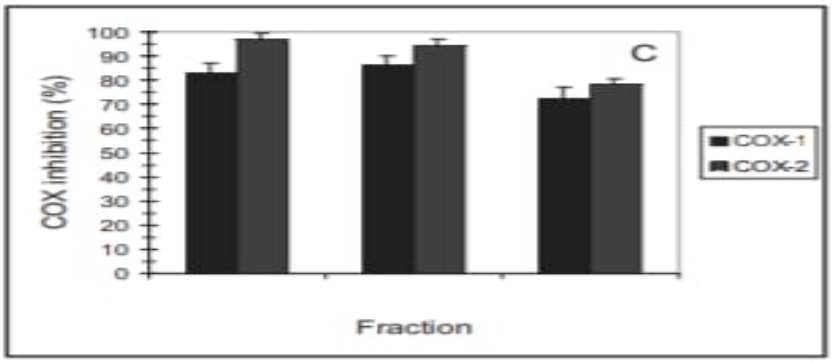
- COX assay.
- Mast cell degranulation.
- Inhibition of NO production induced by TNF- a in mouse macrophages.
- Measurement of NO production in mouse macrophages.
- Adhesion assays.
- Platelet-neutrophils adhesion.
- HRBC membrane stabilization.

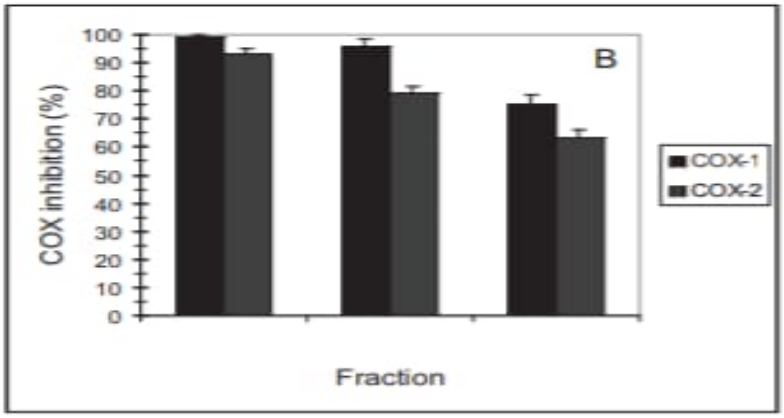
Cox assay:

COX-1:10ml of sample solution added to 19ml of 0.1M of L-adrenaline, dihydrogen tartrate and 10mM of hematin.After adding 0-2 units of COX-1 it is pre incubated for 5 mins by adding 10 mL of 10% formic acid.The concentration PGE2 is measured with a PGE2 enzyme immune assay.

COX-2:Assay mixture consists of 100mm rod phosphate, 1mm of hematin gelation, 2.5mL of compound in DMSO.It is pre incubated for 15 mins at 22 C and the 20 mL of solution of 1mM arachidonic acid and 1mM TPMD in assay buffer is added.The absorbance at 400nm is measured over the fast 36 sec and % inhibition calculated. The enzyme inhibition of TPMD in absence of COX-2 is also observe and subtracted from activity in presence of COX-2



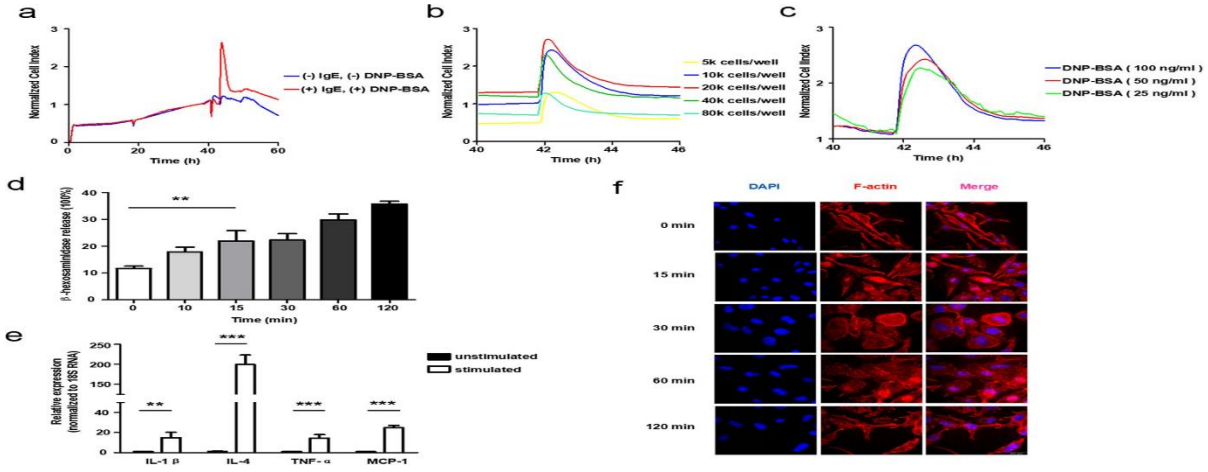




CONCLUSIONS: From the literature review, it can be seen that several in vitro methods are developed for the pharmacological screening of anti-inflammatory activity. Many of the methods reflect in vivo performance. These methods help to understand the real mechanism of inflammation and to identify new compounds possessing the anti-inflammatory activity. It is very difficult to develop single in vitro method for anti-inflammatory activity. Even in future, the mentioned method will accelerate the anti- inflammatory drug development process.

Mast cell degranulation : Heparinized Tyrode's solution is injected into the peritoneal cavity of exsanguinated rat (Sprague dawley after abdominal massage, the cells in peritoneal fluid are harvested and separated through 38% Bovine Serum Albumin. Cells are washed and suspended in Tyrode's solution with 0.1% BSA.The cell suspension is pre incubated with test drugs at 37 C for 3 min. fifteen mins after addition of compound 48/80 (standard compound for mast cell degranulation), glucuronidase (1mM phenolphthalein-D-glucuronide in 0.1 M acetic acid buffer, pH 4.5 is used as a substrate, absorbance monitored at 550nm after alkalization) and histamine (0.2% o-phthalaldehyde condensation in pH 12.5

fluorescence is monitored at 350/450 nm after acidification) in the supernatant are determined. The total content is measured after treatment of the cell suspension with Triton X-100. The percentage release determined is the index of anti-inflammatory activity.



Platelet neutrophil adhesion: Thrombin activated human platelets are incubated with drug at 20 C for 10 mins, and mixed with neutrophils at a ratio of 10:1.Neutrophil with two or more (number positives) and one no adherent or platelets (number negatives) are counted as index of activity. The test drug block the adhesion with respect to controls.

HRBC membrane stabilization:

Plant used:- Abutilon indicum Linn.

Family: - Malvaceae.

Part used:- Leaves.

Common name: - Atibala.

Solvent: - Ethanol, chloroform and water.

Yield: - Ethanol-4.5% w/w, chloroform- 0.45% w/w and distilled water-3,7% w/w

Dose:- 50 mg/100ml.

Route of administration: Not mentioned.

Chemical used:- Diclophenac, phosphate buffer, HRBC suspension.

Procedure: The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Alsever solution (2%) dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. The assay mixture contained the drug. 1 ml of phosphate buffer (0.15M.pH 7.4),2ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclophenac was used as reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixture were incubated at 370C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%.

Mechanism of action: HRBC membrane similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs.

Conclusion: They increasing activity at low concentration levels but decreasing activity with high concentration. They have a critical concentration (50 mg/100ml) at which their activities are maximum. The activities of all extracts are comparable to that of Diclophenac at concentration of 50 mg/100ml. The variation of activity with time was studied at different concentration, the activities in general decreased with time.

CONCLUSION:

This research underscores the promising anti-inflammatory properties of the herbal emulsion. The F4 formulation comprising ashwagandha, cinnamon, turmeric, ginger, and stevia in an emulsion, demonstrate the most effective herbal anti-inflammatory effects. Through meticulous formulation and precise incorporation of these ingredients, F4 exhibits promising potential for anti-inflammatory properties. Potentially offering a natural alternative or complimentary strategy in managing inflammatory conditions.

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